| Volume: 21 | Issue: 11 | November 2023

Experimental and Clinical Transplantation

OFFICIAL JOURNAL OF THE MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION

| www.ectrx.org | Print ISSN 1304-0855 | Online ISSN 2146-8427

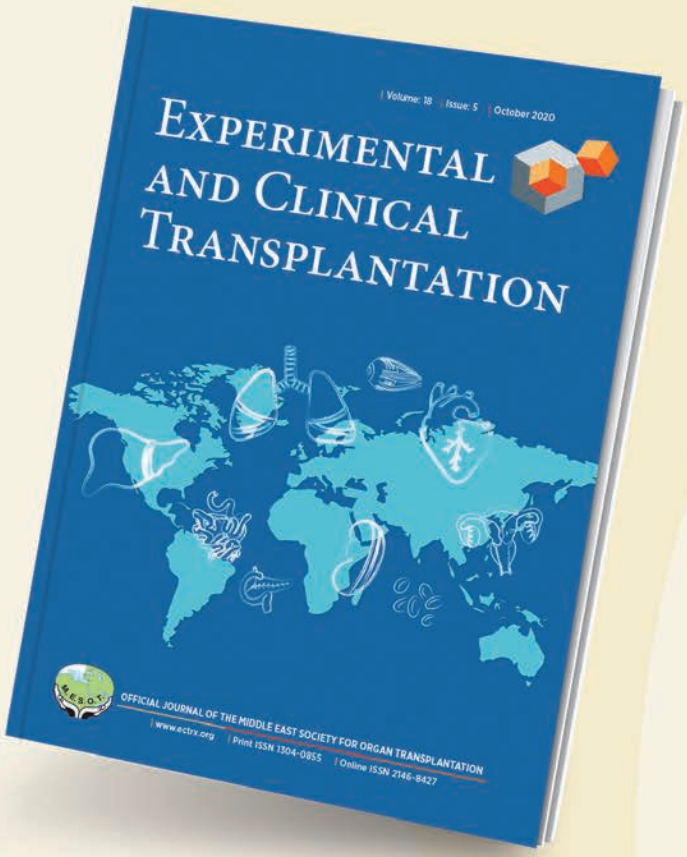
now 12 issues per year

www.ectrx.org

To Our Esteemed Colleagues,

Because of a rising interest in **Experimental and Clinical Transplantation** as well as an increased number of manuscript submissions, we are expanding our publication schedule **following October 2020 from 6 issues to 12 issues per year.** Please look for the journal to be published in **January, February, March, April, May, June, July, August, September, October, November and December.**

We would like to extend our sincere thanks for your continuing support, and we look forward to receiving your manuscripts.



http://mc.manuscriptcentral.com/ect.

Experimental and Clinical Transplantation

Official Journal of the Middle East Society for Organ Transplantation

Editor-in-Chief

Mehmet HABERAL, *Ankara, TURKEY*

Honorary Board Members

*Thomas E STARZL, *Pittsburgh, PA, USA*
*John S NAJARIAN, *Minneapolis, MN, USA*
*J Wesley ALEXANDER, *Cincinnati, OH, USA*
*George M ABOUNA, *Radnor, PA, USA*
*Nevzat BİLGİN, *Ankara, TURKEY*
*Nebil BÜYÜKPAMUKÇU, *Ankara, TURKEY*

Associate Editors

Mustafa AL-MOUSAWI, *Kuwait, KUWAIT*
Hassan ARGANI, *Tehran, IRAN*
Antoine BARBARI, *Beirut, LEBANON*
Nadey S HAKIM, *London, UK*
Refaat R KAMEL, *Cairo, EGYPT*
S Ali MALEK HOSSEINI, *Shiraz, IRAN*
Marwan MASRI, *Beirut, LEBANON*
S A Anwar NAQVI, *Karachi, PAKISTAN*
Bassam SAEED, *Damascus, SYRIA*

Senior Editorial Board Members

Roy CALNE, *Cambridge, UK*
Jeremy CHAPMAN, *Westmead, NSW, AUSTRALIA*
Iradj FAZEL, *Tehran, IRAN*
Ahad J GHODS, *Tehran, IRAN*
*Pekka HAYRY, *Helsinki, FINLAND*
Josep LLOVERAS, *Barcelona, SPAIN*
Ignazio R MARINO, *Philadelphia, PA, USA*
*Anthony P MONACO, *Boston, MA, USA*
S Adibul Hasan RIZVI, *Karachi, PAKISTAN*
Faissal A M SHAHEEN, *Riyadh, SAUDI ARABIA*
David E R SUTHERLAND, *Minneapolis, MN, USA*

Editorial Board Members

*Abdulla A AL-SAYYARI, *Riyadh, SAUDI ARABIA*
Gülnaz ARSLAN, *Ankara, TURKEY*
Hande ARSLAN, *Ankara, TURKEY*
*Mohamed Adel BAKR, *Mansoura, EGYPT*
Behrooz BROUMAND, *Tehran, IRAN*
Marcelo CANTAROVICH, *Montreal, CANADA*
J Michael CECKA, *Los Angeles, CA, USA*
Elias DAVID-NETO, *São Paulo, BRAZIL*
Gabriel E. GONDOLESI, *Buenos Aires, ARGENTINA*
William T. MERRITT, *Maryland, USA*
Nabil MOHSIN, *Muscat, OMAN*
Shahrzad OSSAREH, *Tehran, IRAN*
B. Handan OZDEMIR, *Ankara, TURKEY*
Nasser SIMFOROOSH, *Tehran, IRAN*
Jean-Paul SQUIFFLET, *Brussels, BELGIUM*
Alexander H TOLEDO, *Chapel Hill, NC, USA*
Andreas G TZAKIS, *Miami, FL, USA*
Magdi YAQOOB, *London, UK*
*Hasan YERSIZ, *Los Angeles, CA, USA*

*Deceased

BAŞKENT UNIVERSITY
publisher
www.ectrx.org

Printed by Elma Teknik Basım Matbacılık Tic. Ltd. Şti.
İvedik OSB Matbaacılar Sitesi 1516 / 1 Sokak No: 35
Yenimahalle 06378, Ankara-Türkiye (+90.312) 229 92 65

Date Printed 25 November 2023

Print ISSN 1304-0855
Online ISSN 2146-8427

EDITORIAL POLICY

MISSION

Experimental and Clinical Transplantation (ECT) is the official journal of the Middle East Society for Organ Transplantation (MESOT). The Society was originally founded in Turkey in 1987, and was subsequently incorporated at Bern, Switzerland, in 1988 as a non-profit, international, scientific organization comprising 20 countries of the Middle East, North Africa, Mid-Asia, and neighboring nations.

The aim of the journal is to provide a medium forum for where clinical scientists, basic scientists, ethicists, and public health professionals to communicate ideas and advances in the field of experimental and clinical organ and tissue transplantation, and to discuss related social and ethical issues. The topics will be of interest to transplant surgeons, clinicians in all major disciplines and subspecialties, basic science researchers, and other professionals involved with sociological aspects of experimental and clinical transplantation.

Experimental and Clinical Transplantation is a peer-reviewed international publication that accepts manuscripts of full-length original articles, case reports, letters to the editor, and invited reviews. It is published in English bimonthly (February, April, June, August, October, and December).

Our editorial team is committed to producing a journal of extremely high standards. The journal is fully indexed in EBSCO, Excerpta Medica, Index Medicus, Journal Citation Reports/ Science Edition, MEDLINE, Science Citation Index Expanded™, and Turkey Citation Index. Full-text articles are available on the Internet via PubMed or at the Journal's Web site, at **http://www.ectrx.org**. ECT is also available as hard-copy bound volumes by subscription, printed on acid-free paper.

SCOPE

The scope of the journal includes the following:

- Surgical techniques, innovations, and novelties
- Immunobiology and immunosuppression
- Clinical results
- Complications
- Infection
- Malignancies
- Organ donation
- Organ and tissue procurement and preservation
- Sociological and ethical issues
- Xenotransplantation

ETHICS

The Journal expects that all procedures and studies involving human subjects have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in **The Helsinki Declaration** as well as **The Declaration of Istanbul on Organ Trafficking and Transplant Tourism**. Manuscripts must contain a statement to this effect.

All authors are required to sign an ethical disclosure form stating that they have not been involved in commercial transactions or other unethical practices in obtaining donor organs, and that no organs or tissues from executed prisoners have been used in this research.

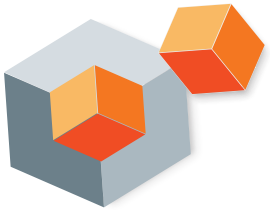
Experimental and Clinical Transplantation adheres to the ethical principles outlined by COPE (Committee on Publication Ethics).

SUBSCRIPTION RATES

MESOT Members*
Single Issue: $20.00
Annual Subscription: $100.00

Non- MESOT Members
Single Issue: $50.00
Annual Subscription: $250.00

Institutions
Annual Subscription: $1000.00



* These rates and terms are not applicable, if membership dues not paid for two consecutive years.

Twelve issues per year, appearing in January, February, March, April, May, June, July, August, September, October, November and December

Shipping Outside Turkey
- Surface Delivery: No additional charge
- Air Mail Delivery: Add $8.00 extra

Yayın Adı: Experimental and Clinical Transplantation
Yayın Türü: Yerel süreli yayın
Yayın Şekli: Aylık-İngilizce
Yayın Sahibi: Başkent Üniversitesi adına Mehmet Haberal
Sorumlu Yazı İşleri Müdürü: Mehmet Haberal
Yayının İdare Adresi: Taşkent Cad. No:77
06490 Bahçelievler, Çankaya, Ankara

Name of the Journal: Experimental and Clinical Transplantation
Type of Publication: Academic Journal
Type of Release: Bimonthly - English
Publisher: Mehmet Haberal on behalf of Baskent University
Editor in Chief: Mehmet Haberal
Address: Taskent Cad. No:77
06490 Bahcelievler, Cankaya, Ankara

EDITORIAL POLICY FOR **LIVING DONOR TRANSPLANTATION**

Dear Colleagues,

Kindly be reminded of our Editorial Policy regarding **Living Donation** in transplantation.

As per our acceptance criteria, donor must be a relative (up to the 4th degree) or spouse of the recipient and over 18 years old. We would like to **remind** all of you that as per our Journal policy, we do not accept any papers that involve transplantation from **living unrelated donors**.

In the recent period (from January 2019 to present), 662 manuscripts have been submitted to our Journal from various countries throughout the world. Out of these 662 manuscripts, a decision has been made for 554 manuscripts and **377 (68%)** of them were **rejected**. Of these 377 rejected manuscripts, **55 (14.6%)** of them have been rejected as they involved transplantation from **unrelated living donors**.

We hope that an increase in such policies will help to underline the importance of the legal and ethical aspects of transplantation. Please feel free to contact us regarding any comments as our aim is to contribute to the transplantation field in the world.

Please keep safe and healthy during these times of Covid-19 pandemic.

Sincerely,

Mehmet Haberal, MD, FACS (Hon), FICS (Hon), FASA (Hon), FIMSA (Hon), Hon FRCS (Glasg)
Editor-in-Chief
Experimental and Clinical Transplantation

INTERNATIONAL SYMPOSIUM:

PAEDIATRIC ORGAN DONATION & TRANSPLANTATION

MARCH 6-7, 2024 • KIZILCAHAMAM-ANKARA-TÜRKİYE

The Turkish Transplantation Society (TOND)
The Turkic World Transplantation Society (TDTD)
The Middle East Society for Organ Transplantation (MESOT)

www.paediatricsymp2024.com

ABSTRACT SUBMISSION DEADLINE
JANUARY 15, 2024

tts2024.org

TTS 2024

ISTANBUL TURKEY

September 22-25

30th International Congress of **The Transplantation Society**

Endorsed by

THE TURKISH TRANSPLANTATION SOCIETY (TOND)

THE TURKIC WORLD TRANSPLANTATION SOCIETY (TDTD)

Venue

ICC ISTANBUL CONGRESS CENTER

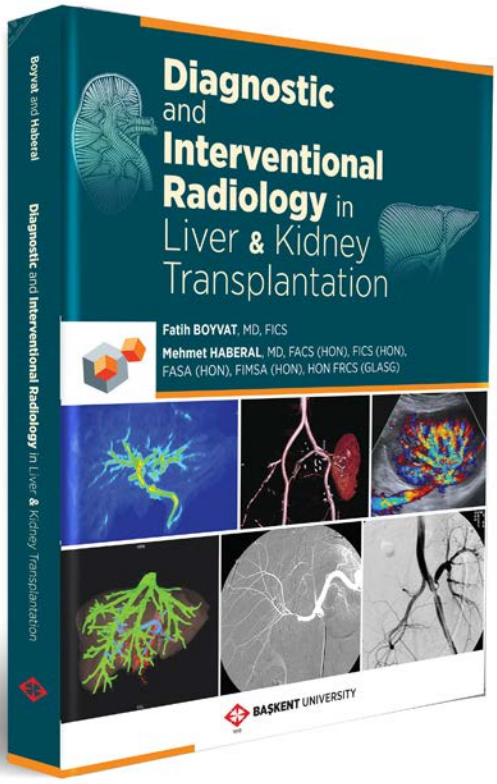
Official Congress Airline

TURKISH AIRLINES

Save the Dates!

Organized in partnership with

MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION



Diagnostic and **Interventional Radiology** in Liver **&** Kidney Transplantation

Fatih BOYVAT, MD, FICS

Mehmet HABERAL, MD, FACS (HON), FICS (HON), FASA (HON), FIMSA (HON), HON FRCS (GLASG)

Transplant medicine remains one of the most challenging and complex areas of modern medicine. Although important medical breakthroughs such as immunosuppressive drugs have allowed for more organ transplants and a longer survival rate, transplant professionals still face serious problems, especially with regard to achieving correct diagnosis and treating postoperative complications.

Advances in imaging techniques, including in computed tomography, magnetic resonance imaging, and ultrasonography, and the use of interventional radiology have allowed transplant professionals to provide more accurate results both for diagnosis and for treatment of complications that occur after liver and kidney transplant. Moreover, with the use of interventional radiology, transplant professionals can now reach deep structures of the body, enabling correct diagnoses and treatment without performing surgery.

Publisher BAŞKENT UNIVERSITY 1993

Editorial Office
Taskent Caddesi, No:77, Kat 4, Bahcelievler
Ankara, 06490 Turkey
Telephone: +90 (312) 212 73 93 Fax: +90 (312) 215 08 35
rectorate@baskent.edu.tr

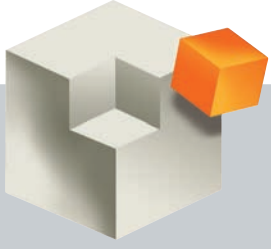
The Middle East Society for Organ Transplantation

MESOT welcomes professionals actively involved in all fields of transplantation.

The benefits of membership:

- The opportunity to be part of a regional network of physicians, surgeons and scientists involved in transplantation
- Free online access to the journal "Experimental and Clinical Transplantation", the official journal of The Middle East Society for Organ Transplantation
- Substantially reduced rates for subscription to print copies of "Experimental and Clinical Transplantation"
- Entitlement to apply to take part in a fellowship program in one of several leading transplantation centers in the Middle East
- Reduced registration fee at the biennial international congresses which provide an innovative and comprehensive overview of the latest research developments in the field transplantation
- Free online access to the "Transplant Library"
- MESOT newsletter updating members about the latest activities in the transplant community
- Access to the online MESOT Member Directory

Apply online today at **http://www.mesot-tx.org**



Experimental and Clinical Transplantation

Official Journal of the Middle East Society for Organ Transplantation

www.ectrx.org

MESOT Fellowship Program in Organ Transplantation

The Middle East Society for Organ Transplantation is pleased to announce the establishment of the MESOT Fellowship Program. The program, which will be 1-2 years in duration, has been created for physicians and surgeons from the Middle East region willing to acquire particular skills related to clinical and medical aspects of organ transplantation.

The objective of this program is to promote and advance organ transplantation in underserved areas of the region by helping physicians to establish new programs or improve already existing ones. In addition to liver, kidney, pancreas, heart and cornea transplant fellowships, training will be offered in various other departments to support the multidisciplinary nature of transplantation, including gastroenterology, nephrology, cardiology, immunology, radiology, pathology, infectious diseases and intensive care.

A limited number of grants will also be available, with recipients being determined by the Fellowship Program Committee.

Further information can be found online at **http://www.mesot-tx.org/home/fellowship.php**, where candidates may also apply online. The application deadline is the 30th of June of each year.

Inquiries may be directed to the Chairman of the MESOT Fellowship Program Committee:

Mustafa Al-Mousawi, MD, FRCS
Chairman, MESOT Fellowship Program Committee
P.O. Box 288, Safat 13003
Kuwait

Fax: +965 24848615
Email: drmosawi@yahoo.com

Realize the potential of your profession.

"As the largest and most robust surgical organization in the world, we have so much to offer surgeons of all specialties, at any point in their careers. From transition to practice support for those just starting out, to ongoing training and education, to advocacy and leadership, we help surgeons advance their careers and elevate the profession in a way no other organization can."

— Patricia L. Turner, MD, FACS,
Director, ACS Division of Member Services

Are you taking advantage of all the American College of Surgeons *has to offer?*

BECOME A MEMBER

Visit *facs.org/member-services/join* to apply for membership today as a Fellow, Associate Fellow, Resident, Medical Student, or Affiliate Member.

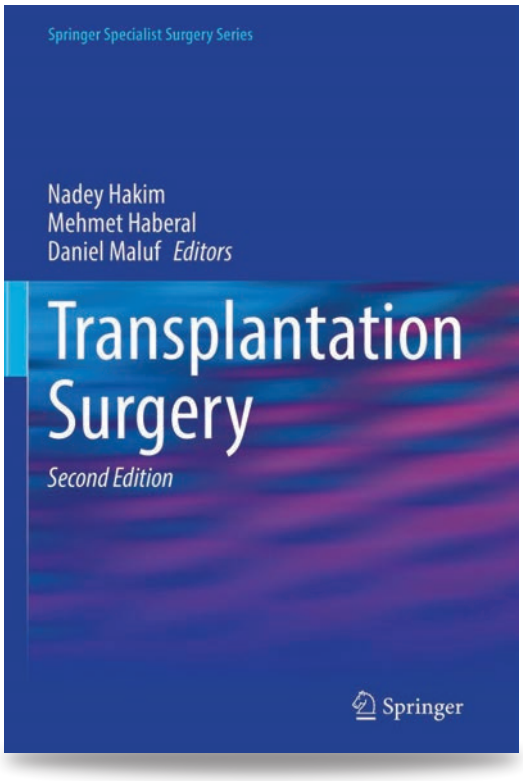
American College of Surgeons
Inspiring Quality:
Highest Standards, Better Outcomes

100+*years*

Nadey Hakim, Mehmet Haberal, Daniel Maluf (Eds.)

Transplantation Surgery

Series: Springer Specialist Surgery Series



- **Each chapter contains key questions to highlight main points**
- **Covers entire topic within a single volume**
- **Written from a global perspective by experts in the field**

This updated volume gives a clear description of transplantation surgery and covers the recent developments and innovations that have occurred within the field. New chapters on the management of graft dysfunction, organ preservation, new immunosuppressive drugs, molecular medicine and transplantation, robotics in transplantation, and organ bio-engineering are included. The book aims to be an authoritative guide to transplantation surgery that will help improve the likeliness of procedures being successful. This book will be relevant to transplant surgeons, physicians, and nephrologists.

Springer
springer.com

Experimental and Clinical Transplantation

Official Journal of the Middle East Society for Organ Transplantation

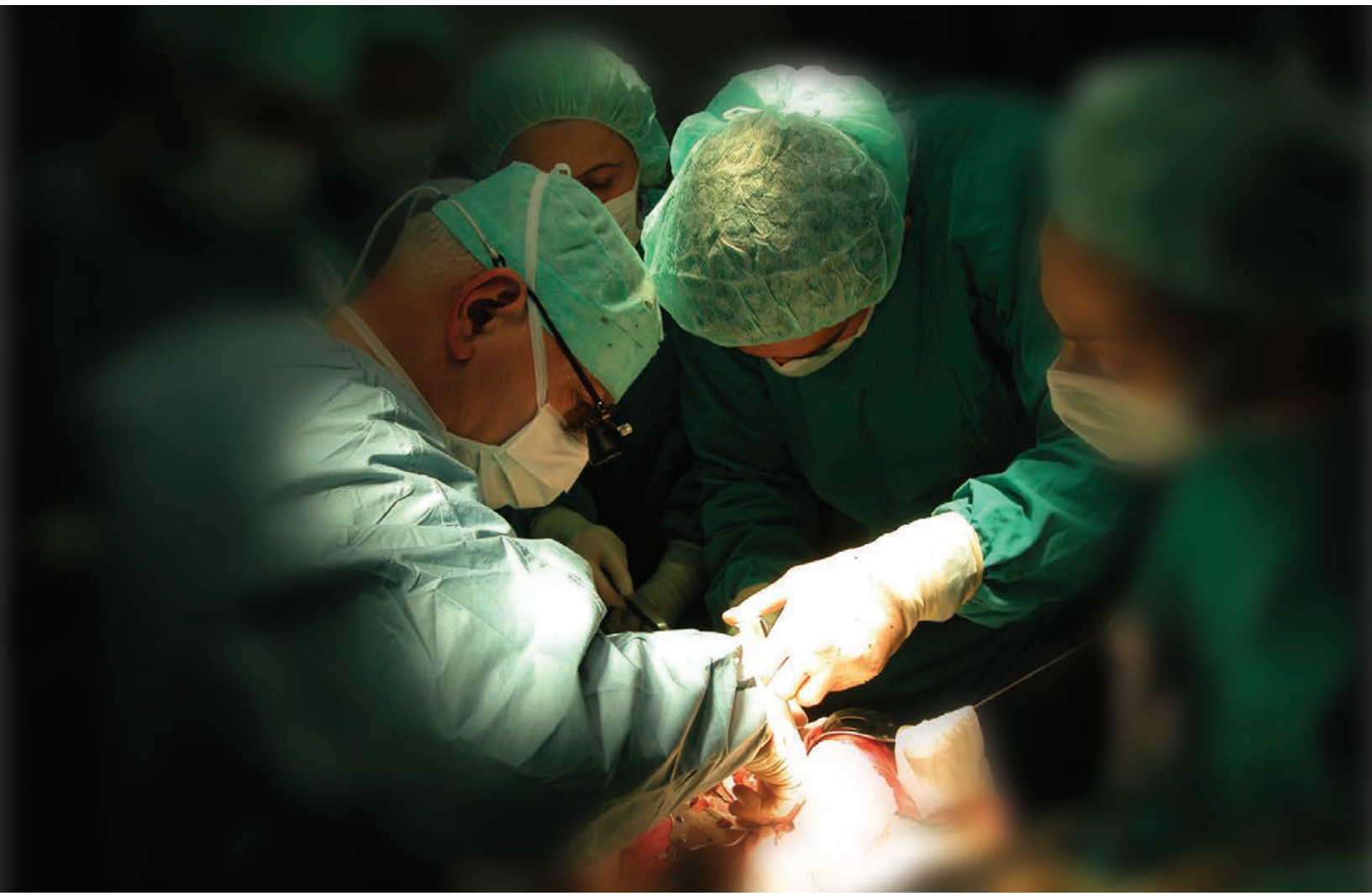
Volume: 21 Issue: 11 November 2023

Contents

BAŞKENT UNIVERSITY
FACULTY OF MEDICINE
DEPARTMENT OF GENERAL SURGERY

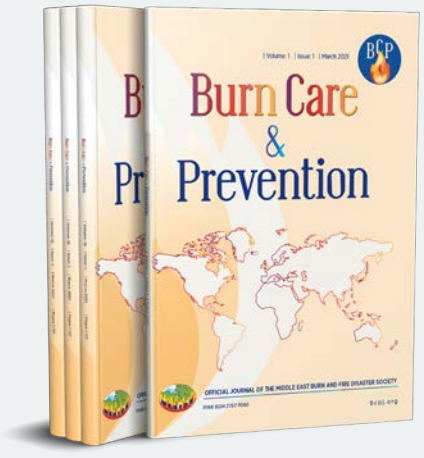
DIVISION OF
TRANSPLANTATION

Kidney & Liver Transplantation Observer & Fellowship Programs



www.baskent.edu.tr

Case Report



bcpj.org

4 issue per year

Supported by

Turkish Burn and Fire Disaster Society

Baskent University Burn and Fire Disaster Institute

WAMBES

To Our Esteemed Colleagues,

We are proud to present you with our new journal, **Burn Care and Prevention,** which will be published 4 issues a year; **March, June, September** and **December**. **Burn Care and Prevention** aims to create an arena to exchange information among all engaged in the field of burn care and prevention.

We would like to extend our sincere thanks for your support, and we look forward to receiving your manuscripts.

https://mc.manuscriptcentral.com/bcpj

BUTO

BASKENT UNIVERSITY
TOURISM & ORGANIZATION

- Group (A) travel agency - member of TURSAB (Association of Turkish Travel Agencies)
- Focus on MICE (Meetings, Incentives, Congresses, Events)
- Committed to high quality service
- A qualified international team with global experience to meet all of your organizational needs

www.buto-org.com

Lymphocele Complication After Kidney Transplant: Current Literature Review and Management Algorithm

Pedro Luis Guachetá-Bomba,[1] Maria Fernanda Sandoval Guerrero,[1] Germán Ramirez,[2,3] Herney Andres Garcia-Perdomo[1,3]

Abstract

Kidney transplant is the best treatment option for patients with end-stage renal disease. It reduces mortality and improves the quality of life. However, kidney transplant presents medical and surgical complications, and one of the most common is the posttransplant lymphocele. Lymphocele complication has an incidence of up to 20% and presents with variable clinical symptoms, which are directly associated with the size and compression effect on the adjacent organs. There are reported risk factors that favor the appearance of lymphocele. Despite known factors, there are more relevant factors (male sex, deceased donor, and corticosteroids) to carry out a stricter follow-up. The treatment of lymphoceles can vary according to the severity of the symptoms, characteristics of the collection, and the patient's clinical status. Despite the high recurrence, percutaneous intervention is the initial approach in this condition. If percutaneous aspiration, drainage, and sclerotherapy are unsuccessful, then open or laparoscopic fenestration can be performed; laparoscopy is the standard of treatment since it is highly effective and has few adverse effects.

Key words: *Kidney transplantation, Laparoscopic fenestration, Laparoscopic marsupialization, Percutaneous sclerotherapy*

Introduction

Kidney transplant is the best treatment option for patients with end-stage kidney disease.[1] This intervention significantly reduces the risk of death (60%), doubles the expected survival time, and improves the quality of life.[2] In 2018, there were 12 832 kidney transplants performed in Latin America, according to the Spanish National Transplant Organization. In Colombia, in 2019, approximately 1000 kidney transplants were performed.

Although kidney transplant is an intervention that improves the quality of life of the recipients, the incidence rate for complications is 15% to 17%.[3] These complications can be divided into medical and surgical aspects. Surgical complications can be vascular or urological problems, lymphoceles, wound infections, and hernias.[4] Medical complications are not a part of this review.

Lymphocele, as the focus of this review, is a common postoperative complication in kidney transplant recipients. Given multiple forms of presentation and treatment, we aimed to describe the current concepts for this complication and propose a management algorithm.

Definition

Lymphoceles correspond to lymphatic fluid accumulation, surrounded by a pseudo membrane, near the transplanted kidney.[5,6] Most lymphoceles develop early after transplant but generally occur 2 weeks to 6 months after the procedure. Lymphoceles have a peak of incidence at 6 weeks and are frequently detected before clinical symptoms.[7,8]

Epidemiology

Previous studies have reported the incidence of symptomatic lymphoceles between 0.6% and 26% after kidney transplant.[2,4,9-11]

From the [1]UROGIV Research Group, Universidad del Valle; the [2]Division of Urology, Clínica Imbanaco, Cali, Colombia and the [3]Division of Urology/Urooncology, Department of Surgery, School of Medicine, Universidad del Valle, Cali, Colombia

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Herney Garcia, Universidad del Valle, Cali, Colombia*
E-mail: *herney.garcia@correounivalle.edu.co*

Experimental and Clinical Transplantation (2023) 11: 855-859

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0037

Pathogenesis

The pathogenesis of a lymphocele has 2 essential steps. First, there is lymph drainage out of the lymphatic vessels, which collects in the retroperitoneal space around the transplanted kidney.[12] Later, a fibrous capsule forms and covers the lymphocele, making its resolution more complex. In such a way, lymphatic vessel injury plays an essential role in lymphocele development.[12]

Risk Factors

Assessment of individual risks before and after transplant could facilitate specific diagnoses and treatment for a patient.[13] Some factors have been implicated in the development of lymphoceles, which include the recipient's age, sex, body mass index, dialysis time, type of donor (deceased), extensive perivascular dissection during native vessel mobilization, capsular tears, episodes of acute rejection, a biopsy of the graft, presence of arteriovenous fistulas in the lower limb, use of diuretics, and high doses of corticosteroids and anticoagulants, among others.[14,15]

Heer and colleagues[8] found that the cumulative prednisone dose at 3 months is a risk factor for lymphocele development. No significant differences were found for age, donor type, single versus multiple arteries, ischemia time, or fluid balance. Pacovsky and colleagues[12] found that male sex is a risk factor for lymphoceles, and they hypothesized that the reason may be differences in protein and albumin values for male versus female sex. Their study did not find meaningful differences in age, body mass index, or dialysis time. Giuliani and colleagues[15] found that age ≥11 years, body mass index ≥95% percentile for age, male sex, and donor type (deceased) are risk factors for lymphocele formation. They also suggested that there could be twice the risk of graft loss in patients who develop lymphoceles versus patients with no lymphoceles. However, there were no statistically significant results to support this hypothesis. Presently, there is a scarcity of studies that adequately evaluate the relationship of these factors with the condition.

Prevention

The development of new technologies and advances in surgical techniques have decreased the risk of lymphocele formation. The use of bipolar electrothermal devices in surgical dissection may prevent lymphocele formation compared with electrocautery methods, creating a fusion of vessels using a combination of pressure and energy. These changes denature the collagen, elastin fibers, and the rest of the connective tissue within the vessels, which allow the proteins to form a seal and thereby fuse the walls. In this way, the lumen is theoretically obliterated.[16]

Other authors have described the use of povidone-iodine immediately after transplant, and it has been shown to reduce lymphocele formation, given its sclerosing action.[17] Use of polyethylene glycol sealants during surgery has been useful to prevent lymphocele formation without significant adverse effects.[18]

Clinical Presentation

The clinical presentation of a lymphocele varies from an asymptomatic liquid collection, to mild symptoms that are easily managed, to severe symptoms that can result in significant morbidity.[19] The symptoms are generally produced by an expansive growth of the fluid, which generates a compression effect on the adjacent organs or the graft itself. Thus, the clinical presentation of lymphoceles can be divided into 3 broad groups of symptoms: urological, vascular, or visceral.[1]

Urological symptoms

The elevation of creatinine values is one of the most frequent signs of lymphocele formation and is most often caused by the ureteral compression of the transplanted kidney, which leads to secondary hydronephrosis, renal dysfunction, and, consequently, elevation of renal function markers. Another pathophysiological mechanism that can lead to elevated creatinine is the compression of the renal vessels at the renal hilum.[5]

Lymphocele displacement of the bladder can lead to symptoms in the lower urinary tract, urinary frequency, dysuria, and urinary retention.

Vascular symptoms

Other symptoms are generated by the compression of vascular structures, iliac vessels, and even the vena cava, as well as edema of the lower limbs (with or without pain). Venous thrombosis and decreased vascular flow from the transplant may also occur.

Visceral symptoms

Visceral compression can cause abdominal discomfort and in some cases may generate compression wound dehiscence and may even present as intestinal obstruction. Another less frequent symptom is fever, which forces to rule out a lymphocele infection.[19]

Diagnosis

Diagnosis is generally made by ultrasonography combined with fluid aspiration to evaluate creatinine, lymphocytes, and gram stain results.[6] Ultrasonography is the established standard for diagnostic imaging and offers the advantages of being noninvasive, safe, easily repeatable at frequent intervals, and unaffected by the degree of kidney dysfunction. Accordingly, follow-up protocols with ultrasonography have been applied immediately after transplant, at the peak of presentation of the lymphocele (4-8 weeks), and later at 6 and 12 months.[20]

With ultrasonography, lymphoceles are visualized as either anechoic or hypoechoic. Fluid collections can be partitioned, small or large, to displace the kidney, and these are sonographically indistinguishable from other collections of simple fluid, such as urinoma and seroma.[20]

Another diagnostic method is abdominal computed tomography with contrast, which can show well-defined collections of hypoattenuating fluids that generally have an attenuation value close to water.[20]

There are no published studies regarding the comparative performance of these imaging techniques.

Treatment and Management Algorithm

Lymphocele treatment depends on its symptoms. Asymptomatic small collections do not require any treatment, and regular monitoring is sufficient. In contrast, large and symptomatic collections justify surgical intervention.

The following factors are considered necessary for proper therapy selection: severity of symptoms, size of the lesion, possible posttherapeutic complications, and the clinical condition of the patient.[1] The management of lymphoceles varies from aspiration or aspiration with sclerotherapy to more invasive techniques such as external or internal drainage. Recurrence in these treatments is high; however, laparoscopic marsupialization is the standard treatment in patients with failure of percutaneous drainage.[5]

Percutaneous Treatment

Ultrasonography-guided percutaneous aspiration is used for diagnosis and treatment. However, recurrence rates are high, up to 75%.[7] A previously published systematic review included 20 studies with 218 patients and reported a 59% rate of recurrence (95% CI, 10%-95%) for aspiration only.[21] Percutaneous drainage with a pigtail catheter has resulted in recurrence rates of 25% to 50%. Prolonged duration (longer than 2 weeks) of the pigtail catheter seems to increase the success rate.[12] However, given the minimally invasive nature of the pigtail technique, it is considered a first-line treatment for lymphoceles.[7] Some limitations that may hinder the success of percutaneous treatment are multilocular collections of fluid that complicate definitive drainage. Also, the puncture can be associated with lymphocele infection, since it converts a collection of sterile fluid to a collection with bacterial colonization.

The use of sclerosing agents for instillation in percutaneous management has produced encouraging results. However, this increases the possibility of surgical exploration in patients who do not respond due to the resultant scarring.[10] Since 1983, povidone-iodine, a protein chelator, has been used as a sclerosing agent. Recurrence of 37.5% has been reported after the initial instillation and 18.7% after the second treatment.[22] Other sclerosing agents include tetracycline, doxycycline, minocycline, fibrin gum, 95% ethanol, factor XIII, and fibrinogen. Lymphocele sclerotherapy is successful in 80% to 90% of cases, regardless of the agent used.[34] In a systematic review, Lucewicz and colleagues analyzed 14 studies and 144 patients and reported 31% recurrence for sclerotherapy.[21] Opponents of sclerotherapy emphasize the possible risk of fibrosis around the transplant, mainly close to the ureter, which may add complexity to possible future procedures.[24]

Surgical Treatment

If minimally invasive management of the lymphocele is unsuccessful, then surgical treatment

is required. This approach has been described as marsupialization. Nevertheless, de-roofing and fenestration are more precise terms. A posttransplant lymphocele can be drained into the intraperitoneal space by opening the peritoneal wall connected to the lymphocele cavity, by either an open approach or laparoscopy.[1] Fenestration is rarely followed by recurrence, and therefore judged to be superior to other treatments, and as such it may become the standard of treatment.

The systematic review by Lucewicz and colleagues also included an analysis of 17 studies with 176 patients for which they reported 16% recurrence after open surgery and a rate of 8% for intrasurgical complications.[21] The authors suggested open surgery as a promising treatment option in those patients for whom percutaneous management fails. In this same systematic review, they analyzed 22 studies and 322 patients with regard to laparoscopic surgery, for which they reported a recurrence rate of 8% and an intrasurgical complication rate of 8% (95% CI, 2%-20%).[21] The length of hospital stay for patients treated with the open technique was 5.5 days, versus 2.5 days for the laparoscopic technique.[21]

Laparoscopic surgery is an adequate and practical option that shortens hospital stays and causes minimal patient discomfort,[10] so it is considered the standard treatment for lymphoceles.

Intervention Proposal

With regard to the details we have reviewed, we suggest the management algorithm shown in Figure 1.

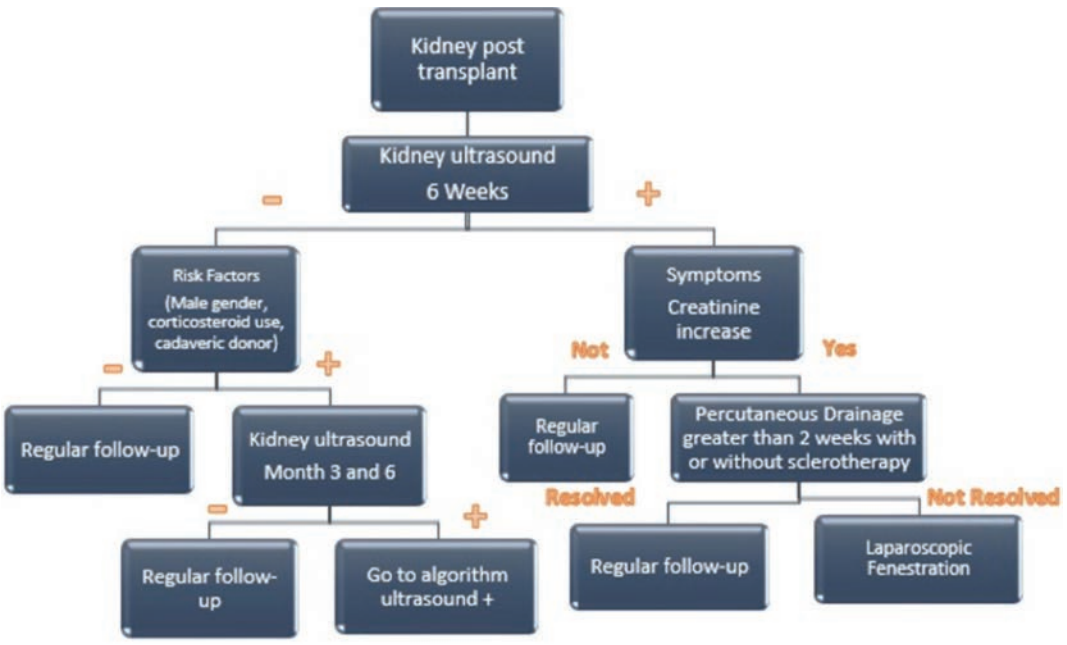
Conclusions

Lymphocele formation is a frequent postoperative complication in kidney transplant recipients, with its most frequent presentation 6 weeks after surgery. Various factors have been implicated in posttransplant lymphocele formation, and some of the most meaningful factors are the use of corticosteroids, male sex, and deceased donor. The clinical presentation is variable, from small and asymptomatic collections to symptomatic collections with a high risk of morbidity that require some intervention. Treatment can begin with percutaneous treatment, and if it there is recurrence, then we suggest laparoscopic fenestration as the standard treatment.

Kidney transplant is the best treatment option for patients with end-stage renal disease, reducing mortality and improving quality of life. However, kidney transplant is associated with medical and surgical complications, and one of the most common is the posttransplant lymphocele.

Posttransplant lymphoceles require various degrees of intervention, and the first step should be

Figure 1. Postoperative Management Algorithm for Kidney Transplant



percutaneous treatment. For subsequent lymphocele recurrence, we suggest laparoscopic fenestration should be the standard treatment. It can be started with percutaneous treatment, and if it recurs, we might offer laparoscopic fenestration as the standard treatment.

References

1. Golriz M, Klauss M, Zeier M, Mehrabi A. Prevention and management of lymphocele formation following kidney transplantation. *Transplant Rev (Orlando)*. 2017;31(2):100-105. doi:10.1016/j.trre.2016.11.001
2. Krajewski W, Dembowski J, Kolodziej A, et al. Urological complications after renal transplantation: a single centre experience. *Cent European J Urol*. 2016;69(3):306-311. doi:10.5173/ceju.2016.833
3. Reyna-Sepulveda F, Ponce-Escobedo A, Guevara-Charles A, et al. Outcomes and surgical complications in kidney transplantation. *Int J Organ Transplant Med*. 2017;8(2):78-84.
4. Ahmed RR, Halabi E, Fathi T, Al-Mousawi M. Postoperative surgical complications of live unrelated renal transplants for patients of end stage renal disease needing renal replacement therapy. *J Coll Physicians Surg Pak*. 2018;28(12):941-944. doi:10.29271/jcpsp.2018.12.941
5. Singh AG, Jai SJ, Ganpule AP, VijayKumar M, Sabnis RB, Desai MR. Critical appraisal of consecutive 36 cases of post renal transplant lymphocele: a proposed algorithm. *World J Urol*. 2017;35(9):1443-1450. doi:10.1007/s00345-016-1997-x
6. Nelson EW, Gross ME, Mone MC, et al. Does ultrasonic energy for surgical dissection reduce the incidence of renal transplant lymphocele? *Transplant Proc*. 2011;43(10):3755-3759. doi:10.1016/j.transproceed.2011.08.079
7. Smyth GP, Beitz G, Eng MP, Gibbons N, Hickey DP, Little DM. Long-term outcome of cadaveric renal transplant after treatment of symptomatic lymphocele. *J Urol*. 2006;176(3):1069-1072. doi:10.1016/j.juro.2006.04.014
8. Heer MK, Clark D, Trevillian PR, Sprott P, Palazzi K, Hibberd AD. Functional significance and risk factors for lymphocele formation after renal transplantation. *ANZ J Surg*. 2018;88(6):597-602. doi:10.1111/ans.14343
9. Dubeaux VT, Oliveira RM, Moura VJ, Pereira JM, Henriques FP. Assessment of lymphocele incidence following 450 renal transplantations. *Int Braz J Urol*. 2004;30(1):18-21. doi:10.1590/s1677-55382004000100004
10. Shaver TR, Swanson SJ 3rd, Fernandez-Bueno C, Kocandrle V. The optimal treatment of lymphoceles following renal transplantation. *Transpl Int*. 1993;6(2):108-110. doi:10.1007/BF00336654
11. Sim A, Ng LG, Cheng C. Occurrence of a lymphocele following renal transplantation. *Singapore Med J*. 2013;54(5):259-262. doi:10.11622/smedj.2013104
12. Pacovsky J, Hyspler R, Husek P, Navratil P, Brodak M. Colloid osmotic pressure participates on the post-transplant lymphocele pathogenesis. *Transplant Proc*. 2018;50(10):3422-3425. doi:10.1016/j.transproceed.2018.06.043
13. Abeling T, Scheffner I, Karch A, et al. Risk factors for death in kidney transplant patients: analysis from a large protocol biopsy registry. *Nephrol Dial Transplant*. 2019;34(7):1171-1181. doi:10.1093/ndt/gfy131
14. Khauli RB, Stoff JS, Lovewell T, Ghavamian R, Baker S. Post-transplant lymphoceles: a critical look into the risk factors, pathophysiology and management. *J Urol*. 1993;150(1):22-26. doi:10.1016/s0022-5347(17)35387-9
15. Giuliani S, Gamba P, Kiblawi R, Midrio P, Ghirardo G, Zanon GF. Lymphocele after pediatric kidney transplantation: incidence and risk factors. *Pediatr Transplant*. 2014;18(7):720-725. doi:10.1111/petr.12341
16. Lucan CV, Jurchis I, Suciu M, Selicean SE, Buttice S. Modern lymphatic dissection techniques for preventing post renal transplant lymphocele. *Clujul Med*. 2017;90(4):416-419. doi:10.15386/cjmed-716
17. Chandrasekaran D, Meyyappan RM, Rajaraman T. Instillation of povidone iodine to treat and prevent lymphocele after renal transplantation. *BJU Int*. 2003;91(3):296. doi:10.1046/j.1464-410x.2003.04028.x
18. Berardinelli L, Raiteri M, Pasciucco A, Carini M. The use of a polymeric sealant for prevention of posttransplantation lymphocele. *Transplant Proc*. 2011;43(4):1072-1073. doi:10.1016/j.transproceed.2011.03.049
19. Pollak R, Veremis SA, Maddux MS, Mozes MF. The natural history of and therapy for perirenal fluid collections following renal transplantation. *J Urol*. 1988;140(4):716-720. doi:10.1016/s0022-5347(17)41795-2
20. Kim N, Juarez R, Levy AD. Imaging non-vascular complications of renal transplantation. *Abdom Radiol (NY)*. 2018;43(10):2555-2563. doi:10.1007/s00261-018-1566-4
21. Lucewicz A, Wong G, Lam VW, et al. Management of primary symptomatic lymphocele after kidney transplantation: a systematic review. *Transplantation*. 2011;92(6):663-673. doi:10.1097/TP.0b013e31822a40ef
22. Rivera M, Marcen R, Burgos J, et al. Treatment of posttransplant lymphocele with povidone-iodine sclerosis: long-term follow-up. *Nephron*. 1996;74(2):324-327. doi:10.1159/000189329
23. Choudhrie AV, Kumar S, Gnanaraj L, Devasia A, Chacko N, Kekre NS. Symptomatic lymphocoeles post renal transplant. *Saudi J Kidney Dis Transpl*. 2012;23(6):1162-1168. doi:10.4103/1319-2442.103554
24. Williams G, Howard N. Management of lymphatic leakage after renal transplantation. *Transplantation*. 1981;31(2):134. doi:10.1097/00007890-198102000-00009

Exploring the Role of an Implantable Doppler Probe as a Blood Flow-Monitoring Device in Kidney Transplant: A Feasibility Randomized Controlled Trial

Muhammad Shahzar Malik,[1,2] Kris Houlberg,[1] Jacob A. Akoh[1]

Abstract

Objectives: **Vascular thrombosis is a disastrous postoperative complication resulting in the loss of 3.5% to 5.7% of all kidney transplants. The use of blood flow-sensing technology in the early postoperative period may help in the early identification of vascular complications crucial to reducing graft loss. This study aimed to assess the feasibility of an implantable Doppler probe as a blood flow-monitoring device in kidney transplant recipients and to evaluate its usefulness in the prevention of early graft loss.**

Materials and Methods: **This 2-arm feasibility randomized controlled trial compared the demographic characteristics and surgical outcomes of kidney transplant recipients who received implantable Doppler probe monitoring (intervention group; n = 30) with those who had standard clinical care (control group; n = 30). Surgical outcomes compared between the groups included the number of early vascular complications identified, the number of departmental ultrasonography scans requested in the first 72 hours postoperatively, and month 3 graft loss.**

Results: **Both groups were similar in demographic characteristics. In the intervention group versus the control group, fewer ultrasonography scans were requested in the first 24 hours postoperatively (56% vs 91%) and lower graft loss (0% vs 6.6%) was recorded. The results addressed uncertainties around the feasibility study's research methods and required resources for a future pragmatic trial.**

Conclusions: **An implantable Doppler probe may be a beneficial adjunct for graft monitoring after kidney transplants. This feasibility study provided the necessary preliminary information and filled initial gaps in the evidence that can inform future research. The prespecified progression criteria of the study were fulfilled. The study template used can be transferable to other transplant centers across the United Kingdom. A pragmatic large-scale randomized controlled trial is warranted to evaluate the effectiveness of implantable Doppler probes in clinical practice.**

Key words: *Blood flow-sensing technology, Graft loss, Renal transplant*

Introduction

Chronic kidney disease (CKD) is responsible for the deaths of 5 to 10 million people annually.[1] Moreover, the prevalence of CKD is increasing globally, with approximately 800 million individuals worldwide with CKD, accounting for 10% of the world's population.[2] The leading cause of CKD-associated mortality is adverse cardiac events, with rates ranging from 20% to 50% over 2 years.[2] The mortality rates of CKD patients on hemodialysis are 17 times higher than age-matched controls from the general population.[3] Patients with CKD on hemodialysis have a worse prognosis than patients with most cancers, except lung and pancreas.[4]

A successful kidney transplant is considered the best treatment for patients with end-stage renal failure.[3,5] The advantages include enhanced generalized well-being, raised quality of life, improved long-term survival, and reduced cost for healthcare systems.[3,5] Although the demand for kidney transplants has increased markedly over the years, the number of suitable grafts available for donation has only slightly increased.[6] Consequently, patients with CKD on hemodialysis needing a kidney transplant typically wait for 3 to 5 years, depending on the geographic area.[7] Different counterstrategies, like encouraging living kidney donors, accepting

From the [1]Southwest Transplant Centre, University Hospitals Plymouth, National Health Service Trust, Plymouth, and the [2]University of Plymouth, Plymouth, United Kingdom

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Muhammad Shahzar Malik, Southwest Transplant Centre, Derriford Hospital, University Hospitals Plymouth NHS Trust, PL6 8DH, United Kingdom*
E-mail: *shahzar.malik@nhs.net*

Experimental and Clinical Transplantation (2023) 11: 860-867

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0272

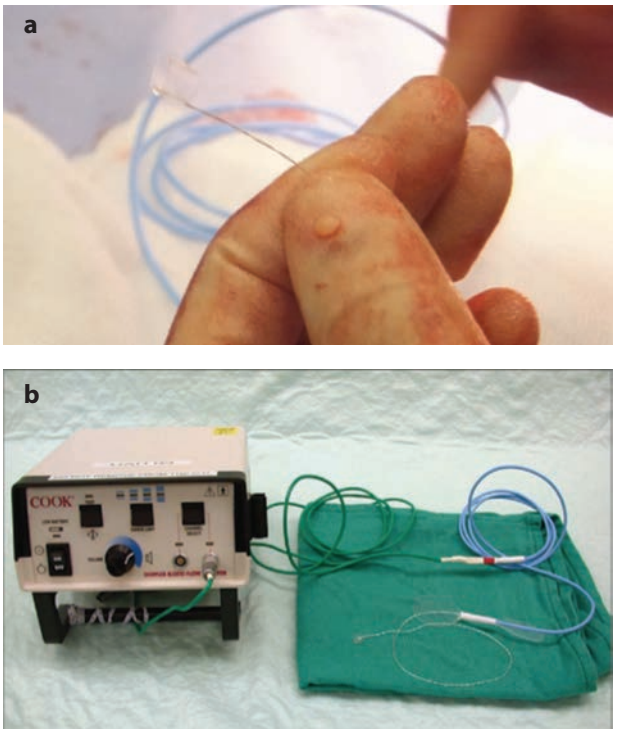
ABO-incompatible kidney transplants, and accepting extended criteria donors, have been implemented to increase the donor pool.[7] Similarly, endeavors must be made to improve the utilization of available grafts.[8]

Vascular complications result in the loss of 3.5% to 5.7% of all kidney transplants.[9,10] Graft losses due to arterial and venous complications are 0.2% to 7.5% and 0.1% to 8.2%, respectively.[10] Graft loss has serious implications for kidney transplant recipients, with 30-day and 90-day mortality rates of 5.2% and 11.1%, respectively.[11] The timely identification of vascular complications is a critical step in reducing early graft loss, as only a prompt surgical intervention can rescue a compromised graft.[12]

Novel blood flow-sensing technologies like the implantable Doppler (ID) probe, which assists in the early detection of vascular complications, have been used successfully for the surveillance of microvascular anastomosis in liver transplant, breast, plastic, and reconstructive surgery.[13] The ID probe (Cook-Swartz Doppler Probe, Cook Medical) consists of 3 parts attached together: a 1-mm^2 piezoelectric crystal, a 20-MHz transducer, and a silicon cuff[14] (Figure 1). Intraoperatively, the probe is placed around the artery supplying the grafted tissues.[14] The transducer in the probe converts the kinetic energy of the blood flowing toward the graft into electric energy.[15] The probe is linked to an external monitor through a thin connecting wire[14] (Figure 2). The external monitor translates the electrical impulses into audible Doppler signals.[15] Continuous audible signals indicate normal blood flowing toward the graft.[16] A pause in the audible signals represents a disrupted blood supply to the graft. Cessation of signals is the early warning sign that allows the opportunity for prompt intervention, as a delay would likely result in graft loss due to irreversible ischemic injury.[16] If the patient is still in the operating room, an immediate reexploration is warranted. Otherwise, if the patient has returned to the hospital ward, urgent radiological investigations (ie, duplex ultrasonography, computed tomography angiography) are organized.[17] Similar to other specialties, the ID probe may be used as a blood flow-monitoring device in kidney transplants.[16]

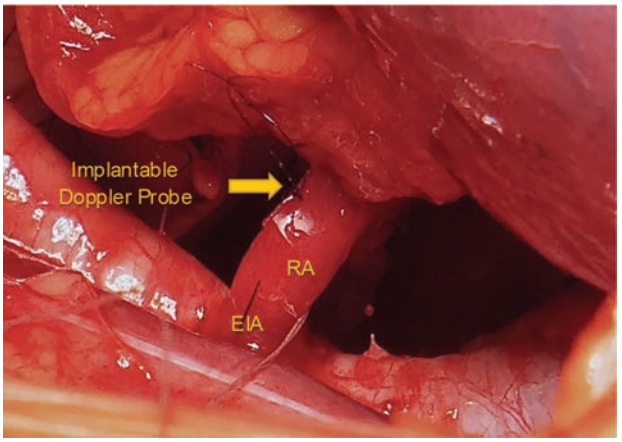
Feasibility studies are increasingly being conducted to investigate potential areas of uncertainty and support the development of future pragmatic studies.[18] This study aimed to assess the feasibility of an ID probe as a blood flow-monitoring device in kidney transplant recipients and to evaluate its usefulness in the prevention of early graft loss. The preliminary information and outcome parameters acquired in the study will inform the development of the future pragmatic large-scale randomized clinical trials.

Figure 1. Cook-Swartz Doppler Probe



a, Cook-Swartz Implantable Doppler flow probe displaying silicon cuff and flexible wire in the background. **b**, Cook-Swartz Implantable Doppler probe connecting wire and external monitoring device.

Figure 2. Cook-Swartz Implantable Doppler Flow Probe In Situ Around the Renal Artery



Abbreviations: EIA, external iliac artery; RA, renal artery

Materials and Methods

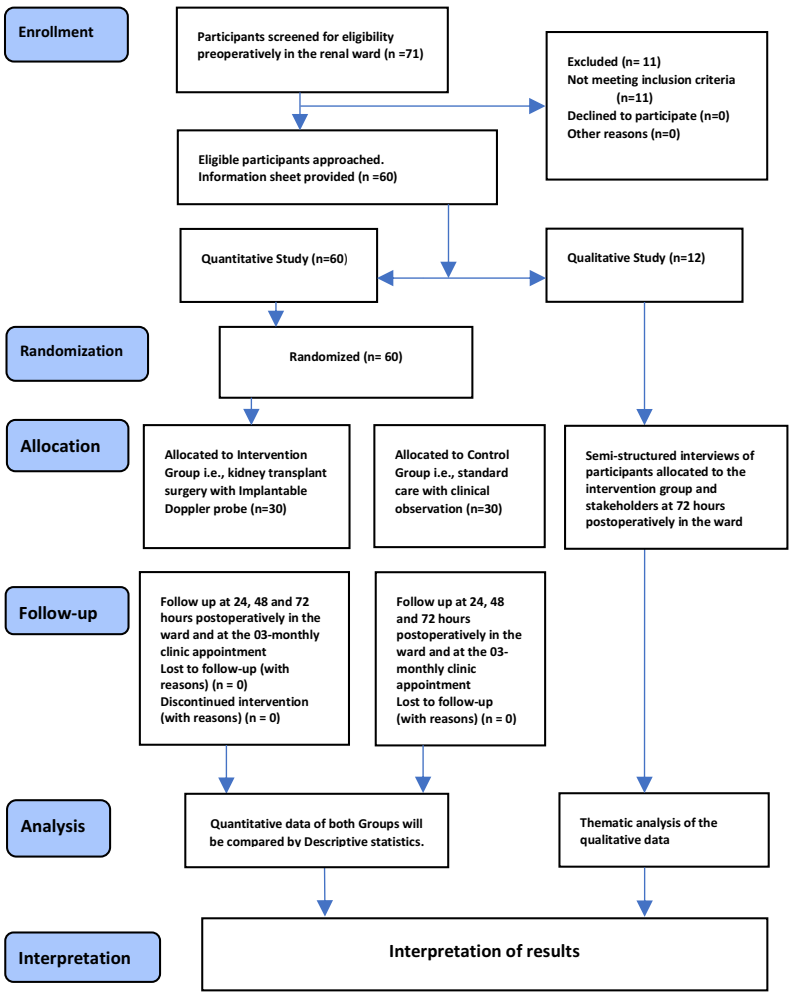
The CONDOR (Continuous Implantable Doppler Probe Monitoring in Renal Transplant) study (ClinicalTrials.gov identifier NCT05634863), a mixed method, 2-arm feasibility, randomized, controlled trial with an embedded qualitative study, was conducted at the Southwest Transplant Centre (SWTC), University Hospitals of Plymouth NHS Trust, United Kingdom, from April 2022 through July 2023 (Figure 3). The ID probe is used routinely for postoperative vascular surveillance of kidney transplant recipients at the SWTC. This allowed local recruitment of patients who had kidney transplant surgery with or without an ID probe monitoring device during the study duration at the SWTC.

Eligibility criteria

Inclusion criteria comprised patients who had deceased or living kidney donor transplants, patients aged 18 years or above, and patients able and willing to comply with the trial requirements. Exclusion criteria consisted of patients who had kidney transplants with 2 or more arteries (evident at the time of surgery), patients aged below 18 years, and patients lacking capacity or unwilling to give consent. Patients who fulfilled the eligibility criteria and consented to study participation were enrolled in the trial.

The on-call transplant surgeons obtained informed consent from eligible participants. Participants were given a detailed explanation of the research process and the functioning of the ID probe monitoring device. An information leaflet was offered.

Figure 3. Condor Study Flow Diagram



Participants received allografts from donors after circulatory death and brain death and from living kidney donors related up to the fourth degree.

Sample size

A formal power calculation is not mandatory to determine the sample size of a feasibility study.[19,20] We selected a realistic recruitment figure of 60 participants to meet the objectives of the study, in line with other similar feasibility studies in the literature.[21,22]

Randomization

We randomly assigned 60 participants into the 2 trial arms (ie, intervention and control groups) in 1:1 ratio random permuted blocks by using an online computer sequence generator (https://www.randomizer.org/#randomize).

Trial arms

The 2 trial arms (intervention and control) consisted of 30 participants each. Participants in the intervention group had kidney transplants with ID probe monitoring for the first 72 hours postoperatively, in addition to the standard clinical care as part of their postoperative management.

Intraoperatively, intervention group participants had the ID probe attached to the renal artery of their transplanted kidneys. A continuous audible signal, generated by blood flow in the renal artery, was used as an indicator of graft perfusion. Audible signals were monitored by the transplant surgeons until wound closure in the operation theatre. Postoperatively, monitoring was continued by the duty nurse during recovery and by the on-call clinician after the patient was transferred to the renal ward.

Cessation of audible signals was considered a warning sign, warranting immediate exploration or radiological investigation, depending on whether the participant was in the operating theatre or renal ward, respectively. All ID probes were removed 72 hours after the surgery.

Participants in the control group received standard clinical care as their postoperative management, in line with the SWTC protocols.

Blinding

The nature of the intervention did not allow blinding of the participants or healthcare professionals to the outcomes of randomization. However, there were no differences in the recruitment, randomization, or postoperative care of participants in the intervention or control groups. The SWTC declared no conflict of interest with the ID probe monitoring device and acted in the best interest of all kidney transplant patients.

Data collection

The feasibility study identified 3 objectives referring to relevant domains required to address potential uncertainty around a pragmatic full-scale trial.[18] Table 1 lists the feasibility objectives along with their relevant outcome measures. Objective 1 assessed the capability of an ID probe as a blood flow-monitoring device in kidney transplant patients. The outcome measures of objective 1, in both the intervention and control groups, were recorded and compared for differences.

Objectives 2 and 3 evaluated the suitability of the feasibility study's research methods and available resources for a future pragmatic trial. The outcome measures of objectives 2 and 3 highlighted barriers and challenges encountered during the conduct of this study.

Table 1. Pretransplant Demographic, Clinical, and Laboratory Data of All Study Patients

Objective 1: To assess the capability of the ID probe as a blood flow monitoring device in kidney transplant patients

- Outcome measures for objective 1
  1. Number of early vascular complications identified in each group
  2. Number of departmental ultrasonogram scans requested in the first 24, 48, and 72 hours postoperatively in each participant
  3. Number of grafts lost due to vascular complications in each group

Objective 2: To assess the research methods used in the feasibility study to inform the future pragmatic trial

- Outcome measures for objective 2
  1. Difficulties encountered with eligibility criteria and recruitment process (measured as binary variable [Yes or No])
  2. Difficulties encountered with randomization or allocation concealment (measured as binary variable [Yes or No])
  3. Difficulties encountered with retention/follow-up/compliance of participants (measured as binary variable [Yes or No])
  4. Variation or fidelity encountered in the delivery of the intervention marked against a fidelity checklist (measured as binary variable [Yes or No])

Objective 3: To assess the availability of research resources and management support in the feasibility study to inform future pragmatic trial

- Outcome measures for objective 3
  1. Availability of participant documentation (ie, participant information sheet, consent form, data collection sheet) (measured as binary variable [Yes or No])
  2. Availability of medical equipment and healthcare staff in the host center required to handle the number of participants and the research procedures (measured as binary variable [Yes or No])
  3. Availability of technological capacity for communication and adequate software to randomize, record, process, and store research data (measured as binary variable [Yes or No])
  4. Availability of management support for the research project and backup plans for any extenuating circumstances (measured as a binary variable [Yes or No])

Attainment of the 3 feasibility objectives comprised the prespecified progression criteria that would guide the trial advancement decision to the next stage.[19,20]

Prospective data collection was conducted independently for both groups. Data included participants' demographic characteristics (Table 2) and relevant outcome measures for the feasibility objectives (Table 1). Measures, time points, and location of the data collection were elaborated (Table 3).

Table 2. Collected Demographic Characteristics of Participants

1. Age at the time of transplant (in years and months; not date of birth), sex, and BMI
2. Cause of renal deterioration
3. Dialysis modality at the time of transplant
4. Time on the kidney transplant waiting list
5. Source of the donated kidney (living vs deceased donor)
6. Prior renal transplant
7. Surgeon undertaking the procedure (coded to maintain anonymity)
8. Recipient risk factors for transplant surgery (age >70 years, BMI >30, cardiovascular disease, smoking, peripheral vascular disease, thromboembolic disease, hypertension, diabetes mellitus, and urological obstructive symptoms)
9. Donor risk factors for transplant surgery (donor age >60 years, BMI >30, cardiovascular disease, smoking, peripheral vascular disease, hypertension, diabetes mellitus, cold ischemia time, cause of death, HLA mismatch, and number of vessels on the graft)

Abbreviations: BMI, body mass index (in kilograms divided by height in meters squared)

Table 3. Data Collection Measures, Time Points, and Location

Measure	Time Points After Transplant				
	Immediately after	24 hours after	48 hours after	72 hours after	3 months after
Demographic characteristics	X				
Outcome measures for feasibility objectives	X	X	X	X	X
Location	Operation theatre recovery	XWard	Ward	Ward	Clinic

Intention to treat analysis

To preserve the benefits of randomization and prevent confounding factors, the data of all participants were summarized separately in their respective groups.[18] We performed data analysis based on descriptive statistics by using IBM SPSS version 28.0. The use of inferential statistics to formally test the effectiveness of an intervention is not appropriate in a feasibility study.[19] We presented continuous variables (ie, the participant's demographic characteristics) as means and standard deviations. We presented categorical variables (ie, outcome measures for the respective feasibility objectives) as frequency distribution and percentage. We compared demographic characteristics and the outcome measures for feasibility objectives to demonstrate any substantial differences between the groups. The relative risk (risk ratio) and risk difference for the outcome measures were calculated using the following formula: risk ratio = percent in the intervention group/percent in the control group; risk difference = percent in the intervention group minus percent in the control group.

We recorded the suitability of the research processes and the availability of research resources and highlighted any difficulties or shortcomings encountered during the feasibility study.

Ethical review

The CONDOR study has been approved prospectively by the regional and national ethical committees, including Health Research Authority UK (approval reference 302833), North of Scotland Research Ethics Committee (approval reference 22/NS/0009), University of Plymouth Faculty Research Ethics and Integrity Committee (approval reference 3358), University Hospitals Plymouth NHS Trust (R&D department sponsorship 21/SUR/626.4863).

Results

Of 60 total participants, 39 (65%) were men and 21 (35%) were women. The mean age and body mass index (in kilograms divided by height in meters squared) of participants were 54 ± 16.22 years and 28.1 ± 5.22, respectively. The average wait time from the participant's activation on the waitlist to the kidney transplant at the SWTC was 601 days.

Comparison of demographic characteristics between groups

The demographic characteristics of participants in both groups were similar, allowing further comparison of their surgical outcomes (Table 4).

Comparison of the outcome measures between the groups

We found 2 vascular complications (ie, external iliac artery dissections) in the control group. Unfortunately, both complications resulted in graft loss. No complications or graft loss occurred in the intervention group. Therefore, graft loss was

0% (0/30) in the intervention group versus 6.6% (2/30) in the control group. Similarly, fewer ultraso-nography scans were requested in the first 24 hours postoperatively in the intervention group versus the control group (56% vs 91%) (Table 5). No limitations regarding the suitability of the research processes or availability of research resources were encountered. We reported results following the CONSORT updated guidelines for reporting feasibility and pilot trials.[20]

Table 4. Comparison of Demographic Characteristics of Intervention and Control Groups

Demographic Characteristic	Intervention Group (with ID probe monitoring) (n = 30)	Control Group (with standard clinical care) (n = 30)
Recipient age, mean ± SD, y	54 ± 11.12	52 ± 15.70
Recipient BMI	27.22	26.71
Sex, %		
Male	56	62
Female	44	38
Mode of dialysis, %		
Hemodialysis	52	58
PD	13	19
CKD etiology	APKD, DM, IgA nephropathy	APKD, DM, IgA nephropathy
Donor age, mean ± SD, y	57 ± 17.21	53 ± 14.44
Type of donor kidney, %		
DCD	32	33
DBD	34	31
LKD	34	36

Abbreviations: APKD, autosomal polycystic kidney disease; BMI, body mass index (in kilograms divided by height in meters squared); CKD, chronic kidney disease; DBD, donor after brain death; DCD, donor after circulatory death; DM, diabetes mellitus; ID, implantable Doppler; IgA, immunoglobulin A; LKD, living kidney donor; PD, peritoneal dialysis

Table 5. Comparison of the Surgical Outcomes of Intervention and Control Groups

Surgical Outcome	Intervention Group (with ID probe monitoring) (n = 30), No. (%)	Control Group (with standard clinical care) (n = 30), No. (%)
Ultrasonography scans performed in first 24 h posttransplant (RR = 0.62)	17 (56%)	27 (90%)
Ultrasonography scans performed in first 48 h posttransplant (RR = 1.3)	9 (30%)	7 (23%)
Ultrasonography scans performed in first 72 h posttransplant (RR = 1.7)	11 (36%)	6 (21%)
Graft loss due to vascular complications in the first 3 months posttransplant (RR = 0)	0 (0%)	2 (6.6%)

Abbreviations: ID, implantable Doppler; RR, risk ratio

Discussion

Blood flow-sensing technology with the ability to monitor the patency of microvascular anastomoses may have a beneficial role in the postoperative care of kidney transplant recipients.[17] Following the UK Medical Research Council framework, evaluating the theoretical basis of an intervention is a key step in the assessment of interventions.[18,19] This study assessed the feasibility of an ID probe as a blood flow-monitoring device and its potential role in the prevention of early graft loss. A qualitative study was embedded with the trial that aimed to test the acceptability of ID probes in clinical practice (Figure 3). Our mixed methods research design is similar to earlier studies evaluating the feasibility of interventions along with stakeholders' perceived values and potential barriers to adoption.[21,22]

Our results revealed lower graft loss and fewer early (first 24 hours) ultrasonography scans in the intervention group (with ID probe) compared with the control group (standard clinical care). In a previous retrospective cohort single-center study of 324 kidney transplant recipients, similar results were shown, with lower graft loss (1.5% vs 3.1%) and fewer first 24-hour ultrasonography scans requested (71.1% vs 83.7%) in kidney transplant recipients with ID probe monitoring compared with patients who had standard clinical care.[8]

The ID probe could have played a valuable monitoring role in the reduction of graft loss. Similarly, continuous ID probe signals may have reassured the on-call clinicians of graft perfusion, who then requested fewer ultrasonography scans. A public-patient involvement consultation involving 12 healthcare professionals with experience in the use of ID probes advocated the ID probe as a reliable postoperative blood flow-monitoring device with the potential to conserve ultrasonography resources.[23] It is worth mentioning that a compensatory rise was noted in the first 48 hours and 72 hours of ultrasonographic scans requested in the participants with ID probes; however, the difference was not as significant as in the first 24 hours (Table 5).

Some shortcomings in the diagnostic accuracy of ID probe monitoring have been reported.[17] The probe offers an indirect flow assessment of the renal vein as it can reliably detect vascular occlusion only in the vessel to which it is attached (ie, renal artery).[13] Despite the limitations, a postoperative blood flow-monitoring device may be beneficial in high-risk kidney transplant cases.[15] Crane and Hakim studied ID probe monitoring in 15 consecutive living kidney

transplant recipients and described it as a useful monitoring device.[12] Hakim and colleagues also reported a case of an ischemic graft that was rescued in time by the ID probe.[16]

Our study participants resemble the target population of a future pragmatic trial (ie, kidney transplant recipients in the UK). No discouraging factors during the recruitment process were identified. Kidney transplant patients expressed interest and willingly consented to participate in the study, as they regarded the ID probe as an additional safety net against possible vascular complications during the procedure. These findings reflect earlier observational studies that reported no recruitment issues while investigating ID probes in kidney and liver transplantation.[8,23]

The template used in this feasibility study is transferable to other transplant centers across the United Kingdom. A multicenter future pragmatic trial would support applicability by investigating the effectiveness of an ID probe across a range of settings.[24] Likewise, broad eligibility criteria used in the study allowed enrollment of both deceased and living kidney transplant recipients.[24] Less restrictive eligibility criteria reduce selection bias and ensure the sample is representative of the target population.[25] However, to maintain standardization of data collection, participants with grafts having 2 or more arteries were excluded. This group comprised 17% of all enrolled kidney transplant recipients. In a study of computed tomography angiogram images of 100 kidney transplant donors, multiple arteries were shown in 18%.[26] This figure should be accounted for during sample size calculations of future pragmatic trials.

All intervention group participants underwent ID probe monitoring for the first 72 hours postoperatively with no loss to follow-up reported. The participants recounted that ID probe signals alleviated their anxiety, and they felt reassured of the transplanted kidney. The monitoring device also did not cause any discomfort or hindrance to patient mobility. There are 2 studies that have reported avulsion of the vascular pedicle during detachment of the ID probe.[27,28] Nevertheless, the ID probes in all 30 participants were removed safely after 72 hours. No technical glitches, malfunctions, or patient complications were encountered during ID probe monitoring. Similarly, the professionals delivering the intervention displayed adherence to the protocol. The likely reason is that the ID probe is simple to attach, needs no additional operating time, and is easy to monitor by the healthcare staff.[12] The use of a probe does not require additional support or disruption to usual care.[17]

During the conduct of this study, no additional resources, provider expertise, or organizational structure were utilized, other than those available in usual practice. This was facilitated because ID probes are already in use by our hospital's plastic and reconstructive surgery department. To encourage the willingness of new sites to participate in future research, the SWTC will train professionals to deliver the intervention. However, the participating units will have to employ specialized resources.

We advocate the outcome measures of objective 1 as the primary outcomes for the future pragmatic trial as they were accurately and conveniently measured during the feasibility study (Table 1). When recorded separately and compared between the intervention and control groups, outcome measures would likely achieve a plausible difference, crucial to future decision-making of effectiveness.[8,24] The previous patient-public involvement consultation showed similar opinions on the choice of primary outcomes, as these can be easily and practically collected as part of usual care.[23] The feasibility study has also generated estimates (ie, effect size) that will be useful to inform the sample size for future research.[24]

This feasibility study was conducted in preparation for a trial with the pragmatic intention of assessing the effectiveness of an ID probe in clinical practice. The study findings of our feasibility study have addressed the uncertainties around the suitability of research processes and the availability of research resources. With attainment of feasibility objectives, we have met the progression criteria for trial advancement. Our feasibility study findings were concordant with the criteria proposed by the Readiness Assessment for Pragmatic Trials (RAPT) model.[30] The ID probe fulfills the RAPT model criteria as it has previously demonstrated to be efficacious,[12] has well-documented protocols,[8] has minimal risk of complications,[13] relies on outcomes that are already routinely measured,[16] is economical,[17] is acceptable to stakeholders,[23] aligns with the clinical requirement,[12] can be implemented within the existing healthcare system,[15] and is likely to inform clinical practice.[24]

Conclusions

An ID probe may be used as a beneficial adjunct for graft monitoring in kidney transplantation. This feasibility study has provided necessary preliminary information and filled initial gaps in the evidence. This knowledge will support the development of future research.

The prespecified progression criteria are fulfilled, and the study template can be transferable to other transplant centers across the United Kingdom. A pragmatic large-scale randomized controlled trial is warranted to evaluate the effectiveness of ID probes in clinical practice.

References

1. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. *Bull World Health Organ*. 2018 ;96(6):414D-422D. doi:10.2471/BLT.17.206441
2. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl*. 2022;12(1):7-11. doi:10.1016/j.kisu.2021.11.003
3. Kramer A, Pippias M, Noordzij M, et al. The European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry Annual Report 2015: a summary. *Clin Kidney J*. 2018;11(1):108-122. doi:10.1093/ckj/sfx149
4. Naylor KL, Kim SJ, McArthur E, Garg AX, McCallum MK, Knoll GA. Mortality in incident maintenance dialysis patients versus incident solid organ cancer patients: a population-based cohort. *Am J Kidney Dis*. 2019;73(6):765-776. doi:10.1053/j.ajkd.2018.12.011
5. Schold JD, Buccini LD, Goldfarb DA, Flechner SM, Poggio ED, Sehgal AR. Association between kidney transplant center performance and the survival benefit of transplantation versus dialysis. *Clin J Am Soc Nephrol*. 2014;9(10):1773-1780 doi:10.2215/CJN.02380314
6. NHS Blood and Transplant. Organ and Tissue Donation and Transplantation Activity report 2020/2021. Assessed May 18, 2023. https://www.organdonation.nhs.uk/helping-you-to-decide/about-organ-donation/statistics-about-organ-donation/transplant-activity-report/
7. Ietto G, Iori V, Inversini D, et al. Indocyanine green angiography for quality assessment of renal graft before transplantation: a pilot study. *Exp Clin Transplant*. 2023;21(2):110-115. doi:10.6002/ect. 2022.0339
8. Malik MS, Akoh J, Aroori S, Latour JM. Implantable Doppler probe as a vascular monitoring device in kidney transplant patients: investigation of use at a single center. *Exp Clin Transplant*. 2022;20(4):355-361. doi:10.6002/ect.2021.0500
9. Keller AK, Jorgensen TM, Jespersen B. Identification of risk factors for vascular thrombosis may reduce early renal graft loss: a review of recent literature. *J Transplant*. 2012;2012:1-9. doi:10.1155/2012/793461
10. Haberal M, Boyvat F, Akdur A, Kırnap M, Özçelik Ü, Yarbuğ Karakayalı F. Surgical complications after kidney transplantation. *Exp Clin Transplant*. 2016;14(6):587-595.
11. Pinto-Ramirez J, Garcia-Lopez A, Salcedo-Herrera S, et al. Risk factors for graft loss and death among kidney transplant recipients: a competing risk analysis. *PLoS One*. 2022;17(7): e0269990. doi:10.1371/journal.pone.0269990
12. Crane J, Hakim N. The use of an implantable Doppler flow probe in kidney transplantation: first report in the literature. *Exp Clin Transplant*. 2011;9(2):118-120.
13. Malik MS, Saif I, Akoh J. Are signals from implantable doppler probes always accurate for prediction of blood flow in kidney transplant? *Exp Clin Transplant*. 2023;21(1): 52-54. doi:10.6002/ect.2022.0318
14. COOK Medical 2021. Cook-Swartz Doppler probe. Assessed May 25, 2023. https://www.cookmedical.com/products/vas_dpsdp_webds/
15. Malik MS, Akoh JA, Houlberg K, Barwell J, Powell R. Iliofemoral polytetrafluoroethylene graft interposition: a rescue procedure in external iliac artery dissection to restore lower limb revascularization and salvage kidney transplant. *Exp Clin Transplant*. 2023;21(5):467-470. doi:10.6002/ect.2023.0062
16. Hakim DN, Nader MA, Sood A, Kandilis A, Hakim NS. Rescue of transplanted kidney thanks to an implantable Doppler probe: is this the future? *Exp Clin Transplant*. 2016;14(4):454-455. doi:10.6002/ect.2014.0135
17. Malik MS, Akoh JA, Houlberg K, The use of the implantable Doppler probe as a blood flow monitoring device in clinical settings: a narrative review of the evidence. *Exp Clin Transplant*. 2023; 21(2):83-92. doi:10.6002/ect.2022.0349
18. Chan CL, Taljaard M, Lancaster GA, Brehaut JC, Eldridge SM. Pilot and feasibility studies for pragmatic trials have unique considerations and areas of uncertainty. *J Clin Epidemiol*. 2021;138:102-114. doi:10.1016/j.jclinepi.2021.06.029
19. National Institute for Health and Care Research. Additional guidance for applicants including a clinical trial, pilot study, or feasibility as part of a personal award application. 2022. Assessed May 26, 2023. https://www.nihr.ac.uk/documents/additional-guidance-for-applicants-including-a-clinical-trial-pilot-study-or-feasibility-as-part-of-a-personal-award-application/11702
20. Eldridge SM, Chan CL, Campbell MJ, et al. CONSORT 2010 statement: extension to randomized pilot and feasibility trials. *BMJ*. 2016;355:i5239. doi:10.1136/bmj.i5239
21. Cooper H, Lancaster GA, Gichuru P, Peak M. A mixed methods study to evaluate the feasibility of using the Adolescent Diabetes Needs Assessment Tool App in paediatric diabetes care in preparation for a longitudinal cohort study. *Pilot Feasibility Stud*. 2018;4(1):1-7. doi:10.1186/s40814-017-0164-5
22. Fiallo-Scharer R, Palta M, Chewning BA, Wysocki T, Wetterneck TB, Cox ED. Design and baseline data from a PCORI-funded randomized controlled trial of family-centered tailoring of diabetes self-management resources. *Contemp Clin Trials*. 2017;58:58-65. doi:10.1016/j.cct.2017.04.007
23. Malik MS, Abideen ZU, Akoh JA, Houlberg K. Exploring views and expectations of clinicians, nurses, and kidney transplant recipients regarding protocol development of a study to investigate feasibility of the implantable doppler probe in kidney transplantation: patient-public involvement consultations. *Exp Clin Transplant*. 2023;21(4):307-316. doi:10.6002/ect.2023.0025
24. Malik MS, Akoh JA, Houlberg K. A study protocol exploring the role of an implantable doppler probe in kidney transplantation: a feasibility randomized controlled trial with an embedded qualitative study. *Exp Clin Transplant*. 2023;21(6):493-503. doi:10.6002/ect.2023.0031
25. Binet I, Bock AH, Vogelbach P, Gasser T, Kiss A, Brunner F, Thiel G. Outcome in emotionally related living kidney donor transplantation. *Nephrol Dial Transplant*. 1997;12(9):1940-1948. doi:10.1093/ndt/12.9.1940
26. Pradhay G, Gopidas GS, Karumathil Pullara S, et al. Prevalence and relevance of multiple renal arteries: a radioanatomical perspective. *Cureus*. 2021;13(10):e18957. doi:10.7759/cureus.18957
27. Anctil V, Brisebois S, Fortier PH. Free flap anastomosis leak after implantable Doppler removal. *OTO Open*. 2017;1(1):2473974X17697057. doi:10.1177/2473974X17697057
28. Hayler R, Low TH, Fung K, et al. Implantable Doppler ultrasound monitoring in head and neck free flaps: balancing the pros and cons. *Laryngoscope*. 2021;131(6): E1854-E1859. doi:10.1002/lary. 29247
29. Malik MS, Akoh JA, Houlberg K. Exploring the risk factors and preventable measures for early graft loss in kidney transplantation: a single-center analysis in the Southwest of the United Kingdom. *Exp Clin Transplant*. 2023; 21(8):639-644.doi:10.6002/ect.2023.0099
30. Baier RR, Jutkowitz E, Mitchell SL, McCreedy E, Mor V. Readiness assessment for pragmatic trials (RAPT): a model to assess the readiness of an intervention for testing in a pragmatic trial. *BMC Med Res Methodol*. 2019;19:156. doi:10.1186/s12874-019-0794-9

Parvovirus in Kidney Transplant Recipients: A Single-Center Experience

Priyash Tambi, Ruchir Dave, Vivek Kute, Himanshu V. Patel, Shivam Shah, Rahul Yadav

Abstract

Objectives: **Parvovirus testing is not done in routine clinical practice; thus, it is possible that reported parvovirus cases are just the tip of the iceberg of total prevalence. We present a single-center retrospective analysis of 22 events of parvovirus B19 anemia in 20 renal transplant recipients, among which 2 patients had recurrence.**

Materials and Methods: **For this descriptive analytical study, parvovirus B19 disease was defined as parvovirus infection (detection by real-time polymerase chain reaction) in the presence of anemia with clinical symptoms or bone marrow biopsy findings consistent with the diagnosis. Study duration was 18 months, from June 2021 through December 2022, and patients were enrolled from a single center.**

Results: **All patients detected with the virus had received induction with thymocyte globulin and were on standard triple drug immunosuppression. Mean age was 32 ± 12 years with median time to diagnosis of 2 months after transplant. Anemia was observed in all patients with mean hemoglobin level at presentation of 6.02 ± 1.28 g/dL. Creatinine at presentation was 1.49 mg/dL (interquartile range, 0.92-2.69 mg/dL). The most common presentation was asymptomatic patient with evaluation for anemia. During therapy, the highest median creatinine level was 2.0 mg/dL (interquartile range, 1.38-3.2 mg/dL), which was significantly higher than that at presentation (*P* < .018). After therapy, median creatinine level was 1.3 mg/dL, which was not significantly higher than the baseline level, demonstrating a mostly transient graft dysfunction.**

Conclusions: **Parvovirus B19 is a relatively underreported disease in renal transplant recipients, with patients presenting with anemia and the disease causing transient graft dysfunction. Parvovirus B19 infection responds well to a decrease in immunosuppression and intravenous immunoglobulin therapy.**

Key words: *Anemia, Graft dysfunction, Renal transplantation*

Introduction

Parvovirus B19 is a single-stranded DNA virus that universally infects humans. In immunocompetent people, the infection is usually cleared by the immune system, with the systemic manifestations being limited to fever and viral exanthem for those presenting in childhood. About 60% to 90% of people have antibodies against parvovirus B19.[1,2] However, this is not the case in immunocompromised patients, with parvovirus B19 causing severe anemia. The first case of parvovirus B19 infection posttransplant was reported in 1986[3]; the infection can be seen in any solid-organ transplant and in hematopoietic stem cell transplant. Anemia is the most common manifestation.[4] Parvovirus B19 infection can also cause allograft dysfunction in solid-organ transplants, like renal transplant.[5] Anemia often occurs after transplant, and there are many contributing factors, such as perioperative blood loss, preexisting chronic inflammatory state, iron deficiency, and induction and maintenance drugs used for transplant.[4] Parvovirus B19 testing is not done in routine clinical practice; therefore, it is possible that reported parvovirus B19 cases are just the tip of the iceberg of total prevalence.

We present a single-center retrospective analysis of 22 events of parvovirus B19 anaemia in 20 renal transplant recipients, among which 2 patients had recurrence.

From the Department of Nephrology and Transplantation, Institute of Kidney Disease and Research Center, Institute of Transplantation Sciences (IKDRC-ITS), Ahmedabad, Gujarat, India

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest. Data are available from the corresponding author on reasonable request.*

Corresponding author: *Priyash Tambi, Department of Nephrology, IKDRC-ITS, Ahmedabad, 380016 Gujarat, India*
Phone: *+91 954 938 76 57* ***E-mail:*** *priyashtambi@gmail.com*

Experimental and Clinical Transplantation (2023) 11: 868-871

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0127

Materials and Methods

Case definition

Parvovirus P19 infection is diagnosed by either serology or direct viral detection in clinical specimens, such as blood, bone marrow, and other solid organs like kidney, liver, and lung.[6] Parvovirus B19 serology may not be completely reliable for diagnosis in immunocompromised patients due to inadequate immune response.[7,8] Only patients with positive polymerase chain reaction test confirmation of parvovirus P19, with corroborative clinical findings cases, were enrolled in our study. Study duration was 18 months, from June 2021 through December 2022, and patients were enrolled from single center.

Statistical analyses

We used descriptive statistics for data analyses, including presentation in mean ± SD, median, and interquartile range (IQR).

Results

During the study period, 540 kidney transplant procedures were done at our center. Of these, 20 kidney transplant recipients (with total of 22 events) with parvovirus B19 disease were enrolled; 1 patient had simultaneous kidney and pancreatic transplant. Of the 20 patients, 6 (27.3%) were deceased donor transplant recipients (all others were living donor transplant recipients). Ten patients (45.5%) received grafts from parents, 2 (9.1%) from spouses, and 1 from grandparent (4.6%). Three patients (13.6%) received grafts through a living related kidney paired donation program.

All patients detected with the virus had received induction therapy with thymocyte globulin (either rATG or Grafalon) and were on standard triple drug immunosuppression. None of included patients had received interleukin 2 (Simulect) as induction therapy. Among study patients, mean age was 32 ± 12 years, and 15 (75.0%) were male patients (the patient representation may be biased because of the higher number of renal transplant recipients among males in our country).

Median time to onset of symptoms was 2 months (IQR, 1.0-7.5 mo) after transplant. Anemia was observed in all patients, with mean hemoglobin level at presentation of 6.02 ± 1.28 g/dL. Mean hematocrit value at presentation was 19.57 ± 4.28. Leukopenia was observed in 6 patients (with 1 patient having recurrence). The mean leukocyte count at presentation was $6.27 \pm 3.84 \times 10^9$ cells/L, with lowest white blood cell count (WBC) of 2.1×10^9 cells/L. Two patients had thrombocytopenia, with 1 patient having blood stream infection/sepsis as etiology and the other patient having pancytopenia. Mean platelet count was $230 \pm 131 \times 10^9$ cells/L. Patients had a median baseline creatinine level of 1.33 mg/dL (IQR, 1.00-1.61 mg/dL) before infection. At time of presentation with infection, median creatinine level was 1.49 mg/dL (IQR, 0.92-2.69 mg/dL), showing no significant difference.

The most common presentation was asymptomatic presentation with evaluation for anemia in 11 patients (50.0%) followed by dyspnea on exertion in 6 patients (27.3%). The common presentation of asymptomatic with anemia evaluation may be because the median time to parvovirus B19 illness was about 2 months posttransplant, when patients were under regular and more intense follow-up. Patients were immunosuppressed during the initial 3 months posttransplant after induction therapy and on higher doses of maintenance immunosuppression. Six events of infection (27.3%) occurred after antirejection therapy: 5 patients had antibody-mediated rejection and 1 patient had T-cell-mediated rejection. One patient (4.5%) was treated with plasmapheresis for posttransplant recurrence of glomerulonephritis (focal segmental glomerulosclerosis).

One patient with parvovirus B19 who had disease recurrence came with complaints of severe abdominal pain. On evaluation, the patient had acute pancreatitis and epididimo-orchitis with generalized sepsis. He later developed acute respiratory distress syndrome and needed intensive care unit hospitalization. All patients were treated with 2 g/kg intravenous immunoglobulin therapy, with decrease in antiproliferative therapy by about 50% as per institutional protocol and further reduction if required as per clinical indication. Packed red blood cell or packed cell volume was transfused if needed per clinician discretion.

During therapy, mean hemoglobin was 5.9 ± 1.09 g/dL, which was not significantly lower than hemoglobin at presentation. After therapy (after an average of 5.4 ± 2.4 weeks), hemoglobin level increased to 10.9 ± 1.6 g/dL, which was significantly higher than level at baseline or presentation ($P < .005$).

During therapy, the highest median creatinine level was 2.0 mg/dL (IQR, 1.38-3.2 mg/dL), which was significantly higher than the level at presentation ($P < .018$). After therapy, creatinine level decreased, with median posttherapy level of 1.3 mg/dL (IQR, 1.00-1.72 mg/dL), which was not significantly higher than the baseline or presentation creatinine level ($P = .2$). This demonstrates a mostly transient graft dysfunction, with graft function returning to baseline posttherapy.

Two patients experienced graft loss, with 1 loss because of renal vein thrombosis and the other loss because of severe sepsis-induced graft dysfunction. This patient had prolonged intensive care unit and hospital stays and eventual renal allograft loss.

Discussion

The overall estimated incidence of positive parvovirus B19 DNA among kidney transplant recipients has been reported as 10.3%. Among kidney transplant patients with anemia, the incidence rate of positive parvovirus B19 DNA has been reported as 27.4%.[9]

In a prospective study by Huang and colleagues, 118 kidney transplant recipients were followed up and their plasma samples were screened for parvovirus B19 DNA copies by polymerase chain reaction and serology for immunoglobulin M (IgM) and IgG weekly for 1 month and then monthly for 6 months. Among the study population, 10.17% had parvovirus infection and showed a significant drop in hemoglobin and reticulocyte count but no significant renal dysfunction.[10]

In another study on posttransplant parvovirus B19 infections, Eid and colleagues studied patients over a 16-year period (1990-2005) and documented 7 patients with parvovirus B19 (6 were renal transplant recipients and 1 was a heart transplant recipient); median time to onset of symptoms was 1.5 months.[11] This result was comparative to our study, where median time to onset of symptoms was 2 months (IQR, 1.0-7.5 mo) after transplant (Table 1). Anemia was observed in all patients in their study (hemoglobin level ranging from 4.9-6.8 mg/dL), which was also comparative to our study, where we observed mean hemoglobin level at presentation of 6.02 ± 1.28 g/dL, ranging from 4.0 to 8.9 mg/dL. Leukopenia was observed in 5 patients (71.4% of patients) in the study from Eid and colleagues, with WBC ranging from 2.0 to 2.5 × 10^9 cells/L. In our study, only 6 cases (27.3%) had leukopenia, of which 1 was recurrence. In our study, the mean leukocyte count at presentation was 6.27 ± 3.84 × 10^9 cells/L, with lowest WBC count of 2.1 × 10^9 cells/L.

Allograft dysfunction was observed at the time of parvovirus B19 infection in 1 patient (14.28%) in the study from Eid and colleagues. In our study, 6 patients (27.3%) had no allograft dysfunction, 13 patients (59.1%) had transient rise of creatinine, which returned to baseline after therapy and rationalization of medication, and 3 patients (13.6%) had renal allograft dysfunction that was persistent in nature. Two of our patients experienced graft loss (9.1%); however, Eid and colleagues reported all patients alive with functioning allografts.

Eid and colleagues also compared their cases with other 91 cases in the literature (98 cases, all solid-organ transplant and hematopoietic stem cell transplant recipients with parvovirus B19). The investigators showed the median time to onset of parvovirus B19 disease was 1.75 months (range, 1 week to 96 months) after transplant. In 65% of patients, the onset was within 3 months post-transplant. The most common manifestation was anemia (98.8% of all patients), with patients

Table 1. Comparison of Patients With Parvovirus Infection

Patient Characteristics	Present Study	Hegde et al[12]	Eid et al[11]	Kidney Transplant Recipients*	All Patients*
Age, means ± SD, y	32 ± 12			36.7 ± 15.5	35.3 ± 17.1
Median time to onset, mo	2	1.3	1.5	1.25	1.75
Anemia, %	100	100	100	98.1	98.8
Lowest hemoglobin level, mean ± SD, mg/dL	6.02 ± 1.28	6.49 ± 1	4.9-6.8 (range)	6.2 ± 1.2	6.4 ± 1.5
Leukopenia, %	26.2	20%	71.4	64.2	38.3
Lowest WBC level, mean ± SD, mg/dL	6.27 ± 3.84		2.0-2.5 (range)	1.2 ± 1.2	2.1 ± 1
Graft loss/dysfunction, %	72.7	50	14.28	15.6	10.6

Abbreviations: WBC, white blood cell

*Cases reviewed by Eid and colleagues were separated into kidney transplant recipients and all solid-organ and hematopoietic stem cell transplant patients combined.

presenting clinically with weakness, dyspnea, and orthostasis. Leukopenia was observed in 37.5% of patients, and thrombocytopenia was observed in 21.0% of patients. Allograft loss, rejection, or dysfunction occurred in 10.4% of patients. Among these cases, 53 were renal transplant recipients (54%) with mean age of 36.7 ± 15.5 years, 63.2% male patients, and median time to onset posttransplant of 1.25 months. Mean low hemoglobin level was 6.2 ± 1.2 g/dL. Leukopenia was observed in 34 patients (64.2%), with lowest mean WBC of $1.2 \pm 1.2 \times 10^9$ cells/L and allograft dysfunction observed in 15.6% of patients. None of the renal transplant recipients died of parvovirus B19. These results were similar to our study, except our patients had less frequent leukopenia.

Hegde and colleagues evaluated renal transplant from 2013 to 2020 with persistent anemia and reported 71 patients who were tested for parvovirus B19, of which 20 patients had positive tests. Median time to detection of parvovirus B19 was 1.3 months posttransplant. Mean hemoglobin was 6.49 ± 1 g/dL. Leukopenia and thrombocytopenia were present in 20% and 10% of patients, respectively. Graft dysfunction was observed at the time of parvovirus B19 disease in 10 patients (50.0%) (Table 1).[12]

Our study was similar to a Korean study from Baek and colleagues, which demonstrated no difference in long-term allograft outcome that could be attributed to parvovirus B19 infection.[13] In our study, the cases of graft loss had other causative factors, such as severe sepsis leading to acute allograft dysfunction in 1 of the cases and renal vein thrombosis in the other case.

Conclusions

A high index of suspicion is needed for detection of parvovirus B19 infection in posttransplant patients presenting with anemia. The risk of parvovirus B19 infection is significantly higher in patient groups with higher immunosuppression, particularly in those who had treatment for graft rejection and more so if in the immediate posttransplant period. The typical course is benign, with only transient graft dysfunction that responds well to a decrease in immunosuppression and intravenous immunoglobulin treatment. If resources permit, a routine parvovirus B19 screening in all transplant recipients is recommended, with close follow-up to further study the prevalence of this disease, which might be missed if only screening and testing patients at high risk of infection.

References

1. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. *Lancet*. 1975;1(7898):72-73. doi:10.1016/s0140-6736(75)91074-0
2. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus 19 in England and Wales. *J Med Microbiol*. 1988;25(2):151-153. doi:10.1099/00222615-25-2-151
3. Neild G, Anderson M, Hawes S, Colvin BT. Parvovirus infection after renal transplant. *Lancet*. 1986;2(8517):1226-1227. doi:10.1016/s0140-6736(86)92245-2
4. Rerolle JP, Morelon E, Helal I, Peraldi MN, Mamzer-Bruneel MF, Kreis H. Parvovirus B19-related anaemia after renal transplantation. *Scand J Infect Dis*. 2004;36(6-7):513-516. doi:10.1080/00365540410020244
5. Cavallo R, Merlino C, Re D, et al. B19 virus infection in renal transplant recipients. *J Clin Virol*. 2003;26(3):361-368. doi:10.1016/s1386-6532(02)00104-x
6. Eid AJ, Ardura MI; AST Infectious Diseases Community of Practice. Human parvovirus B19 in solid organ transplantation: guidelines from the American society of transplantation infectious diseases community of practice. *Clin Transplant*. 2019;33(9):e13535. doi:10.1111/ctr.13535
7. Broliden K. Parvovirus B19 infection in pediatric solid–organ and bone marrow transplantation. *Pediatr Transplant*. 2001;5(5):320-330. doi:10.1034/j.1399-3046.2001.00035.x
8. Kurtzman GJ, Ozawa K, Cohen B, Hanson G, Oseas R, Young NS. Chronic bone marrow failure due to persistent B19 parvovirus infection. *N Engl J Med*. 1987;317(5):287-294. doi:10.1056/NEJM198707303170506
9. Thongprayoon C, Khoury NJ, Bathini T, et al. Epidemiology of parvovirus B19 and anemia among kidney transplant recipients: a meta-analysis. *Urol Ann*. 2020;12(3):241-247. doi:10.4103/UA.UA_89_19
10. Huang Q, Wang Y, Chen R, et al. Parvovirus B19 infection in kidney transplant recipients: A prospective study in a teaching hospital in Shanghai, China. *Transpl Immunol*. 2022;74:101667. doi:10.1016/j.trim.2022.101667
11. Eid AJ, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis*. 2006;43(1):40-48. doi:10.1086/504812
12. Hegde U, Mittal A, Rajapurkar M, et al. Parvovirus disease post renal transplant presenting as refractory anemia-single centre experience (Abstract POS-731). *Kidney Int Rep*. 2021;6(4).
13. Baek CH, Kim H, Yang WS, Han DJ, Park SK. Risk factors and long-term outcomes of parvovirus B19 infection in kidney transplant patients. *Transpl Infect Dis*. 2017;19(5). doi:10.1111/tid.12754

TCF7L2 (rs7903146) But Not CDKAL1 (rs7754840) Gene Polymorphisms Increase the Risk of New-Onset Diabetes After Kidney Transplant

Özant Helvaci,[1] Berfu Korucu,[2] Haci Hasan Yeter,[2] Sevim Gönen,[3] Burçak Cavnar Helvaci,[4] Yavuz Sanisoğlu,[5] Musa Bali,[2] Galip Güz[2]

Abstract

Objectives: **Incidence of new-onset diabetes after transplant negatively affects graft and patient survival. Obesity, impaired fasting glucose before transplant, and a history of diabetes in first-degree relatives are well-defined risk factors. TCF7L2 and CDKAL1 gene polymorphisms have been implicated in the pathogenesis. We investigated the effect of single gene polymorphisms of TCF7L2 (rs7903146) and CDKAL1 (rs7754840) on new-onset diabetes in renal transplant recipients.**

Materials and Methods: **We evaluated 239 renal transplant recipients. TCF7L2 and CDKAL1 gene polymorphisms were assessed by polymerase chain reaction.**

Results: **Mean patient age was 43 ± 13 years. There were 148 male patients (61.9%), and 91 were female (38.1%). New-onset diabetes was detected in 55 patients (23%). In 20 cases (36%), the glycemic disorder was transient; 61% of patients required insulin therapy. In terms of CDKAL1, 108 patients had the wild-type allele, 112 had a single-allele mutation, and 19 had a 2-allele mutation (45.2%, 46.9%, and 7.9%, respectively). In terms of TCF7L2, 163 of the patients had the wild-type allele, 49 had a single-allele mutation, and 27 had a 2-allele mutation (68%, 20%, and 11%, respectively). New-onset diabetes-related factors were age at transplant, body mass index after transplant (calculated as weight in kilograms divided by height in meters squared), tacrolimus, mycophenolate, and TCF7L2 polymorphism but not CDKAL1 polymorphism. After multiple regression analysis, the effect of TCF7L2 polymorphism persisted. A single allelic change resulted in a risk factor 1.4 times higher for new-onset diabetes after transplant (*P* = .043; 95% CI, 1.142-1.874) and a double allelic change was 2.7 times higher (*P* < .01; 95% CI, 1.310-4.073)**

Conclusions: **TCF7L2 (rs7903146) gene polymorphism is an independent risk factor for new-onset diabetes in Turkish renal transplant patients. This study is the first in Turkey to show the distribution and effect of these genes in kidney transplant patients.**

Key words: *Diabetes mellitus, Posttransplant diabetes mellitus, Renal transplant*

Introduction

Renal transplant is the preferred treatment for end-stage renal disease.[1] New-onset diabetes after transplant (NODAT) is an important and frequent complication in kidney transplant recipients. New-onset diabetes after transplant is associated with reductions in patient and organ survival. The prevalence has been reported to range from 10% to 40% in series, dependent on the definition of diabetes.[2-4] The risk factors are age over 40 years, obesity, impaired fasting glucose before transplant, hepatitis C virus (HCV) seropositivity, ethnicity (Black or Hispanic), and family history.[5-8] Furthermore, glucocorticoids, calcineurin inhibitors, and rapamycin also increase risk.[2,9,10]

In the past 3 decades, science has witnessed the discovery of numerous genes related to type 2 diabetes. More recently, genome-wide-association studies revealed the association between several single-nucleotide polymorphisms (SNP) and diabetes, one of the most important of which is the transcription factor 7 like 2 (TCF7L2).[11] In 2008, Kang and colleagues proved the close relation between TCF7L2 SNPs and NODAT for the first time.[12]

From the [1]Yildirim Beyazit University, Yenimahalle Education and Research Hospital, Ankara, Turkey; the [2]Department of Nephrology, Gazi University, Ankara, Turkey; the [3]Department of Immunology, Gazi University, Ankara, Turkey; the [4]Department of Endocrinology, Ankara City Hospital, Ankara, Turkey; and the [5]Department of Biostatistics, Yildirim Beyazit University, Ankara, Turkey

Acknowledgements: *All expenditures of this study were supported by the Gazi University Scientific Research Project Unit. Other than described above, the authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no further declarations of potential conflicts of interest.*

Corresponding author: *Özant Helvaci, Yildirim Beyazit University, Yenimahalle Education and Research Hospital, Kardelen, Ankara, Turkey*

Phone: *+90 533 162 78 21* ***E-mail:*** *drozant@hotmail.com*

Experimental and Clinical Transplantation (2023) 11: 872-878

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2020.0335

Many other polymorphisms are also associated with NODAT to some extent. In a recent meta-analysis, Benson and colleagues evaluated 18 single-gene polymorphisms of 12 genes and found 3 SNPs invariably associated with NODAT.[13] These were TCF7L2 rs7903146, CDK5 regulatory subunit associated protein 1 like 1 (CDKAL1) rs10946398, and potassium voltage-gated channel subfamily Q member 1 (KCNQ1) rs2237892 gene polymorphisms.

TCF7L2 protein functions as a transcription factor encoded by the gene of the same name. The TCF7L2 gene is located on chromosome 10q and is inherited via autosomal dominant fashion. It affects various biological pathways, including the Wnt signaling pathway and insulin metabolism. The β cells of the pancreas extensively express TCF7L2.[14] Individuals with gene polymorphisms have a higher risk of diabetes, NODAT, gestational diabetes, and obesity.[15] These may also increase the risk of coronary artery disease and hyperlipidemia. TCF7L2 rs7903146 C-to-T is the most common polymorphism.

The CDKAL1 gene encodes the protein of the same name. This protein is a methylthiotransferase with a function that is not clearly understood. It is thought to cause susceptibility to diabetes by disruption of insulin secretion. CDKAL1 rs7754840 G-to-C is the most common polymorphism.[16]

Several studies demonstrated the association between TCF7L2 rs7903146 and diabetes and obesity in nontransplanted Turkish patients.[17-19] To our knowledge, no data on CDKAL1 rs7754840 SNP in a Turkish population cohort exist. KCNQ1 rs2237892 SNP has been extensively studied in Asian populations, and its association with diabetes and NODAT in the Caucasian population is questionable.[13,20]

In this study, we investigated the association between TCF7L2 rs7903146 and CDKAL1 rs10946398 polymorphisms with NODAT risk in our kidney transplant recipients.

Materials and Methods

Study population and ethical statement

This study was conducted in our Nephrology and Transplantation department. Kidney transplant recipients who were older than >18 years of age and agreed to participate were included in the study. We recruited 239 recipients of living or deceased donor organs. Patients received transplants between 1990 and 2016. Informed consent was obtained from all patients to be included in the study. Patients who did not want to give informed consent, those who were followed up for less than 2 years, and patients with a diagnosis of pretransplant diabetes were excluded. The clinical features of the patients such as age, sex, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared), primary kidney disease, transplant type, dialysis duration, and modality before transplant and comorbid conditions were recorded. Previous and current medical treatments regarding immunosuppression and NODAT were also noted. Creatinine levels and glomerular filtration rates (GFR) of the patients in the first and the last years after transplant were noted. We used the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) to estimate GFR.

This study protocol conformed to ethical guidelines of the Declaration of Helsinki, and all participants gave written informed consent and willingness to participate. The ethics committee of Zekai Tahir Burak Research and Training Hospital approved the study protocol.

Definition of new-onset diabetes after transplant

American Association of Endocrinology 2016 criteria were used for diagnosis, as follows: (1) fasting plasma glucose ≥126 mg/dL, or (2) incidental plasma glucose + diabetes symptoms ≥200 mg/dL, or (3) oral glucose tolerance test 2-hour plasma glucose = 200 mg/dL, or (4) hemoglobin A1c (HbA1c) ≥6.5%.

The treatments the patients received for NODAT were recorded as diet alone, oral anti-diabetes drug, insulin, and insulin plus oral anti-diabetes drug. Hemoglobin A1c values of patients with NODAT were recorded.

Genotyping and single-gene polymorphism analysis

We prepared genomic DNA extracts from whole blood samples containing EDTA with the automated magnetic dead method (MagPurix, kit ZP02001; Zinexts) according to the manufacturer's instructions; samples were stored at -80 °C until analysis. TCF7L2 rs7903146 and CDKAL1 rs10946398 polymorphisms were genotyped by real-time polymerase chain reaction (PCR) with specific oligonucleotide primers, which are shown in Table 1 and Table 2.

Table 1. DNA Isolation and Primer Design

Primer	Sequence (5′-3′)	Polymorphism	Amplicon Size, bp
CDKAL1_SNP1_F	CAAATTGTCCAGATTTGAGAGTGAGC	SNP1	213
CDKAL1_SNP1_R	CATCAGGCACTATTCTAGAGACCAAGG		
TCF7L2_SNP1_F	GTCAGATGGTAATGCAGATGTGATGAG	SNP1	238
TCF7L2_SNP1_R	CATTGACTAAGTTACTTGCCTTCCCTG		

Abbreviations: PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism

One researcher (SG) performed all the tests. DNA isolation was performed with an automated system by magnetic bead method (MagPurix Blood DNA Extraction Kit 200; catalog No. ZP02001; Zinexts). A pair of PCR primers were designed for each polymorphism to be studied.

Table 2. Polymerase Chain Reaction Conditions

Content	Amount per Reaction, µL
Distilled H_2O	15
5× Buffer (ThermoFisher)	5
dNTP mix, 10 mM each	0.5
Advanced primer (5 µM)	1
Back primer (5 µM)	1
Phire Hot Start II DNA polymerase (ThermoFisher)	0.5
Template DNA (20-50 ng/µL)	2
Total	25

Abbreviations: dNTP, deoxynucleoside triphosphate; PCR, polymerase chain reaction

PCR amplification of the polymorphism regions to be studied was performed using designed primers. Reaction results were visualized with 2% agarose gel electrophoresis. PCR products obtained for each sample were mixed so that each sample was approximately equally represented, considering the reaction efficiency. PCR pools created for each sample were purified by an appropriate method (NucleoFast 96 PCR 96-well ultrafiltration kit, Macherey-Nagel). Purified PCR pools were measured with a spectrophotometer (NanoDrop 1000, ThermoFisher). The measured PCR pools were standardized to 0.2 ng/µL in accordance with the sequencing kit. New-generation sequencing was performed with the MiSeq platform (Illumina), and data were analyzed with MiSeq Reporter software (Illumina) and IGV 2.3 Integrative Genomics Viewer software (Broad Institute). Samples were analyzed, and the genotypes of each sample were extracted.

The PCR pools generated for each sample were purified with the NucleoFast 96 PCR 96-well ultrafiltration kit (Macherey-Nagel). Purified samples were measured with a NanoDrop 1000 spectrophotometer (ThermoFisher). New-generation sequencing was performed on the MiSeq platform (Illumina) with the v2 300 cycle sequencing kit. Single-nucleotide polymorphisms were finally determined by with MiSeq Reporter software (Illumina) and IGV 2.[3] Integrative Genomics Viewer software (Broad Institute).

Statistical analyses

Continuous variables are expressed as means and SD, and categorical variables are expressed as frequency and percentage. The normal distribution pattern was tested with the Kolmogorov-Smirnov test. Independent samples *t* tests and Mann-Whitney U tests detected differences between groups when appropriate. The compliance of the genetic distribution to the Hardy-Weinberg balance was analyzed with the chi-square test. The variations of genotypic distributions between patients with and without NODAT were evaluated with the Fisher exact test. Multivariate logistic regression analysis was used to identify possible risk factors for NODAT. A *P* value of less than .05 was considered statistically significant. All statistical analyses were performed with SSPS software for personal computers (version 20.0)

Results

There were 239 renal transplant recipients included in the study; 148 of the patients were male (61.9%), and 91 were female (38.1%). The mean age at transplant was 32 ± 13 years. The mean follow-up was 10.3 ± 5.6 years. A preemptive transplant was received by 33 patients (13%). The remainder underwent either hemodialysis, peritoneal dialysis, or a combination of both. The mean dialysis vintage was 43 ± 53 months.

There were 184 patients (76%) who had living donors, and 55 patients (23%) who had deceased donors. Of 184 living transplants, 87 donations were from first-degree relatives (47%), 83 were from second-degree relatives (45%), and 14 were from third-degree relatives (7%).

New-onset diabetes after transplant was detected in 55 of the patients (23%). Thirty-four of these 55 patients (61%) required insulin (Table 3). Mean HbA1c was 7.1 ± 1.2.

Patients with NODAT had a higher age at transplant. TCF7L2 mutations to CC and CT were higher in the NODAT group. Sex, preemptive transplant rates, modality, vintage of renal replacement, donor type, second transplant, and HCV infection rates were similar between those with and without NODAT (Table 4).

Posttransplant BMI was higher in patients who developed diabetes. For the first and last year

posttransplant, GFR values were lower in the NODAT group. These patients had a higher rate of tacrolimus use and a lower rate of mycophenolate use. Cytomegalovirus infection was similar between the 2 groups. Patients without NODAT were less likely to have hypertension and coronary artery disease (Table 5).

Age, tacrolimus, BMI, and TCF7L2 polymorphism were risk factors for NODAT in univariate analysis. In multivariate analysis, all parameters except BMI remained significant. Mycophenolate was protective of NODAT (Table 6).

Table 3. New-Onset Diabetes After Transplant and Treatment

Modality	Number of Patients	% of NODAT
Diet alone	4	7.3
OAD	17	30.9
OAD + insulin	27	49.1
Insulin	7	12.7
Total	55	100

Abbreviations: NODAT, new-onset diabetes after transplant; OAD, oral antidiabetes drug

Table 4. Characteristics of Transplant Recipients

	Total (N = 239)[a]	NODAT Absent (n = 184)[b]	NODAT Present (n = 55)[b]	*P*
Age at transplant, mean ± SD, y	32 ± 13	30 ± 12	40 ± 13	<.001
Sex, No. of patients (%)				.53
Male	148 (61%)[a]	116 (78%)[b]	32 (21%)[b]	
Female	91 (38%)	68 (74%)	23 (25%)	
Dialysis vintage, mean ± SD, mo	40 ± 53	41 ± 57	39 ± 50	.52
Preemptive transplant, No. of patients (%)				.49
Yes	33 (13%)	26 (78%)	7 (21%)	
No	206 (87%)	158 (76%)	48 (23%)	
Dialysis modality, No. of patients (%)				.81
HD	164 (79%)	126 (76%)	38 (23%)	
PD	23 (11%)	16 (69%)	7 (31%)	
HD + PD	16 (8%)	13 (81%)	3 (18%)	
HCV infection, No. of patients (%)				.45
No	227 (94%)	174 (76%)	53 (23%)	
Yes	12 (5%)	10 (83%)	2 (16%)	
Donor, No. of patients (%)				.48
Living	184 (76%)	141 (76%)	43 (23%)	
Deceased	55 (23%)	43 (78%)	12 (21%)	
CDKAL1, No. of patients (%)				
GG	110 (46%)	83 (45%)[c]	27 (45%)[c]	.727
GC	110 (46%)	85 (46%)	25 (49%)	
CC	19 (7%)	16 (9%)	3 (6%)	
TCF7L2, No. of patients (%)				
CC	163	136 (74%)[c]	27 (49%)[c]	.001
CT	49	33 (18%)	16 (42%)	
TT	27	15 (8%)	12 (22%)	

Abbreviations: HCV, hepatis C virus; HD, hemodialysis; NODAT, new-onset diabetes after transplant; PD, peritoneal dialysis
[a]Column percentage. [b]Row percentage. [c]Column percentage. Note: additive values may not sum 100 because of rounding.

Table 5. Posttransplant Follow-up Characteristics of Transplant Recipients

	Total (N = 239)	NODAT Absent (n = 184)	NODAT Present (n = 55)	*P*
Follow-up, mean ± SD, y	10.3 ± 5.5	10.5 ± 5.6	9.6 ± 5.1	.295
Posttransplant BMI, mean ± SD,	26 ± 3	25 ± 4	27 ± 3	.018
First year posttransplant				
Creatinine, mean ± SD, mg/dL	1.11 ± 0.32	1.09 ± 0.30	1.16 ± 0.38	.131
GFR, mean ± SD, mL/min	83 ± 23	86 ± 23	74 ± 21	.001
Last year posttransplant				
Creatinine, mean ± SD, mg/dL	1.36 ± 0.84	1.36 ± 0.84	1.36 ± 0.54	.998
GFR, mean ± SD, mL/min	71 ± 26	73 ± 26	62 ± 22	.004
Corticosteroids, No. of patients (%)				.45
Present	226 (94%)[a]	174 (76%)[b]	52 (23%)	
Absent	13 (5%)	10 (76%)	3 (23%)	
Tacrolimus, No. of patients (%)				.002
Present	178 (74%)	146 (82%)	32 (17%)	
Absent	61 (26%)	38 (62%)	23 (37%)	
Mycophenolate, No. of patients (%)				.004
Present	166 (69%)	143 (86%)	23 (13%)	
Absent	73 (31%)	41 (56%)	32 (43%)	
CMV infection, No. of patients (%)				.55
Yes	218 (91%)	168 (77%)	50 (22%)	
No	21 (9%)	16 (76%)	5 (23%)	
Hypertension, No. of patients (%)				.009
Yes	153 (64%)	110 (71%)	43 (28%)	
No	86 (35%)	74 (86%)	12 (13%)	
Coronary artery disease, No. of patients (%)				.001
Yes	222 (92%)	177 (79%)	45 (20%)	
No	17 (7%)	7 (41%)	10 (58%)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CMV, cytomegalovirus; GFR, glomerular filtration rate; NODAT, new-onset diabetes after transplant
[a]Column percentage. [b]Row percentage. Note: additive values may not sum 100 because of rounding.

Table 6. Risk Factors for New-Onset Diabetes After Transplant

	Univariate Logistic Regression		Multivariate Logistic Regression	
	P	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)
TCF7L2 rs7903146				
CT	.031	1.442 (1.182-1.547)	.043	1.413 (1.142-1.874)
TT	<.01	2.868 (1.698-4.030)	<.01	2.758 (1.310-4.073)
Age at transplant	<.01	1.112 (1.059-1.105)	<.01	1.077 (1.046-1.109)
Posttransplant BMI	.02	1.200 (1.003-1.196)	.052	1.093 (0.987-1.284)
Tacrolimus	<.01	2.097 (1.337-3.289)	.013	1.378 (1.175-1.815)
Mycophenolate	<.01	0.784 (0.644-0.854)	.02	0.841 (0.631-0.943)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

Discussion

Our study, conducted in 239 patients, showed that the rs7903146 polymorphism of TCF7L2 was an independent risk factor for NODAT in Turkish renal transplant recipients.

Single-center studies from Turkey have reported NODAT prevalence to be between 15% and 26%.[21-24] Our prevalence of 23.3% complies with these studies.

Common variants in TCF7L2 stand out as the leading polymorphisms associated with type 2 diabetes in the general population in most genome-wide studies performed to date.[25] A meta-analysis by Benson and colleagues also proved the association of rs7903146 with NODAT in different communities with a homogeneous distribution.[13] Our study showed that a C-to-T change of a single allele increases the relative risk of NODAT by 1.4 times; with the change of 2 alleles, the risk increases by 2.7 times. TCF7L2 rs7903146 has been associated with diabetes and obesity in Turkish patients.[17-19] In this respect, our findings are compatible with both global and local literature.

We did not find an association between CDKAL1 rs10946398 G-to-C polymorphisms and NODAT. Kang and colleagues reported CDKAL1 rs10946398 to be a significant risk factor in Korean patients.[26] The statistical power of their study reflects its influence on the review from Benson and colleagues.[13] However, other studies have disagreed with their results.[27,28] Interestingly, another study from the Korean transplant database, published in 2019,[29] did not replicate the results of Kang and colleagues. Genetic differences between the populations may explain this phenomenon. Overall, our results are not discordant with current literature. Knowledge of CDKAL1 SNPs in the Turkish people does not exist; hence, it is not possible to comment on local data.

According to our data, the presence, duration, and type of dialysis treatment before transplant do not affect NODAT. This subject has not been well elucidated. A single study from Poland reported peritoneal dialysis to be a risk factor for NODAT,[30] whereas 2 other studies did not.[31,32] Studies with prospective cohorts may help resolve these conflicting reports. The current literature does not include sex as a major risk factor for NODAT; our study has yielded similar results.[33]

Age at the time of transplant, posttransplant BMI, and tacrolimus use are associated with the risk of NODAT. In multivariate regression, the interaction between NODAT and BMI lost its significance. Obesity increases NODAT risk; however, depending on the definition of obesity and statistical technique, the odds ratio may be between 1.04 and 1.7.[9,34,35] Age is a well-known and repeatable risk factor.[7] In this respect, our findings are compatible with the literature.

Tacrolimus and cyclosporine disrupt insulin secretion by interference with the nuclear factor of active T cell signaling in pancreatic β islets. This toxicity is more severe for tacrolimus.[36] Several studies have repeatedly proved that tacrolimus is a potent diabetogenic and independent risk factor for NODAT.[4,37]

Corticosteroids trigger diabetes via multiple mechanisms[10]; however, in our study, we were unable to establish an association between steroid use and NODAT. In our clinic, we do not have "steroid-free regimens." Therefore, the total number of patients who were not on corticosteroids was only 13, which may have interfered with statistics.

Retrospective data have suggested that mycophenolate (sodium or mofetil) might reduce the risk of diabetes by about 15% to 25%.[7] This finding has not been validated in a prospective setting. It remains uncertain whether this effect of mycophenolate is the result of a metabolic pathway or the result of its strong immunosuppressive properties that allow lower doses of diabetogenic drugs. Nonetheless, our findings agree with current knowledge.

Cytomegalovirus and HCV infection do not increase the risk of NODAT according to our work. Data for cytomegalovirus infection are variable.[38,39] Hepatitis C virus is expected to be associated with diabetes.[37] However, we had few patients seropositive for HCV (only 12), and their tests were positive for anti-HCV IgG but negative for HCV RNA, ie, without active hepatitis. No patient received anti-HCV treatment after transplant. These characteristics might explain why we failed to demonstrate an association between HCV and NODAT.

Our study also revealed the coexistence of NODAT with hypertension and coronary artery disease. This association is well known.[2,7,23,33] In addition, patients with NODAT had lower GFR both in the first year and in the last year of follow-up. Because we did not recruit patients with failed grafts, it is impossible to comment on the effect of NODAT on renal survival. Nevertheless, given the mentioned interactions, NODAT diagnosis should be warning for premature mortality risk.

Our study is cross-sectional, and this is a limitation. The family history, pretransplant BMI, and glycemic status are notable risk factors for

NODAT.[3,4] Unfortunately, we were not able to analyze these because of a lack of data.

Conclusions

New-onset diabetes after transplant is a prevalent complication associated with hypertension and coronary artery disease. This study proves TCF7L2 (rs7903146) to be an independent and significant risk factor for NODAT in Turkish renal transplant recipients.

References

1. Voora S, Adey DB. Management of kidney transplant recipients by general nephrologists: core curriculum 2019. *Am J Kidney Dis*. 2019;73(6):866-879. doi:10.1053/j.ajkd.2019.01.031
2. Sulanc E, Lane JT, Puumala SE, Groggel GC, Wrenshall LE, Stevens RB. New-onset diabetes after kidney transplantation: an application of 2003 International Guidelines. *Transplantation*. 2005;80(7):945-952. doi:10.1097/01.tp.0000176482.63122.03
3. Porrini EL, Diaz JM, Moreso F, et al. Clinical evolution of post-transplant diabetes mellitus. *Nephrol Dial Transplant*. 2016;31(3):495-505. doi:10.1093/ndt/gfv368
4. Heisel O, Heisel R, Balshaw R, Keown P. New onset diabetes mellitus in patients receiving calcineurin inhibitors: a systematic review and meta-analysis. *Am J Transplant*. 2004;4(4):583-595. doi:10.1046/j.1600-6143.2003.00372.x
5. Boudreaux JP, McHugh L, Canafax DM, et al. The impact of cyclosporine and combination immunosuppression on the incidence of posttransplant diabetes in renal allograft recipients. *Transplantation*. 1987;44(3):376-381. doi:10.1097/00007890-198709000-00010
6. Friedman AN, Miskulin DC, Rosenberg IH, Levey AS. Demographics and trends in overweight and obesity in patients at time of kidney transplantation. *Am J Kidney Dis*. 2003;41(2):480-487. doi:10.1053/ajkd.2003.50059
7. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant*. 2003;3(2):178-185. doi:10.1034/j.1600-6143.2003.00010.x
8. Sumrani N, Delaney V, Ding Z, et al. Posttransplant diabetes mellitus in cyclosporine-treated renal transplant recipients. *Transplant Proc*. 1991;23(1 Pt 2):1249-1250.
9. Bloom RD, Crutchlow MF. New-onset diabetes mellitus in the kidney recipient: diagnosis and management strategies. *Clin J Am Soc Nephrol*. 2008;3 Suppl 2:S38-S48. doi:10.2215/CJN.02650707
10. Hjelmesaeth J, Hartmann A, Kofstad J, et al. Glucose intolerance after renal transplantation depends upon prednisolone dose and recipient age. *Transplantation*. 1997;64(7):979-983. doi:10.1097/00007890-199710150-00008
11. Grarup N, Sandholt CH, Hansen T, Pedersen O. Genetic susceptibility to type 2 diabetes and obesity: from genome-wide association studies to rare variants and beyond. *Diabetologia*. 2014;57(8):1528-1541. doi:10.1007/s00125-014-3270-4
12. Kang ES, Kim MS, Kim YS, et al. A variant of the transcription factor 7-like 2 (TCF7L2) gene and the risk of posttransplantation diabetes mellitus in renal allograft recipients. *Diabetes Care*. 2008;31(1):63-68. doi:10.2337/dc07-1005
13. Benson KA, Maxwell AP, McKnight AJ. A HuGE review and meta-analyses of genetic associations in new onset diabetes after kidney transplantation. *PLoS One*. 2016;11(1):e0147323. doi:10.1371/journal.pone.0147323
14. Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest*. 2007;117(8):2155-2163. doi:10.1172/JCI30706
15. Lou L, Wang J, Wang J. Genetic associations between Transcription Factor 7 Like 2 rs7903146 polymorphism and type 2 diabetes mellitus: a meta-analysis of 115,809 subjects. *Diabetol Metab Syndr*. 2019;11:56. doi:10.1186/s13098-019-0451-9
16. Liu X, Liang J, Geng H, Xu W, Teng F, Yang M. Association of the CDKAL1 polymorphism rs10946398 with type 2 diabetes mellitus in adults: a meta-analysis. *Medicine (Baltimore)*. 2020;99(30):e21383. doi:10.1097/MD.0000000000021383
17. Erkoc Kaya D, Arikoglu H, Kayis SA, Ozturk O, Gonen MS. Transcription factor 7-like 2 (TCF7L2) gene polymorphisms are strong predictors of type 2 diabetes among nonobese diabetics in the Turkish population. *Turk J Med Sci*. 2017;47(1):22-28. doi:10.3906/sag-1507-160
18. Vural HC. Tcf7l2 gene polymorphism in T2dm with patients in Turkish population. *J Clin Epigenet*. 2017;3:27. doi:10.21767/2472-1158.100061
19. Demirsoy IH, Aras N, Cinkir U. TCF7L2 rs7903146 gene variation is associated with risk of type 2 diabetes in Turkish population. *J Clin Med Genomics*. 2016;4(1):141-143. doi:10.4172/2472-128X.1000141
20. Yu XX, Liao MQ, Zeng YF, et al. Associations of KCNQ1 polymorphisms with the risk of type 2 diabetes mellitus: an updated meta-analysis with trial sequential analysis. *J Diabetes Res*. 2020;2020:7145139. doi:10.1155/2020/7145139
21. Gulsoy Kirnap N, Bozkus Y, Haberal M. Analysis of risk factors for posttransplant diabetes mellitus after kidney transplantation: single-center experience. *Exp Clin Transplant*. 2020;18(Suppl 1):36-40. doi:10.6002/ect.TOND-TDTD2019.O8
22. Sinangil A, Celik V, Barlas S, et al. The incidence of new onset diabetes after transplantation and related factors: single center experience. *Nefrologia*. 2017;37(2):181-188. doi:10.1016/j.nefro.2016.11.022
23. Demirci MS, Toz H, Yilmaz F, et al. Risk factors and consequences of post-transplant diabetes mellitus. *Clin Transplant*. 2010;24(5):E170-E177. doi:10.1111/j.1399-0012.2010.01247.x
24. Tatar E, Kircelli F, Demirci MS, et al. Pre-transplant HbA1c level as an early marker for new-onset diabetes after renal transplantation. *Int Urol Nephrol*. 2013;45(1):251-258. doi:10.1007/s11255-012-0304-z
25. Grant SFA. The TCF7L2 locus: a genetic window into the pathogenesis of type 1 and type 2 diabetes. *Diabetes Care*. 2019;42(9):1624-1629. doi:10.2337/dci19-0001
26. Kang ES, Kim MS, Kim CH, et al. Association of common type 2 diabetes risk gene variants and posttransplantation diabetes mellitus in renal allograft recipients in Korea. *Transplantation*. 2009;88(5):693-698. doi:10.1097/TP.0b013e3181b29c41
27. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. *J Am Soc Nephrol*. 2014;25(5):1037-1049. doi:10.1681/ASN.2013040383
28. Chakkera HA, Hanson RL, Raza SM, et al. Pilot study: association of traditional and genetic risk factors and new-onset diabetes mellitus following kidney transplantation. *Transplant Proc*. 2009;41(10):4172-4177. doi:10.1016/j.transproceed.2009.08.063
29. Hwang HS, Hong KW, Kim JS, et al. Validation of identified susceptible gene variants for new-onset diabetes in renal transplant recipients. *J Clin Med*. 2019;8(10). doi:10.3390/jcm8101696
30. Madziarska K, Weyde W, Krajewska M, et al. The increased risk of post-transplant diabetes mellitus in peritoneal dialysis-treated kidney allograft recipients. *Nephrol Dial Transplant*. 2011;26(4):1396-1401. doi:10.1093/ndt/gfq568
31. Seifi S, Rahbar M, Lessan-Pezeshki M, et al. Posttransplant diabetes mellitus: incidence and risk factors. *Transplant Proc*. 2009;41(7):2811-2813. doi:10.1016/j.transproceed.2009.07.043
32. Courivaud C, Ladriere M, Toupance O, et al. Impact of pre-transplant dialysis modality on post-transplant diabetes mellitus after kidney transplantation. *Clin Transplant*. 2011;25(5):794-799. doi:10.1111/j.1399-0012.2010.01367.x
33. Jenssen T, Hartmann A. Post-transplant diabetes mellitus in patients with solid organ transplants. *Nat Rev Endocrinol*. 2019;15(3):172-188. doi:10.1038/s41574-018-0137-7

34. Parikh CR, Klem P, Wong C, Yalavarthy R, Chan L. Obesity as an independent predictor of posttransplant diabetes mellitus. *Transplant Proc.* 2003;35(8):2922-2926. doi:10.1016/j.transproceed.2003.10.074
35. Chowdhury TA. Post-transplant diabetes mellitus. *Clin Med (Lond).* 2019;19(5):392-395. doi:10.7861/clinmed.2019-0195
36. Luan FL, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. *Transplantation.* 2011;91(3):334-341. doi:10.1097/TP.0b013e318203c25f
37. Shah T, Kasravi A, Huang E, et al. Risk factors for development of new-onset diabetes mellitus after kidney transplantation. *Transplantation.* 2006;82(12):1673-1676. doi:10.1097/01.tp.0000250756.66348.9a
38. Hjelmesaeth J, Sagedal S, Hartmann A, et al. Asymptomatic cytomegalovirus infection is associated with increased risk of new-onset diabetes mellitus and impaired insulin release after renal transplantation. *Diabetologia.* 2004;47(9):1550-1556. doi:10.1007/s00125-004-1499-z
39. Dedinska I, Laca L, Miklusica J, Kantarova D, Galajda P, Mokan M. Correlation between CMV infection and post-transplantation new-onset diabetes mellitus. *Int J Organ Transplant Med.* 2016;7(3):173-182.

Impact and Consequences of Recipient Gastroduodenal Artery Ligation Before Hepatic Artery Anastomosis During Orthotopic Liver Transplantation

Aman Kumar,[1] Simran Kaur Singh,[2] Navkaran Singh,[2] Melissa Chen,[1] Randall Watkins,[1] Sorabh Kapoor,[1] Chirag S. Desai[1]

Abstract

Objectives: **The recipient's gastroduodenal artery is often ligated before the hepatic artery anastomosis during orthotopic liver transplant, to gain either mobility or length of recipient's hepatic artery, potentially protecting the anastomosis by preventing "steal syndrome." In this study, our aim was to evaluate the consequences of gastroduodenal artery ligation and its effect on prevention of hepatic artery thrombosis.**

Materials and Methods: **We retrospectively analyzed deceased-donor orthotopic liver transplant procedures (n = 210) performed at a single center between January 2016 and July 2021 to compare outcomes between recipients with (group 1) and recipients without (group 2) gastroduodenal artery ligation. Group 1 included 78 patients (37%), in which the recipient's common hepatic artery was used for arterial anastomosis; group 2 included 132 patients (63%), in which the right hepatic artery or the proper hepatic artery was used for arterial anastomosis. Occurrences of hepatic artery thrombosis, posto-perative hyperamylasemia, nausea and vomiting, and delayed feeding were compared between the groups.**

Results: **There was no incidence of hepatic artery thrombosis reported in either group. In group 1, 31 patients (39.7%) were reported to have postoperative hyperamylasemia, ranging from 200 to 4700 U/L accompanied by delayed feeding, whereas, in group 2, only 16 of 132 patients (12%) had postoperative hyperamylasemia, ranging from 200 to 1400 U/L (*P* < .01).**

Conclusions: **Ligation of recipient's gastroduodenal artery is not associated with decreased risk of hepatic artery thrombosis compared with nonligation. However, the procedure does have consequences in the form of possible postoperative hyperamylasemia, leading to delayed feeding probably due to decreased oral tolerance.**

Key words: *Hepatic artery thrombosis, Postoperative hyperamylasemia, Splenic artery steal syndrome*

Introduction

Hepatic artery (HA) anastomosis is the most critical component of liver transplant with immediate and long-term implications on graft and patient survival based on its patency. Documented incidence of hepatic artery thrombosis (HAT) after liver transplant has been as high as 12% of adult liver transplant recipients in earlier series.[1] However, with improvement of techniques, the current incidence ranges from 2% to 4%.[2] Of the multiple factors responsible for early HAT, the anastomotic technique still remains an important factor. Various techniques used by different surgeons are end-to-end anastomosis between celiac axis stem of graft and recipient common hepatic artery (CHA), graft right hepatic artery (RHA) to the recipient CHA, use of donor HA with splenic patch, use of a patch at the division of donor gastroduodenal artery (GDA), use of donor HA with aortic patch to the recipient CHA, use of infrarenal conduit, and use of supraceliac conduit.[3] However, HAT has been shown to be associated with all of the techniques, with varying degrees, and no particular technique has shown a statistically significant result.[3]

Performing tension-free anastomosis by ensuring adequate length and mobilization of artery, ligation of branch vessels to ensure good blood flow at the anastomotic site to prevent steal, and meticulous

From the [1]Division of Abdominal Transplant, Department of Surgery, UNC School of Medicine, Chapel Hill, North Carolina; and the [2]Department of Global Health, University of North Carolina, Chapel Hill, North Carolina, USA

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Chirag S. Desai, Department of Surgery, UNC School of Medicine 4024 Burnett Womack Building, Chapel Hill, NC 27599, USA*
E-mail: *chirag_desai@med.unc.edu*

Experimental and Clinical Transplantation (2023) 11: 879-882

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0270

anastomosis techniques are important technical considerations for prevention of HAT.[4-7] When HA anastomosis is performed during orthotopic liver transplant, the recipient's GDA is often ligated to increase the length of the recipient's artery for anastomosis and also to potentially avoid the steal phenomenon.[8,9] There are reports of graft hypoperfusion requiring procedures at times due to these steal phenomena, which are reported to be due to either GDA or splenic artery steal syndrome and may require interventions to augment HA flow.[10-12] However, these reports are anecdotal and limited by a small numbers of cases. There has been no large series, to our knowledge, evaluating the effects of GDA ligation during liver transplant, specifically with whole organ deceased-donor liver allografts. We aimed to evaluate the effects of GDA ligation in recipients of orthotopic liver transplant. The primary outcome evaluated was the incidence of HAT. Secondary outcomes assessed included the effects on recipients' postoperative parameters, namely, hyperamylasemia, nausea and vomiting and delayed feeding.

Materials and Methods

Patients included in this retrospective study had provided detailed and informed consent. This study received approval from our institutional review board. All deceased-donor orthotopic whole liver transplants performed at a single center between January 2016 and July 2021 were retrospectively analyzed. The surgical technique of liver transplant included both bicaval replacement and the piggyback technique. End-to-end HA anastomosis was performed either in a continuous or an interrupted fashion using a fine 7-0 Prolene suture based on surgeon's preference. In the case of replaced vessels, especially right replaced HA, an arterial reconstruction was performed on the bench where donor-replaced right HA was anastomosed to the graft HA's GDA or splenic stump. All recipients had single anastomosis to the recipient artery.

For evaluation of outcomes, we divided recipients into 2 groups: group 1 included patients who had GDA ligation, and group 2 included patients who did not have GDA ligation during the transplant. Occurrence of HAT, postoperative hyperamylasemia (POHA), postoperative nausea and vomiting (PONV), and delayed feeding were assessed in both groups. Feeding was considered delayed if patients were not started on a diet until 5 days postsurgery, which was considered standard in liver transplant recipients. Continuous variables were compared using the *t* test, and categorical variables were compared using the Pearson chi-square test.

Results

During the study period, our center performed 210 deceased-donor orthotopic liver transplants. Demographic data of orthotopic liver transplant recipients are listed in Table 1. Group 1 consisted of 78 recipients, in which the common HA was used for arterial anastomosis. Group 2 consisted of 132 recipients, in which anastomosis was performed using either the right HA or the proper HA. Among the total patients, 162 patients (77.1%) did not have replaced or accessory vessels. Of 48 patients (22.9%) with replaced vessels, 28 (13.3%) had right replaced/accessory, 18 (8.6%) had left replaced/accessory, and 2 (0.8%) had both right and left replaced/accessory vessel. Of these 48 patients with replaced vessels, 18 (23.1%) were in group 1 and 30 (22.7%) were in group 2. We found no incidence of HAT in either of the 2 groups.

Table 1. Demographic Data of Liver Transplant Recipients and Donors (N = 210)

	Group 1 (n = 78; 37.1%)	Group 2 (n = 132; 62.9%)
Median age at transplant (IQR), y	57 (48.25-63)	55.5 (47.75-63)
Median MELD score (IQR)	34 (24-35)	30 (27-31)
Recipient sex, No. (%)		
Male	42 (53.8%)	92 (69.7%)
Female	36 (46.2%)	40 (30.3%)
Most common etiology	NASH	NASH and ALD
Coronary artery disease, No. (%)	3 (0.038%)	5 (0.037%)
Median age of donor (IQR), y	33.5 (26-45.75)	34 (26-44.25)
Median BMI of donor (IQR)	25 (22.3-30.5)	25.9 (23.2-30.4)
Donor sex, %		
Male	58.9%	59.8%
Female	41.1%	40.2%
Donor criteria		
DBD	77 (99.99%)	125 (99.955)
DCD	1 (0.01%)	7 (0.05%)

Abbreviations: ALD, alcoholic liver disease; BMI, body mass index (in kilograms divided by height in meters squared); DBD, donation after brain death; DCD, donation after circulatory death; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis

Group 1 had gastroduodenal artery ligation, and group 2 did not.

Group 1 had 31 patients (47%) with POHA (ranging from 200 to 4700 U/L), and group 2 had 30 patients (25.4%) with POHA (ranging from 200 to 1400 U/L) (P = .003). Nineteen patients (24.4%) in

group 1 required tube feeds compared with 14 patients (10.6%) in group 2 (P = .01). Thirty-eight patients (4.7%) in group 1 had PONV in the early postoperative period compared with 17 patients (12.9%) in group 2 (P = .001). Feeding was delayed in 23 patients (29.5%) in group 1 compared with 9 patients (6.8%) in group 2 (P = .001) (Table 2).

Table 2. Comparison Between Group 1 and Group 2

	Group 1 (n = 78; 37.1%)	Group 2 (n = 132; 62.9%)	Total (N = 210)	*P*
Patient age, mean (SD), y	53.8 (13.6)	53.0 (12.9)	53.3 (13.1)	.7
Multiorgan transplant, No. (%)	14 (18.0)	14 (10.6)	28 (13.3)	.1
Hyperamylasemia, No. (%)	31 (47.0)	30 (25.4)	61 (33.2)	.003
Required tube feeds, No. (%)	19 (24.4)	14 (10.6)	33 (15.7)	.01
PONV, No. (%)	38 (48.7)	17 (12.9)	55 (26.2)	<.001
Delayed feeding, No. (%)	23 (29.5)	9 (6.8)	32 (15.2)	<.001

Abbreviations: PONV, postoperative nausea and vomiting
Group 1 had gastroduodenal artery ligation, and group 2 did not.

Discussion

Hepatic artery thrombosis and hepatic artery stenosis are dreaded complications of liver transplant surgery that can occur in the early and the late phase. Hepatic artery thrombosis can lead to fulminant liver failure, delayed biliary leak and biliary sepsis, relapsing bacteremia, ischemic strictures, cholangitis, graft dysfunction, and multiorgan failure.[13] It leads to bile duct injury and necrosis, which can frequently be followed by bacterial translocation, sepsis, and death.[3,13] The incidence of HAT has significantly decreased over the years from 42% (in children) and 12% (in adults) to 4.4% and 2%, respectively,[3,14] likely because of improvements in surgical technique, organ preservation, and graft and patient selection. However, there are other causes of early HAT, like rejection and decreased flow through the HA.[15] Various authors have described the occurrence of steal syndrome with both GDA steal and splenic artery steal responsible for poor flow across HA anastomosis.[8-12] The reported incidence of HAT is low but may be up to 0.6%.[16] Our study did not show any significant change in incidence of HAT in patients with either ligation of GDA or no ligation. Replaced or accessory vessels present in the donor liver and bench reconstruction may increase the risk of arterial thrombosis.[17,18] However, in our analysis, we did not see an increased incidence of HAT with bench reconstruction to enable single anastomosis to the recipient's artery, irrespective of whether recipient GDA was ligated or not.

Among our patients, GDA ligation led to higher incidence of hyperamylasemia and delay in enteral nutrition. Delayed feeding observed in the cohort was most likely attributed to this subtle pancreatitis with resultant pyloric spasm that resulted in delay in ability to start enteral nutrition. Patients with GDA ligation had increased incidence of nausea and vomiting, contributing to increased length of stay in the hospital among other factors. Our results suggest that recipient GDA ligation for HA anastomosis is not a mandatory step in liver transplant and can be avoided with the benefit of decreased POHA, PONV, and early feeding.

Limitations to our study include other factors that may contribute toward POHA. With dissection in the HA bed to expose the common HA or to gain extra length on the artery, placement of a retractor blade in that vicinity may agitate the pancreas, leading to some degree of inflammation in that region and associated pancreatitis. There may be other causes of PONV and delayed feeding in our orthotopic liver transplant recipients, such as medications, frailty, delayed gastric emptying, prolonged encephalopathy, or postoperative intensive care or delirium; however, correlation with hyperamylasemia cannot be overlooked for reasons stated above. In addition, this symptomatology has a broad etiology base, making it difficult to do any multivariate analysis to have accurate reason.

Conclusions

Patients who did not have ligation of recipient GDA before HA anastomosis during liver transplant did not have increased incidence of HAT. However, GDA ligation may lead to significant hyperamylasemia, which can be a cause of upper gastrointestinal symptoms in the early postoperative period and can be a subject of interest for future studies.

References

1. Wozney P, Zajko AB, Bron KM, Point S, Starzl TE. Vascular complications after liver transplantation: a 5-year experience. *AJR Am J Roentgenol.* 1986;147(4):657-663. doi:10.2214/ajr.147.4.657
2. Bekker J, Ploem S, de Jong KP. Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant.* 2009;9(4):746-757. doi:10.1111/j.1600-6143.2008.02541.x

3. Mourad MM, Liossis C, Gunson BK, et al. Etiology and management of hepatic artery thrombosis after adult liver transplantation. *Liver Transpl.* 2014;20(6):713-723. doi:10.1002/lt.23874
4. Abbasoglu O, Levy MF, Testa G, et al. Does intraoperative hepatic artery flow predict arterial complications after liver transplantation? *Transplantation*. 1998;66(5):598-601. doi:10.1097/00007890-199809150-00008
5. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol.* 2010;16(48):6046-6057. doi:10.3748/wjg.v16.i48.6046
6. Sevmis S, Boyvat F, Aytekin C, et al. Arterial steal syndrome after orthotopic liver transplantation. *Transplant Proc.* 2006;38(10):3651-3655. doi:10.1016/j.transproceed.2006.10.145
7. Nussler NC, Settmacher U, Haase R, Stange B, Heise M, Neuhaus P. Diagnosis and treatment of arterial steal syndromes in liver transplant recipients. *Liver Transpl.* 2003;9(6):596-602. doi:10.1053/jlts.2003.50080
8. Jiménez-Rodríguez R, Martínez JA, Artacho GS, Bravo MG. Liver graft necrosis caused by a gastroduodenal arterial steal syndrome. *Rev Esp Enferm Dig.* 2009;101(6):446-447.
9. Nishida S, Kadono J, DeFaria W, et al. Gastroduodenal artery steal syndrome during liver transplantation: intraoperative diagnosis with Doppler ultrasound and management. *Transpl Int*. 2005;18(3):350-353. doi:10.1111/j.1432-2277.2004.00065.x
10. Lima CX, Mandil A, Ulhoa AC, Lima AS. Splenic artery steal syndrome after liver transplantation: an alternative technique of embolization. *Transplant Proc.* 2009;41(5):1990-1993. doi:10.1016/j.transproceed.2009.01.086
11. Vogl TJ, Pegios W, Balzer JO, Lobo M, Neuhaus P. [Arterial steal syndrome in patients after liver transplantation: transarterial embolization of the splenic and gastroduodenal arteries]. *Rofo.* 2001;173(10):908-913. doi:10.1055/s-2001-17592
12. Langer R, Langer M, Scholz A, Felix R, Neuhaus P, Keck H. [The splenic steal syndrome and the gastroduodenal steal syndrome in patients before and after liver transplantation]. *Aktuelle Radiol.* 1992;2(2):55-58.
13. Crossin JD, Muradali D, Wilson SR. US of liver transplants: normal and abnormal. *Radiographics*. 2003;23(5):1093-1114. doi:10.1148/rg.235035031
14. Tzakis AG, Gordon RD, Shaw BW, Jr., Iwatsuki S, Starzl TE. Clinical presentation of hepatic artery thrombosis after liver transplantation in the cyclosporine era. *Transplantation.* 1985;40(6):667-671. doi:10.1097/00007890-198512000-00019
15. Abou El-Ella K, Al Sebayel M, Ramirez C, Hussien R. Outcome and risk factors of hepatic artery thrombosis after orthotopic liver transplantation in adults. *Transplant Proc.* 2001;33(5):2712-2713. doi:10.1016/s0041-1345(01)02157-1
16. Stange BJ, Glanemann M, Nuessler NC, Settmacher U, Steinmuller T, Neuhaus P. Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl.* 2003;9(6):612-620. doi:10.1053/jlts.2003.50098
17. Soliman T, Bodingbauer M, Langer F, et al. The role of complex hepatic artery reconstruction in orthotopic liver transplantation. *Liver Transpl*. 2003;9(9):970-975. doi:10.1053/jlts.2003.50167
18. Xue Z, Chen M, Zhang X, et al. Analysis of early hepatic artery thrombosis after liver transplantation. *ANZ J Surg.* 2018;88(3):172-176. doi:10.1111/ans.13911

Busulfan-Based and Treosulfan-Based Myeloablative Conditioning for Allogeneic Transplantation in Children with Thalassemia Major: a Single-Center Experience From Southern Turkey

Utku Aygüneş,[1] Barbaros Sahin Karagun,[1] Defne Ay Tuncel,[2] Hatice Ilgen Sasmaz,[1] Bülent Antmen[1]

Abstract

Objectives: **Allogeneic hematopoietic stem cell transplant is the only curative treatment for patients with transfusion-dependent thalassemia major. In recent years, a number of novel approaches have improved patient outcomes and quality of life by minimizing the toxicity of conditioning regimens. The objective of this study was to compare the role of treosulfan- and busulfan-based conditioning in transfusion-dependent thalassemia.**

Materials and Methods: **Data were collected retrospectively on 121 children with beta thalassemia major who underwent hematopoietic stem cell transplant using treosulfan-based (n = 37) or busulfan-based (n = 84) conditioning regimens between 2012 and 2022.**

Results: **Two-year overall survival was 87.5% in the busulfan-based conditioning group and 91.1% in the treosulfan-based conditioning group. The group given the busulfan regimen compared with treosulfan regimen had significantly increased number of side effects (58.3% vs 21.6%, respectively; *P* < .001). When the busulfan-based regimen by level was evaluated, we observed no significant differences between the frequency of side effects according to drug serum levels. In addition, no significant differences were shown between the 2 regimen groups for cumulative incidence of acute and chronic graft-versus-host disease.**

Conclusions: **The safety and effectiveness of a treosulfan-based myeloablative conditioning regimen has been confirmed by our retrospective investigation of pediatric patients with beta thalassemia.**

Key words: *Cooley anemia, Hematopoietic stem cell transplant, Toxicity, Transfusion-dependent thalassemia major*

From the [1]Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation, Acibadem Adana Hospital, Adana; and the [2]Department of Pediatric Hematology, Oncology, Adana City Training and Research Hospital, Adana, Turkey

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Utku Aygüneş, Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation Unit, Acibadem Adana Hospital, 01130 Adana, Turkey*
Phone: *+90 5062225522* ***E-mail:*** *utkuayg@gmail.com*

Experimental and Clinical Transplantation (2023) 11: 883-892

Introduction

Thalassemia major (TM), also known as Cooley anemia, is a prevalent monogenic inherited hemoglobin disorder. Patients with thalassemia consequently require lifelong blood transfusions and suffer significant organ dysfunction as a result of primary and secondary iron overload.[1]

For patients with TM, allogeneic hematopoietic stem cell transplantation (HSCT) is the only current curative option for patients to save patients from the long-term consequences of the disease and/or treatment and from the lifelong, difficult treatment itself.[2,3]

For patients with TM undergoing HSCT, iron overload is a significant risk factor for poor prognosis, even with the best available management.[4,5] The use of Pesaro risk stratification, increased donor selection, supportive care, and modified conditioning regimens have reduced transplant-related mortality and improved HSCT results in TM patients.[6] However, because of the significant risk of treatment-related problems or graft failure, the clinical prognosis following HSCT in children with TM who are categorized as Pesaro class 3 remains unsatisfactory.[7] Treatment-related mortality, as determined by the standard Pesaro criteria, was 3% among patients in Pesaro classes 1 and 2 compared with 8% in class 3.8

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0143

According to the literature, 2.7% to 8.8% of pediatric patients with TM who underwent HSCT experienced graft rejection.[9,10] In addition, many serious treatment-related complications such as sepsis, seizure and veno-occlusive disease can be seen.[11]

Over time, outcomes have improved with improvements in our knowledge and management of transplant-related problems. However, transplant-related acute and long-term complications are still frequently seen and depend on the severity of the conditioning regimen. The most widely used conditioning regimens in HSCT for thalassemia include busulfan, treosulfan, fludarabine, and antithymocyte globulin. Since the 1950s, busulfan has been in use as an alkylating anticancer drug. Busulfan usage is frequently correlated with certain adverse effects. Intestinal mucosal damage, hepatotoxicity, sinusoidal obstruction syndrome, and pancytopenia are all frequent side effects connected to busulfan. Treosulfan, also known as L-threitol-1,4-bismethanesulphonate, is a water-soluble alkylating agent and a prodrug of L-epoxybutane.[12] Even without drug-level monitoring, treosulfan-based conditioning has reduced regimen-related toxicity and follows linear pharmacokinetic properties.[13] In comparison with a busulfan-based regimen, treosulfan has been used as an HSCT preparation and has shown decreased risk of hepatic, pulmonary, and nervous system damage.[14,15] High doses of busulfan reduce the risk of relapse; however, busulfan can increase long-term late complication rates, morbidity rates, and mortality rates.[16]

Treosulfan-based conditioning has been used more frequently in children since the first report of it in pediatric allo-HSCT in 2002, largely because it is thought to have a broad therapeutic index and a lower tendency to cause veno-occlusive disease than busulfan.[17] Several studies have shown better results in terms of toxicity, complete donor chimerism, graft-versus-host disease (GVHD), treatment-related mortality, and relapse rates.[17-19]

All of these features make using a treosulfan-based preparative regimen desirable for thalassanemia patients, lowering the risk of potentially fatal complications and boosting the proportion of patients who are successfully treated. We conducted a retrospective analysis to assess results in patients with TM who received either a busulfan-fludarabine-based or treosulfan-fludarabine-based conditioning regimen for HSCT.

Materials and Methods

Study design and characteristics of the study cohort
In a retrospective, single-center cohort study, we retrospectively evaluated consecutive HLA-matched HSCTs with treosulfan-based or busulfan-based conditioning regimens (n = 121) at the Department of Pediatric Bone Marrow Transplantation, Acibadem Adana Hospital (Adana, Turkey). Up to 28 days after HSCT, early regimen-related toxicity was assessed. We collected patient data from hospital medical records by using electronic case report forms. Data safety was ensured by a confidential disclosure agreement signed by the hospital for each clinician in Turkey, as required by law and regulations. We collected data on patient demographics, diagnosis, donor matching, HLA compatibility, stem cell source, conditioning regimen, transplant-related complications, early regimen-related toxicities, GVHD, donor chimerism, outcome, and time of follow-up. Informed consent from parent or guardian was obtained before patients were enrolled. The Research Ethics Committee of the Acibadem University, Faculty of Medicine, approved the use of the data for the study.

Children under the age of 18 who underwent their first allogeneic HSCT for transfusion-dependent TM (TDT) between January 2012 and November 2022 were included in this study. Data for 121 patients who underwent HSCT using a myeloablative busulfan-fludarabine-based conditioning regimen (busulfan group) were analyzed retrospectively and compared with patients who received a treosulfan-fludarabine-based regimen (treosulfan group). Both groups had undergone their first HSCT with bone marrow or peripheral blood stem cells from a matched sibling donor, other related donor, or unrelated donor. According to high-resolution HLA allele typing at loci A, B, C, DRB1, and DQ, unrelated donors were categorized as being matched (10/10 loci) or mismatched at 9/10 or 8/10 loci.

Study endpoints

We analyzed demographics and baseline variables for the entire study population and for each conditioning regimen group. The main objectives of the study were to quantify the frequency of early regimen-related toxicities, graft failure, and treatment-related mortality following HSCT. Cumulative incidence of acute GVHD (aGVHD) and chronic GVHD (cGVHD) were secondary objectives.

The first of 3 days with an absolute neutrophil count of $<0.5 \times 10^9$/L was defined as neutrophil recovery. Platelet engraftment was defined as platelet count $>20 \times 10^9$/L, without platelet support for 3 consecutive days. Chimerism was evaluated in peripheral blood granulocytes and mononuclear cells by fluorescein in situ hybridization in sex-mismatched transplants and by polymerase chain reaction in others. Time to aGVHD was defined as first occurrence (date of diagnosis) of aGVHD after HSCT. Time to cGVHD was defined as the first episode of cGVHD (date of diagnosis) after HSCT. Severity of aGVHD and cGVHD was graded according to the Seattle criteria.[20]

Sinusoidal obstruction syndrome, also known as veno-occlusive disease, was diagnosed according to clinical criteria as the presence within 3 weeks after HCT of (1) hyperbilirubinemia (bilirubin >2.0 mg/dL), (2) painful hepatomegaly, and (3) weight gain.

All patients in the treosulfan group received the same conditioning regimen, comprising intravenous (IV) thiotepa 8 mg/kg on day -6, treosulfan 14 g/m^2/-day on day -5 to day -3, and fludarabine 40 mg/m^2/-day on day -5 to day -2. Patients in the busulfan group received oral busulfan 3.5 mg/kg/day on day -9 to day -6, cyclophosphamide 50 mg/kg/day on day -5 to day -2, and antithymocyte globulin 30 mg/kg/day on day -4 to day -2. Prophylaxis for GVHD included cyclosporine (2.5 mg/kg IV twice daily) and methotrexate (10 mg/m^2 IV on day -1 and 7 mg/m^2 on days +3, +6, and +11). All patients received cyclosporine for 9 to 12 months post-HSCT, with plasma cyclosporine levels maintained at 200 to 350 ng/mL. Adjusted doses for both busulfan and treosulfan according to plasma drug levels were obtained from the patient records.

The Common Terminology Criteria for Adverse Events version 4.01 were used to analyze toxicity events. All patients were given standard oral care. Patients with neurological complaints were diagnosed with radiological imaging methods, including magnetic resonance imaging.

Statistical analyses

Data were analyzed with IBM SPSS version 23. Conformity to normal distribution was evaluated with Shapiro-Wilk and Kolmogorov-Smirnov tests. We used the chi-square test and the Fisher exact test to compare categorical variables between the treosulfan group and the busulfan group. Cumulative incidence estimates were used to determine the incidences of primary rejection, treatment-related mortality, and aGVHD and cGVHD (comprising disease-free survival). We used the Mann-Whitney U test to compare the quantitative data that were not normally distributed according to the paired groups. The probability of overall survival (OS) was calculated using the Kaplan-Meier method. A log-rank (Mantel-Cox) test was used to compare the survival outcome and OS according to the groups. Results are shown as mean ± SD for quantitative data and as median (minimum to maximum) and frequency (percentage) for categorical data. Results are shown with 95% confidence interval (95% CI). We used the Gray test (cmprsk package) to evaluate the overall differences among cumulative incidence functions. $P < .05$ was considered statistically significant.

Results

We examined 121 consecutive TM patients (76 males, 45 females) who had allogeneic HSCT using a treosulfan-based or busulfan-based conditioning regimen. The median patient age was 13.6 ± 6.0 years (range, 2-18 years). Table 1, Table 2, and Table 3 list the patient and the donor characteristics, conditioning regimen, source of the stem cells, GVHD prophylaxis, and other laboratory results. The busulfan-based conditioning group included 84 patients, and the treosulfan-based conditioning group included 37 patients. Median age at HSCT was 14.0 ± 5.8 years (range, 3.0-18.0 years) in the busulfan group and 12.8 ± 6.5 years (range, 2.0-17.6 years) in the treosulfan group ($P < .001$). Based on the size of the liver, the degree of chelation, and the presence of hepatic fibrosis, patients were categorized using the Pesaro categorization system, with 43 patients in Pesaro class 1, 50 patients in Pesaro class 2, and 27 patients in Pesaro class 3 ($P < .001$). For Pesaro class 2, 50.6% of patients were in the busulfan group and 21.6% of patients were in the treosulfan group. For Pesaro class 3, 12% were in the busulfan group and 45.9% were in the treosulfan group.

Most patients in the busulfan group received a combination of busulfan plus cyclophosphamide without thiotepa (46/84 patients, 54.8%). Most patients in the treosulfan group received treosulfan plus fludarabine plus thiotepa for conditioning (34/37 patients, 91.6%). Both the busulfan group and the treosulfan group relied on calcineurin inhibitors

for GVHD prophylaxis (n = 43 [51.2%] and n = 20 [54%], respectively).

Table 1. Comparison of Demographic and Laboratory Data by Regimen Group

	Busulfan Based	Treosulfan Based	Total	Test Statistic	*P*
Sex, No. (%)					
Male	56 (66.7)	20 (54.1)	76 (62.8)	1.749	.186*
Female	28 (33.3)	17 (45.9)	45 (37.2)		
Pesaro class , No. (%)					
1	31 (37.3)	12 (32.4)	43 (35.8)	18.401	<.001*
2	42 (50.6)[a]	8 (21.6)[b]	50 (41.7)		
3	10 (12)[a]	17 (45.9)[b]	27 (22.5)		
Donor, No. (%)					
Sibling	42 (50)	12 (32.4)	54 (44.6)	23.604	<.001*
Mother	7 (8.3)	1 (2.7)	8 (6.6)		
Father	9 (10.7)[a]	0 (0)[b]	9 (7.4)		
Relative	13 (15.5)	3 (8.1)	16 (13.2)		
Nonrelative	13 (15.5)[a]	21 (56.8)[b]	34 (28.1)		
HLA compatibility, No. (%)					
10/10	75 (89.3)	32 (86.5)	107 (88.4)		.759†
9/10	9 (10.7)	5 (13.5)	14 (11.6)		
Stem cell source, No. (%)					
Bone marrow	42 (50)	21 (56.8)	63 (52.1)	4.984	.289*
Peripheral blood stem cells	32 (38.1)	14 (37.8)	46 (38)		
Umbilical cord blood	2 (2.4)	0 (0)	2 (1.7)		
Bone marrow + umbilical cord blood	8 (9.5)	1 (2.7)	9 (7.4)		
Bone marrow + peripheral stem cells	0 (0)	1 (2.7)	1 (0.8)		
Conditioning regimen, No. (%)					
Bu + Cy	7 (8.3)	0 (0)	7 (5.8)	117.4	<.001*
Tre + Flu + TT	0 (0)[a]	34 (91.9)[b]	34 (28.1)		
Bu + Cy + Flu	46 (5.8)[a]	0 (0)b	46 (38)		
Bu + Flu + Cy + TT	14 (16.7)[a]	0 (0)[b]	14 (11.6)		
Tre + Flu	1 (1.2)[a]	3 (8.1)[b]	4 (3.3)		
Bu	16 (19)[a]	0 (0)b	16 (13.2)		
Serotherapy, No. (%)					
No	45 (53.6)	4 (10.8)	49 (40.5)	19.491	<.001*
ATG	39 (46.4)	33 (89.2)	72 (59.5)		
GVHD prophylaxis, No. (%)					
CNI monotherapy	43 (51.2)	20 (54.1)	63 (52.1)	2.930	.231*
CNI plus prednisolone	29 (34.5)	8 (21.6)	37 (30.6)		
CNI plus MMF	12 (14.3)	9 (24.3)	21 (17.4)		

Abbreviations: ATG, antithymocyte globulin; Bu, busulfan; CNI, calcineurin inhibitor; Cy, cyclophosphamide; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; TT, thiotepa; Tre, treosulfan
*Chi-square test. †Fisher exact test. [a,b]No difference in results with the same letter in each row.

A significant difference in the distribution of donor type was shown between the groups ($P < .001$). Thirteen patients (15.5%) in the busulfan group and 21 patients (56.8%) in the treosulfan group received an HSCT from a matched unrelated donor. The stem cell source was bone marrow in 63 patients, filgrastim (granulocyte colony-stimulating factor)-mobilized peripheral blood in 46 patients, and bone marrow plus cord blood in 9 patients ($P = .289$).

Table 2. Comparison of Toxicity, Graft-Versus-Host Disease, and Transplant Status by Regimen

	Busulfan Based	Treosulfan Based	Total	Test Statistic	*P*
Toxicity, No. (%)					
Skin	7 (14.3)	2 (25)	9 (15.8)		
Cardiac	5 (10.2)	1 (12.5)	6 (10.5)		
Respiratory	2 (4.1)	1 (12.5)	3 (5.3)		
Renal	11 (22.4)	0 (0)	11 (19.3)		
Hepatobiliary	6 (12.2)	0 (0)	6 (10.5)		
Neurologic	2 (4.1)	2 (25)	4 (7)		
Hematologic	2 (4.1)	1 (12.5)	3 (5.3)		
Mucositis	7 (14.3)	1 (12.5)	8 (14)		
Vomiting	5 (10.2)	0 (0)	5 (8.8)		
Hemorrhage/ bleeding	2 (4,1)	0 (0)	2 (3,5)		
Toxicity, No. (%)					
No	35 (41.7)	29 (78.4)	64 (52.9)	13.894	<.001*
Yes	49 (58.3)	8 (21.6)	57 (47.1)		
Grade of toxicity, No. (%)					
1	22 (44.9)	5 (62.5)	27 (47.4)	1.533	.821*
2	20 (40.8)	2 (25)	22 (38.6)		
3	4 (8.2)	1 (12.5)	5 (8.8)		
4	1 (2)	0 (0)	1 (1.8)		
5	2 (4.1)	0 (0)	2 (3.5)		
aGVHD grade, No. (%)					
1	3 (3.6)	6 (16.7)	9 (7.6)	9.068	.059*
2	11 (13.3)	2 (5.5)	13 (12.6)		
3	5 (6)	3 (8.3)	8 (6.7)		
4	7 (8.4)	2 (5.5)	9 (7.5)		
No	57 (68.7)	23 (63.9)	80 (67.2)		
cGVHD, No. (%)					
No	67 (79.8)	33 (89.2)	100 (82.6)	1.889	.596*
Yes, mild	4 (4.8)	1 (2.7)	5 (4.1)		
Yes, moderate	7 (8.3)	1 (2.7)	8 (6.6)		
Yes, severe	6 (7.1)	2 (5.4)	8 (6.6)		
Improvement in cGVHD symptoms, No. (%)					
Yes	8 (47.1)	2 (50)	10 (47.6)		1.000†
No	9 (52.9)	2 (50)	11 (52.4)		
Transplant status, No. (%)					
Complete chimerism	73 (86.9)	36 (97.3)	109 (90.1)	7.423	.060*

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease
*By chi-square test. †By Fisher exact test.

Table 3. Comparison of Last Status by Regimen

	Busulfan -Based	Treosulfan Based	Total	Test Statistic	*P*
Last status, No. (%)					
Alive, without thalassemia	71 (84.5)	33 (89.2)	104 (86)	1.133	.769*
Alive, thalassemic	3 (3.5)	0 (0)	3 (2.5)		
Deceased	9 (10.7)	3 (8.1)	12 (9.9)		
2nd transplant, deceased	2 (2.4)	0 (0)	2 (1.7)		
Late status group, No. (%)					
Alive	73 (86.9)	34 (91.9)	107 (88.4)		.547†
Deceased	11 (13.1)	3 (8.1)	14 (11.6)		

*By chi-square test. †By Fisher exact test.

Table 4. Comparison of Quantitative Data by Regimen

	Busulfan-Based		Treosulfan-Based		Total		Statistic*
	Mean ± SD	Median (range)	Mean ± SD	Median (range)	Mean ± SD	Median (range)	
Age, y	14.0 ± 5.8	13.0 (3.0-29.0)	12.8 ± 6.5	14.0 (2.0-23.0)	13.6 ± 6.0	14.0 (2.0-29.0)	0.000
Busulfan (mg/kg) or treosulfan (g/m^2) dose	3.9 ± 0.7	4.0 (0.8-4.8)	14.0 ± 0.0	14.0 (14.0-14.0)	7.0 ± 4.7	4.4 (0.8-14.0)	682.000
Busulfan, (µmol/L/min) or treosulfan (mg/L/h) level	1256.1 ± 320.6	1228.0 (667.0-2775.0)	1690.7 ± 634.8	1547.0 (781.0-3363.0)	1371.5 ± 465.6	1280.0 (667.0-3363.0)	1430.000
Time to neutrophil engraftment, day	15.4 ± 3.6	15.0 (11.0-31.0)	15.0 ± 2.6	15.0 (11.0-23.0)	15.3 ± 3.3	15.0 (11.0-31.0)	1534.000
Time to platelet engraftment, day	15.9 ± 3.5	15.0 (9.0-27.0)	15.5 ± 3.2	15.0 (12.0-26.0)	15.8 ± 3.4	15.0 (9.0-27.0)	1432.500

*By Mann-Whitney U test.

Engraftment, chimerism and survival

In the treosulfan group, the median times to neutrophil and platelet engraftment were 15.0 days (range, 11.0-23.0 days) and 15.0 days (range, 12.0-26.0 days), respectively. In the busulfan group, the median times to neutrophil and platelet engraftment were 15 days (range, 11.0-31.0 days) and 15.0 days (range, 9.0-27.0 days), respectively. There was no noticeable difference between platelet and neutrophil engraftment in the 2 groups (Table 4).

Median follow-up time among both groups was 4.3 years (95% CI, 3.2-7.5). Mean survival time was 94.7 months (95% CI, 87.4-102.1) in the busulfan group and 125.3 months (95% CI, 112.3-138.3) in the treosulfan group, with survival for both groups of 120.9 months (95% CI, 112.8-129.0). Two-year OS was 87.5% in the busulfan group and 91.1% in the treosulfan group; groups were not significantly different with regard to OS ($P = 0.72$) (Table 5, Figure 1). Among 121 patients, 16 died as a result of complications of TDT or HSCT (Table 6).

Table 5. Comparison of Survival Time by Regimen

	Mean Survival (95% CI), months	2-Year Cumulative Survival Rate (SE)	*P**
Busulfan-based regimen	94.7 (87.4-102.1)	0.875 (0.037)	.721
Treosulfan-based regimen	125.3 (112.3-138.3)	0.911 (0.049)	
Overall	120.9 (112.8-129.0)		

*By log-rank (Mantel-Cox).

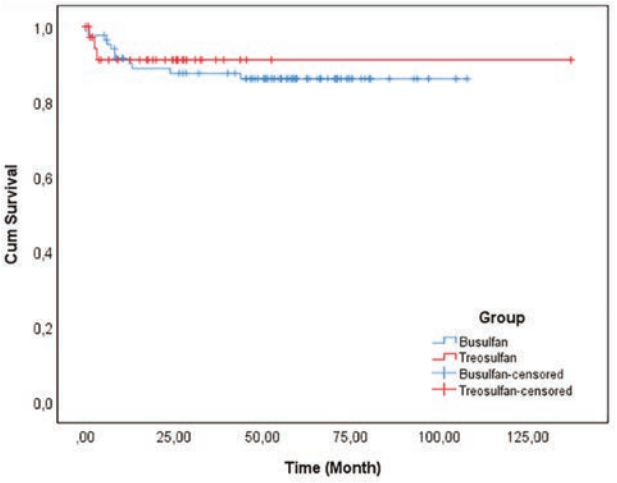
Table 6. Distribution of Causes of Death by Regimen

Cause of Death	No. (%)		
	Busulfan-Based	Treosulfan-Based	Total
Infection	3 (23.1)	1 (33.3)	4 (25)
Hemorrhage	1 (7.7)	0 (0)	1 (6.3)
aGVHD + infection	2 (15.4)	1 (33.3)	3 (18.8)
cGVHD	4 (30.8)	1 (33.3)	5 (31.3)
Nontransplant cause	1 (7.7)	0 (0)	1 (6.3)
Toxicity	2 (15.4)	0 (0)	2 (12.5)

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease

Statistical comparisons could not be made because of the low number of observations.

Figure 1. Overall Survival



A total of 107 patients were evaluable at 1 year following HSCT, 111 patients were at day +100 chimerism, and 121 patients were evaluable at day +30 chimerism. In 73 patients (86.9%) in the busulfan group and 36 patients (97.3%) in the treosulfan group, donor chimerism was between 90% and 100% at year 1, respectively. No relationship was seen with treosulfan or busulfan exposure at year 1 ($P = .060$).

Cumulative incidence of treatment-related mortality at day +100 was 2.4% (n = 2) (95% CI, 5%-20%) in the busulfan group and 5.4% (n = 2) in the treosulfan group, with no relationship found between treatment-related mortality and conditioning regimen ($P = .585$) (Table 7).

Table 7. Comparison of Treatment-Related Mortality by Regimen

Treatment-Related Mortality	Busulfan-Based	Treosulfan-Based	Total	*P**
Alive, No. (%)	82 (97.6)	35 (94.6)	117 (96.7)	.585
Deceased, No. (%)	2 (2.4)	2 (5.4)	4 (3.3)	

*By Fisher exact test.

Early regimen-related toxicity

Incidences of mucosal, liver, cutaneous, and renal damage were the most frequently observed toxicities.

Table 2 lists toxicities according to the conditioning regimen group. Eight patients (6.6%) experienced mucositis (developing grade 3 or 4). Hepatobiliary toxicity was not observed in the treosulfan group. Six patients (4.9%) experienced hepatobiliary toxicity in the busulfan group, with 2 having severe veno-occlusive disease/sinusoidal obstruction syndrome, as determined by modified Seattle criteria. Although significant, significance could not be mentioned because there were not enough cases. Skin toxicity, including erythematous rash and skin exfoliation, was observed in 7 patients in the busulfan group and 1 patient in the treosulfan group. No significant association was shown between busulfan exposure and skin toxicity according to drug plasma levels. Convulsions and posterior reversible encephalopathy syndrome were among the neurological symptoms that were observed in 2 patients in each group (total of 4 patients, 3.3%). Neurological problems and busulfan exposure were not significantly related. Although there was no one-by-one difference between system involvements, we observed a significant increase in the busulfan group (n = 49, 58.3%) compared with the treosulfan group (n = 8, 21.6%) in terms of total side effects ($P < .001$).

When we analyzed toxicities in the treosulfan group by treosulfan level, the number of side effects was low and an evaluation could not be made (Table 8). However, with regard to busulfan-based regimens, no significant difference was shown between the frequency of side effects and drug serum levels ($P = .879$) (Table 9).

Graft-versus-host disease

Grade III or IV aGVHD cumulative incidence was 14%, with 12 patients in the busulfan group and 5 patients in the treosulfan group. When we compared these 2 group with the Fisher exact test, we found no significant different. Of 121 patients, 39 experienced grade I-IV aGVHD, with 5-year cumulative incidence of 25.4% in busulfan group and 60.7% in the treosulfan group. Among 121 patients at risk, 8 developed extensive severe cGVHD, with 5-year cumulative incidence of 17.4 % in busulfan group and 24.6% in the treosulfan group. We also analyzed the estimated aGVHD cumulative incidence with the Gray test method. Among 84 patients in the busulfan group and 37 patients in the treosulfan group, estimated 5-year cumulative incidence was 25.4% in the busulfan group (95% CI, 16.4%-39.4%) and 60.7% in the treosulfan group (95% CI, 41.6%-88.4%) (Table 10). The estimated 8-year cumulative incidence was 71.4% (95% CI, 49.8%-100%) and 60.7% (95% CI, 41.6%-88.4%), respectively. The Gray test showed a significant difference between the busulfan and treosulfan groups for the 5-year estimated cumulative incidence for aGVHD ($P < .001$). Chronic GVHD occurred in 17 patients in the busulfan group and in 4 patients in the treosulfan group, with estimated 5-year cumulative incidence of 17.4% (95% CI, 10%-30.5%) and 24.6% (95% CI, 10.1%-60.1%) and estimated 8-year cumulative incidence of 59.7% (95% CI, 32.4%-100%) and 24.6% (95% CI, 10.1%-60.1%) in the busulfan and treosulfan groups, respectively. We found no significant difference in the 5-year and 8-year estimated cumulative incidence of cGVHD between the groups ($P = .988$) (Table 10, Figure 2, Figure 3).

The estimated 5-year cumulative incidence of treatment-related mortality was 2.4% (95% CI, 0.06%-9.4%) and 5.9% (95% CI, 1.5%-%22.6) for the busulfan and treosulfan groups, respectively. The estimated 8-year cumulative incidence rate was 2.4% (95% CI, 0.06%-9.4%) and 5.9% (95% CI, 1.5%-22.6%) for the busulfan and treosulfan groups, respectively (Table 10, Figure 4).

Table 8. Toxicities in Patients Who Received the Treosulfan-Based Regimen by Level

	No. (%) of Patients Receiving Regimen (in mg/L/h)			Total
	<1130	1130-2450	>2450	
Skin	0 (0)	0 (0)	2 (66.7)	1 (14.3)
Cardiac	1 (33.3)	0 (0)	0 (0)	1 (14.3)
Respiratory	0 (0)	0 (0)	1 (33.3)	1 (14.3)
Neurologic	2 (66.7)	0 (0)	0 (0)	2 (28.6)
Hematologic	0 (0)	1 (50)	0 (0)	1 (14.3)
Mucositis	0 (0)	1 (50)	0 (0)	1 (14.3)

Statistical comparisons could not be made because of the low number of observations.

Table 9. Toxicities in Patients Who Received the Busulfan-Based Regimen by Level

	No. (%) of Patients Receiving Regimen (in µmol/L/min)				Test Statistic	*P**
	<900	900-1350	>1350	Total		
Skin	1 (14.3)	2 (9.1)	4 (20)	7 (14.3)	11.35	.879
Cardiac	0 (0)	3 (13.6)	2 (10)	5 (10.2)		
Respiratory	0 (0)	1 (4.5)	1 (5)	2 (4.1)		
Renal	3 (42.9)	5 (22.7)	3 (15)	11 (22.4)		
Hepatobiliary	1 (14.3)	3 (13.6)	2 (10)	6 (12.2)		
Neurologic	0 (0)	1 (4.5)	1 (5)	2 (4.1)		
Hematologic	1 (14.3)	1 (4.5)	0 (0)	2 (4.1)		
Mucositis	0 (0)	4 (18.2)	3 (15)	7 (14.3)		
Vomiting	1 (14.3)	2 (9.1)	2 (10)	5 (10.2)		
Hemorrhage/ bleeding	0 (0)	0 (0)	2 (10)	2 (4.1)		

*By chi-square test.

Figure 2. Estimated 8-Year Cumulative Incidence of Acute Graft-Versus-Host Disease Over Time, in Months

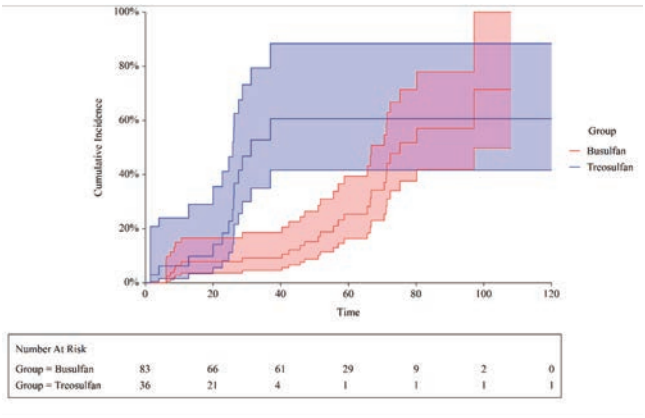
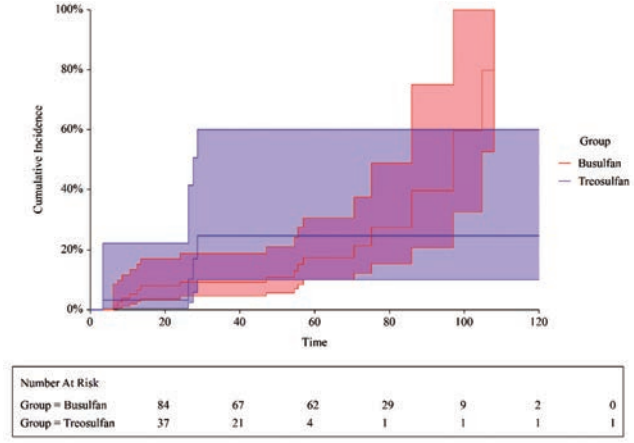


Figure 3. Estimated 8-Year Cumulative Incidence of Chronic Graft-Versus-Host Disease Over Time, in Months



Estimated 5-year cumulative incidence of primary rejection was 3.2% (95% CI, 0.8%-12.5%) and 0%, respectively, for the busulfan and treosulfan groups. Estimated 8-year cumulative incidence rate of primary rejection was 27.4% (95% CI, 6.1%-100%) and 0%, respectively, for the busulfan and treosulfan groups (Table 10, Figure 5).

Figure 4. Estimated 8-Year Cumulative Incidence of Treatment-Related Mortality Over Time, in Months

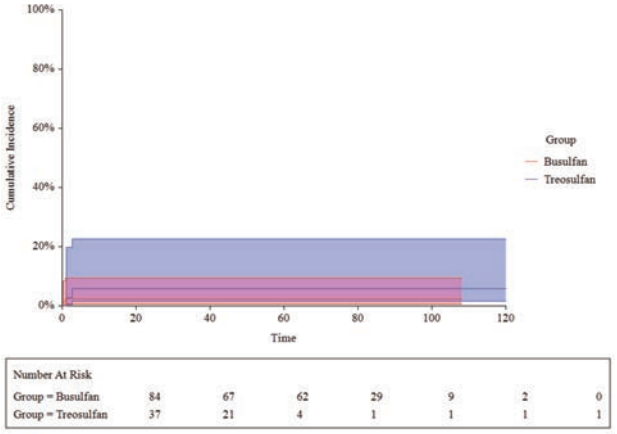
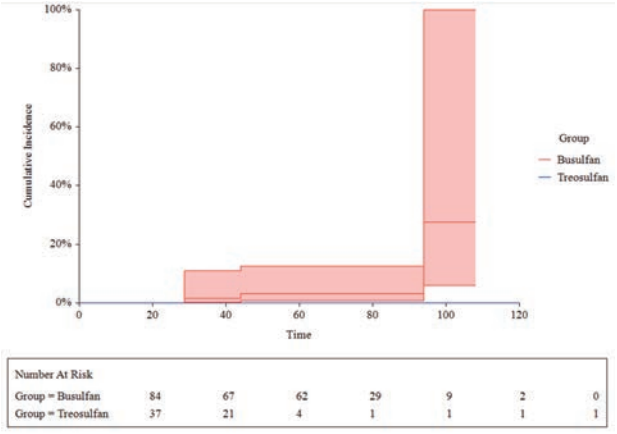


Figure 5. Estimated 8-Year Cumulative Incidence of Primary Rejection Over Time, in Months



Discussion

Allogeneic HSCT remains the solely recognized and broadly available curative standard of therapy for patients with TDT. Treosulfan-based conditioning has been shown to be efficient and well-tolerated in patients with both malignant and nonmalignant diseases, and it is being utilized more frequently in

Table 10. Results of Gray Test for Events Comprising Disease-Free Survival

Event	Regimen Group	No. of Events	5-Year Cumulative Incidence (95% CI)	8-Year Cumulative Incidence (95% CI)	*P*
aGVHD	Busulfan (n = 84)	26	25.4% (16.4-39.4)	71.4% (49.8-100)	<.001
	Treosulfan (n = 37)	13	60.7% (41.6-88.4)	60.7% (41.6-88.4)	
cGVHD	Busulfan (n = 84)	17	17.4% (10-30.5)	59.7% (32.4-100)	.988
	Treosulfan (n = 37)	4	24.6% (10.1-60.1)	24.6% (10.1-60.1)	
TRM	Busulfan (n = 84)	2	2.4% (0.06-9.4)	2.4% (0.06-9.4)	.374
	Treosulfan (n = 37)	2	5.9% (1.5-22.6)	5.9% (1.5-22.6)	
Primary rejection	Busulfan (n = 84)	3	3.2% (0.8-12.5)	27.4% (6.1-100)	.490
	Treosulfan (n = 37)	0	0	0	

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; TRM, treatment-related mortality

pediatric HSCT. Our study showed that, overall, conditioning based on treosulfan was well-tolerated, and limited toxicity was observed compared with the busulfan-based regimen. Treosulfan appears to be safe and effective even for infantile ages, according to preliminary reports.[17] The fact that there were no deaths due to toxicity in the patients who received treosulfan also supports this argument. However, we must accept that treosulfan has minimized but not eliminated treatment-related toxicity. Although the overall toxicity rate decreased in the group receiving a treosulfan-based conditioning regimen, treosulfan exposure is linked to mucositis, skin toxicity, and a risk of experiencing multiple toxicities within the first 28 days following HSCT at all plasma drug levels, suggesting that treosulfan dose regulation may lessen transplant-related morbidity when administered as an individualized treatment dose in each patient. Liver toxicity, pulmonary hypertension, skin toxicity, mucositis, and seizures have also been reported regarding the toxicity of treosulfan, similar to our study.[17,18,21,22] In addition, it should be kept in mind that early regimen-related toxicity rates were higher in the group receiving busulfan, although dose adjustment was also made in the group receiving the busulfan-based regimen according to the plasma drug level.

Although the mean age of the treosulfan group was significantly higher than the busulfan group (taking into account the Pesaro risk classification), the lack of significant difference in OS, treatment-related mortality, and mortality rates can be interpreted as low treatment-related toxicity. As previously shown, risk class is expected to increase in thalassemia patients with a high mean age and the success and benefits of HSCT would decrease inversely.[23-25]

We reported an association between treosulfan exposure and early toxicities (particularly severe mucositis and skin toxicity), but we have not identified a link with neutrophil or platelet engraftment, chimerism at 1 year, and development of severe acute GVHD. The heterogeneity of the main diseases in our group may, however, be to blame for this. Similarly, no significant difference was found between neutrophil and platelet engraftment times in a study conducted by Chaudry and colleagues[26] among 40 patients diagnosed with TM, in which treosulfan- and busulfan-based conditioning regimens were compared ($P = .68$). However, in another study conducted with 189 patients with TM in which the same 2 conditioning regimens were also compared, no significant difference was found between neutrophil engraftment, but platelet engraftment time was found to be significantly shorter in the treosulfan group ($P = .242$ and .000, respectively).[27] The authors explained this situation by change in graft source (peripheral blood stem cells) in some patients.

Overall, only a small percentage of patients experienced severe aGVHD, with no statistical difference between conditioning groups (grade III-IV aGVHD occurred in 14% of patients in the busulfan group vs 8% in the treosulfan group), consistent with previous data.[8] In the busulfan group, 7.1% of patients (n = 7) had 2-year severe cGVHD compared with 5.4% (n = 2) in the treosulfan group ($P > .05$). The fact that treatment-related mortality rates are similar in patients who receive busulfan-based (n = 2, 2.4%) and treosulfan-based (n = 2, 5.4%) regimens also supports this result. However, the drug has a conditional ability to reduce the likelihood of GVHD compared with other conditioning regimens.[11,22,28] The estimated cumulative incidence of aGVHD is significantly lower (Gray test, $P < .001$). Nevertheless, this result may be associated with the small number of patients. Randomized prospective clinical studies are needed to identify differences between the 2 conditioning regimens.

In patients with TDT, HSCT protects against disease-related organ damage and enhances quality of life in terms of health.[2] Because HSCT is usually performed under elective conditions in TDT patients, it is important to choose suitable agents with minimal toxic effects and to consider additional HSCT-related issues, including graft rejection and GVHD, when selecting the conditioning regimen. On the basis of these factors, treosulfan-based conditioning has been shown to be efficient and well-tolerated in patients with malignant and nonmalignant diseases, and it is being used more frequently in pediatric HSCT.[22,27-29] We mentioned that the lower toxicity profile of treosulfan compared with busulfan-cyclophosphamide may have prompted treating physicians to select treosulfan-based preparative regimens.

The busulfan-fludarabine-cyclophosphamide regimen was given to 54.8% of the busulfan group, whereas the treosulfan-fludarabine-thiotepa preparation regimen was applied to 91.9% of the treosulfan group. Treosulfan was administered at a dose of

14 g/m^2, which was widely considered myeloablative, had low toxicity, and produced notable chimerism (97.2%) in addition to reducing regimen-related toxicity and graft failure. This was also demonstrated by a higher 2-year OS in the treosulfan group and no patients needing a second HSCT, similar to the excellent OS rates achieved in a similar study.[18] The high rate of OS and low incidence of secondary HSCT of the conditioning regimen are thought to be due to the combination of treosulfan with fludarabine and an alkylating agent, thiotepa. Similar studies have supported this claim.[18,21] Because of its potent immunosuppressive properties, fludarabine is the major component of the most current reduced intensity conditioning regimens for both adults and children.[30,31]

Our research also revealed that patients in the treosulfan group had more matched unrelated donors than those in the busulfan group ($P = .001$). With the consideration that the OS rates in both the treosulfan and busulfan groups are excellent, it may be an advantage to give patients who do not have a matched sibling donor the chance to have HSCT from a matched unrelated donor. Matched unrelated donors are important in terms of creating a new and extensive resource for HSCT to be performed on patients with TDT.[25]

An advantage of our study is it is more comprehensive because it includes a toxicity profile for busulfan and treosulfan plasma drug levels. A limitation is that the long-term side effects of treosulfan-based and busulfan-based regimens should be handled with an individual approach for each patient in terms of endocrine system problems and the risk of secondary cancers. Such data will only be available with longer follow-up in larger cohorts.

Conclusions

The safety and effectiveness of a treosulfan-based myeloablative conditioning regimen has been confirmed by our retrospective investigation of patients with TDT. Treosulfan-based conditioning regimens are well tolerated and increasingly becoming accepted as the gold standard of care globally, and they appear to be suitable for reducing the risk of short-term life-threatening HSCT complications in patients with TDT.

References

1. Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010;5:1-15. doi:10.1186/1750-1172-5-11
2. Strocchio L, Locatelli F. Hematopoietic stem cell transplantation in thalassemia. *Hematol Oncol Clin North Am*. 2018;32(2):317-328. doi:10.1016/j.hoc.2017.11.011
3. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica*. 2014;99(5):811-820. doi:10.3324/haematol.2013.099747
4. El-Beshlawy A, El-Ghamrawy M. Recent trends in treatment of thalassemia. *Blood Cells Mol Dis*. 2019;76:53-58. doi:10.1016/j.bcmd.2019.01.006
5. Pilo F, Angelucci E. Iron toxicity and hemopoietic cell transplantation: time to change the paradigm. *Mediterr J Hematol Infect Dis*. 2019;11(1):e2019030. doi:10.4084/MJHID.2019.030
6. Angelucci E, Pilo F, Coates TD. Transplantation in thalassemia: revisiting the Pesaro risk factors 25 years later. *Am J Hematol*. 2017;92(5):411-413. doi:10.1002/ajh.24674
7. Choudhary D, Doval D, Sharma SK, Khandelwal V, Setia R, Handoo A. Allogenic hematopoietic cell transplantation in thalassemia major: a single-center retrospective analysis from India. *J Pediatr Hematol Oncol*. 2019;41(5):e296-e301. doi:10.1097/MPH.0000000000001475
8. Luftinger R, Zubarovskaya N, Galimard JE, et al. Busulfan-fludarabine- or treosulfan-fludarabine-based myeloablative conditioning for children with thalassemia major. *Ann Hematol*. 2022;101(3):655-665. doi:10.1007/s00277-021-04732-4
9. Mustafa M, Qatawneh M, Al Jazazi M, et al. Hematopoietic stem cell transplantation in thalassemia patients: a Jordanian single centre experience. *Mater Sociomed*. 2020;32(4):277-282. doi:10.5455/msm.2020.32.277-282
10. Yesilipek MA, Uygun V, Kupesiz A, et al. Thalassemia-free and graft-versus-host-free survival: outcomes of hematopoietic stem cell transplantation for thalassemia major, Turkish experience. *Bone Marrow Transplant*. 2022;57(5):760-767. doi:10.1038/s41409-022-01613-w
11. Anurathapan U, Pakakasama S, Mekjaruskul P, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. *Biol Blood Marrow Transplant*. 2014;20(12):2066-2071. doi:10.1016/j.bbmt.2014.07.016
12. van der Stoep M, Bertaina A, Ten Brink MH, et al. High interpatient variability of treosulfan exposure is associated with early toxicity in paediatric HSCT: a prospective multicentre study. *Br J Haematol*. 2017;179(5):772-780. doi:10.1111/bjh.14960
13. Vellaichamy Swaminathan V, Uppuluri R, Patel S, et al. Treosulfan-based reduced toxicity hematopoietic stem cell transplantation in X-linked agammaglobulinemia: a cost-effective alternative to long-term immunoglobulin replacement in developing countries. *Pediatr Transplant*. 2020;24(1):e13625. doi:10.1111/petr.13625
14. Romanski M, Wachowiak J, Glowka FK. Treosulfan pharmacokinetics and its variability in pediatric and adult patients undergoing conditioning prior to hematopoietic stem cell transplantation: current state of the art, in-depth analysis, and perspectives. *Clin Pharmacokinet*. 2018;57(10):1255-1265. doi:10.1007/s40262-018-0647-4
15. ten Brink MH, Zwaveling J, Swen JJ, Bredius RG, Lankester AC, Guchelaar HJ. Personalized busulfan and treosulfan conditioning for pediatric stem cell transplantation: the role of pharmacogenetics and pharmacokinetics. *Drug Discov Today*. 2014;19(10):1572-1586. doi:10.1016/j.drudis.2014.04.005
16. Mehdizadeh M, Parkhideh S, Salari S, Hajifathali A, Rezvani H, Mabani M. Adverse effects of busulfan plus cyclophosphamide versus busulfan plus fludarabine as conditioning regimens for allogeneic bone marrow transplantation. *Asian Pac J Cancer Prev*. 2021;22(5):1639-1644. doi:10.31557/APJCP.2021.22.5.1639

17. Slatter MA, Rao K, Abd Hamid IJ, et al. Treosulfan and fludarabine conditioning for hematopoietic stem cell transplantation in children with primary immunodeficiency: UK experience. *Biol Blood Marrow Transplant*. 2018;24(3):529-536. doi:10.1016/j.bbmt.2017.11.009
18. Beier R, Schulz A, Honig M, et al. Long-term follow-up of children conditioned with treosulfan: German and Austrian experience. *Bone Marrow Transplant*. 2013;48(4):491-501. doi:10.1038/bmt.2012.188
19. Klaudel-Dreszler MA, Kalwak K, Kurenko-Deptuch M, et al. Treosulfan-based conditioning regimen in a second matched unrelated peripheral blood stem cell transplantation for a pediatric patient with CGD and invasive aspergillosis, who experienced initial graft failure after RIC. *Int J Hematol*. 2009;90(5):571-575. doi:10.1007/s12185-009-0433-z
20. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18(4):295-304. doi:10.1097/00007890-197410000-00001
21. Dinur-Schejter Y, Krauss AC, Erlich O, et al. Bone marrow transplantation for non-malignant diseases using treosulfan-based conditioning. *Pediatr Blood Cancer*. 2015;62(2):299-304. doi:10.1002/pbc.25267
22. Bernardo ME, Piras E, Vacca A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. *Blood*. 2012;120(2):473-476. doi:10.1182/blood-2012-04-423822
23. Peters C. Allogeneic hematopoietic stem cell transplantation to cure transfusion-dependent thalassemia: timing matters! *Biol Blood Marrow Transplant*. 2018;24(6):1107-1108. doi:10.1016/j.bbmt.2018.04.023
24. Giardini C, Lucarelli G. Bone marrow transplantation for beta-thalassemia. *Hematol Oncol Clin North Am*. 1999;13(5):1059-1064. doi:10.1016/s0889-8588(05)70109-x
25. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica*. 2014;99(5):811-820. doi:10.3324/haematol.2013.099747
26. Choudhary D, Sharma SK, Gupta N, et al. Treosulfan-thiotepa-fludarabine-based conditioning regimen for allogeneic transplantation in patients with thalassemia major: a single-center experience from north India. *Biol Blood Marrow Transplant*. 2013;19(3):492-495. doi:10.1016/j.bbmt.2012.11.007
27. Mathews V, George B, Viswabandya A, et al. Improved clinical outcomes of high risk beta thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. *PLoS One*. 2013;8(4):e61637. doi:10.1371/journal.pone.0061637
28. Bernardo ME, Zecca M, Piras E, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major. *Br J Haematol*. 2008;143(4):548-551. doi:10.1111/j.1365-2141.2008.07385.x
29. Burroughs LM, Nemecek ER, Torgerson TR, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. *Biol Blood Marrow Transplant*. 2014;20(12):1996-2003. doi:10.1016/j.bbmt.2014.08.020
30. Strullu M, Rialland F, Cahu X, et al. Allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning regimen in children: a single-center experience. *Eur J Haematol*. 2012;88(6):504-509. doi:10.1111/j.1600-0609.2012.01776.x
31. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. *Blood*. 2014;124(3):344-353. doi:10.1182/blood-2014-02-514778

Current Status and Trends in Lung Transplant Research Funded by the National Natural Science Foundation of China

Xianyu Xie,[1] Zijun Zhao,[2]* Qinde Wu[3]**

Abstract

Objectives: **This study aimed to analyze research projects on lung transplant funded by the National Natural Science Foundation of China from 1986 to 2022 and to provide a scientific reference for lung transplant research.**

Materials and Methods: **We identified research hotspots and frontiers in the field of lung transplant research using CiteSpace visualization.**

Results: **From 1986 to 2022, the National Natural Science Foundation of China funded 93 projects related to lung transplant, with an average of 2.51 projects and ¥0.94 million annually. The National Natural Science Foundation of China funded 30 institutions across 20 provinces, with general and youth science foundation projects comprising 45.16% and 41.93% of the total projects, respectively. The main categories of disciplines included H0113 respiratory intervention, tracheal reconstruction, and lung transplantation; H1105 organ transplantation and transplant immunization; and H0109 acute lung injury and acute respiratory distress syndrome. The research hotspots mainly included ischemia-reperfusion injury, gene regulation, obliterative bronchiolitis, rejection reaction, T cells, and stem cells. The 6 main research clusters were ischemia-reperfusion injury, immune tolerance, obliterative bronchiolitis, stem cells, pulmonary fibrosis, and rejection reaction. The main key word bursts in the past 5 years were "vein endothelial" and "ex vivo lung perfusion."**

Conclusions: **In the past 37 years, National Natural Science Foundation of China-funded projects have significantly advanced the clinical application and basic research of lung transplantation. However, compared with developed countries and other solid-organ transplantations, several problems still require attention and improvements in lung transplant research in China.**

Key words: *Clinical research, Fundamental research, Research frontiers, Research hotspots*

Introduction

Lung transplantation is the only effective treatment for end-stage lung disease.[1] Over 70000 lung transplants have been performed worldwide since 1990.[2] The data analysis report on thoracic organ transplant cohorts by the International Society for Heart and Lung Transplantation focuses on a topic each year, such as donor and recipient age, retransplantation, transplant indications, and graft ischemia time.[3-7] Recently, marginal donor lung, ex vivo lung perfusion (EVLP), and extracorporeal membrane oxygenation have become the focus of lung transplantation. Nevertheless, survival rates after lung transplant are still lower than rates for other solid-organ transplants. Problems, such as donor shortage, donor lung maintenance, primary graft dysfunction, and chronic graft dysfunction, still hinder the development of lung transplantation. Therefore, studies on lung transplant and its basic medicine are important.

The National Natural Science Foundation of China (NSFC) was established in 1986. It is one of the most important and most high-level research funding institutions in China and has greatly promoted scientific research, innovation, and discipline. Analysis of projects funded by NSFC can reflect the nation's support for research and identify

From the [1]Department of Medical Administration, Fujian Medical University Union Hospital, Fuzhou City; the [2]School of Public Administration & Law, Fujian Agriculture and Forestry University, Fuzhou City; and the [3]Department of Education Administration, Fujian Medical University Union Hospital, Fuzhou City, Fujian Province, China

Acknowledgements: *This work was supported by the Startup Fund for Scientific Research (Fujian Medical University) (2021QH1053). The funding source had no role in the study design; the collection, analysis, or interpretation of the data; the writing of the report; or in the decision to submit the article for publication. The authors have no declarations of potential conflicts of interest. We thank those who have helped us throughout the research process and writing of this paper. Our sincere gratitude also goes to our professors and teachers who have greatly helped us in our study and our lives. Finally, we thank our friends and families who have given us much needed encouragement and financial support.*

** X. Xie, Z. Zhao, and Q Wu contributed equally to this work.*

Corresponding author: *Qinde Wu, Department of Education Administration, Fujian Medical University Union Hospital, 29 Xinquan Road, Fuzhou City, Fujian Province, China*

Phone: *+86 13365917369* ***E-mail:*** *qdwu88@126.com*

Experimental and Clinical Transplantation (2023) 11: 893-900

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0220

research frontiers and hotspots in this field.[8] This study aimed to analyze NSFC-funded projects on lung transplantation from 1986 to 2022, provide insights into scientific research trends, guide the direction of research, and assist lung transplant professionals globally and in China.

Materials and Methods

Data sources

Using the NSFC network information system, we used the key word "lung transplantation" as the search strategy to identify funded projects from 1986 to 2022. Lung transplant experts were invited to review all key words, abstracts, or concluding reports, and the funding projects that met the study criteria were selected. No ethical approval was required, as there were no human participants in this study.

Quality control and statistical methods

We gathered data on various parameters, such as project leaders, supporting units, project names, key words, funding type, project year and duration, and funding amount. We entered parameters into an EXCEL 2019 form to establish a database. To ensure quality control, 2 researchers independently collected and entered the data, cross-checking until consistency was achieved. We used CiteSpace software (6.2. R2) to perform research frontier and hotspot analysis.[9]

Results

Number and amount of funded projects

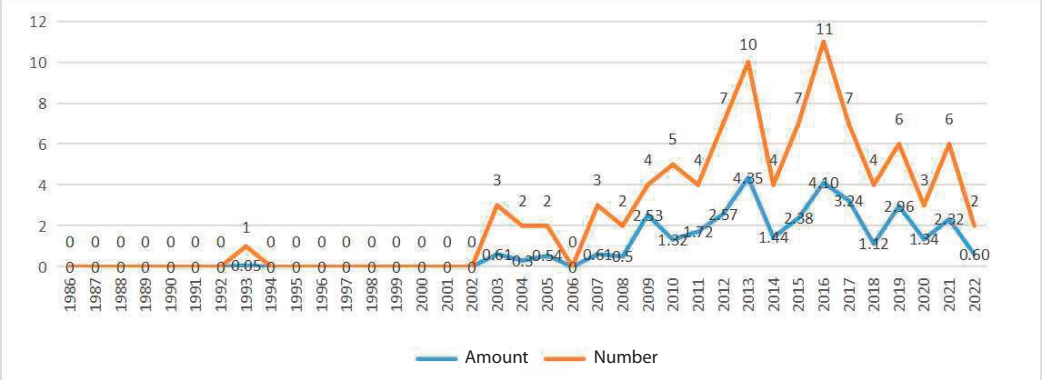
Between 1986 and 2022, NSFC funded a total of 93 projects in the field of lung transplantation, with funding for an average of 2.51 projects annually. The cumulative funding amounted to ¥34.60 million, averaging ¥0.94 million annually. Project numbers exhibited an initial increase, peaking in 2016 with 11 funded projects, whereas, prior to 2003, there were almost no projects, except for 1 in 1993. However, project numbers gradually declined after 2016, with only 2 funded by 2022. A similar trend was observed from the yearly analysis of the funding amount, reaching a peak of ¥4.35 million in 2013. Subsequently, the funding amount gradually decreased to ¥0.60 million in 2022 (Figure 1).

Funded project institutions and geographical distribution

From 1986 to 2022, the NSFC funded 30 institutions across 20 provinces in the field of lung transplantation. Regarding the geographical distribution of funding, Shanghai and Hubei emerged as the top 2 funded regions. Both provinces had over 18 projects and received over ¥5 million in funding. Shanghai ranked first with 33 funded projects, accounting for over one-third of the total number of funded projects and receiving over one-third of the total funds. Overall, the regional distribution of project funding was unbalanced (Table 1).

The top 7 funded institutions in terms of funding distribution were Tongji University, Huazhong University of Science and Technology, Harbin Medical University, Shanghai Jiao Tong University, Capital Medical University, and Fudan University. Tongji University and Huazhong University of Science and Technology ranked among the top 2 regarding project quantity and funding amount (Table 2). Tongji University ranked first with 21 funded projects and ¥8.32 million in funding, surpassing other institutions. This accounted for

Figure 1. Amount of Funding and Number of Studies Funded by the National Natural Science Foundation of China in the Field of Lung Transplantation From 1986 to 2022



22.58% of the total number of projects and 24.05% of the total funding, highlighting the institution's strong research capabilities in the field of lung transplantation.

Table 1. Regional Ranking and Distribution of Funded Projects

Ranking	Province	Number	Amount (¥, million)	No. of Institutions
1	Shanghai	33	13.86	4
2	Hubei	18	5.75	2
3	Beijing	8	3.87	3
4	Heilongjiang	8	1.92	1
5	Fujian	2	0.31	1
6	Guangdong	2	0.88	2
7	Guangxi	2	0.85	2
8	Jiangsu	2	0.52	2
9	Jiangxi	2	0.83	1
10	Liaoning	2	0.21	1
11	Shandong	2	0.46	1
12	Shanxi	2	0.13	2
13	Tianjin	2	0.36	1
14	Zhejiang	2	0.14	1
15	Hainan	1	0.34	1
16	Jilin	1	0.60	1
17	Inner Mongolia	1	0.36	1
18	Sichuan	1	0.32	1
19	Xinjiang	1	0.30	1
20	Yunnan	1	0.25	1

Table 2. Ranking of Institutions That Received Funding

Ranking	Institution	Province	Number	Amount (¥, million)
1	Tongji University	Shanghai	21	8.32
2	Huazhong University of Science and Technology	Hubei	15	4.37
3	Harbin Medical University	Heilongjiang	8	1.92
4	Shanghai Jiao Tong University	Shanghai	8	3.10
5	Capital Medical University	Beijing	6	2.82
6	Fudan University	Shanghai	3	2.14
7	Wuhan University	Hubei	3	1.38

Types of funded projects

The NSFC funding categories include key projects, general projects, youth science foundation projects, regional science foundation projects, major research plan projects, National Outstanding Youth Science Foundation projects, and special fund projects. Data analysis revealed large differences in the number and funding amounts among these categories (Table 3). General and youth science foundation projects accounted for 45.16% and 41.93% of the total number of projects, respectively. However, the average funding amounts were ¥0.49 million and ¥0.22 million, respectively. Major research plans and key projects had smaller numbers but relatively high average funding, with individual averages of ¥0.60 and ¥1.75 million, respectively.

Table 3. Categories of Funded Projects

Ranking	Funded Project Type	Number (%)	Amount (¥, million)	Average Amount (¥, million)
1	General projects	42 (45.16%)	20.69	0.49
2	Youth science foundation projects	39 (41.93%)	8.43	0.22
3	Regional science foundation projects	8 (8.60%)	2.93	0.37
4	Special fund projects	2 (2.15%)	0.20	0.10
5	Major research plan projects	1 (1.08%)	0.60	0.60
6	Key projects	1 (1.08%)	1.75	1.75

Disciplines of funded project

In the field of lung transplantation, NSFC funding is categorized into 3 main disciplines: H0113 respiratory intervention, tracheal reconstruction, and lung transplantation; H1105 organ transplantation and transplantation immunization; and H0109 acute lung injury and acute respiratory distress syndrome. Among these, the category of H0113 respiratory intervention, tracheal reconstruction, and lung transplantation received funding for 47 projects, accounting for 50.54% of the total projects. The average funding amount for H0109 acute lung injury and acute respiratory distress syndrome was the highest, reaching ¥0.45 million (Table 4).

Analysis of research hotspots and frontiers

Key word co-occurrence analysis. We used CiteSpace to construct a key word co-occurrence network graph. The comprehensive high-frequency and high-

Table 4 Disciplines of Funded Projects

Ranking	Discipline of Funded Project	Number	Amount (¥, million)	Average Amount (¥, million)
1	H0113 respiratory intervention, tracheal reconstruction, and lung transplantation	47 (50.54%)	17.24	0.37
2	H1105 organ transplantation and transplantation immunization	8 (8.60%)	3.07	0.38
3	H0109 acute lung injury and acute respiratory distress syndrome	7 (7.53%)	3.12	0.45
4	H01 respiratory system	5 (5.38%)	1.98	0.40
5	H0108 interstitial pulmonary disease	4 (4.30%)	1.20	0.30
6	Other	22 (23.66%)	7.99	0.36

centrality key word distribution and the co-occurrence relationship graph are shown in Table 5 and Figure 2. The research hotspots of lung transplantation included ischemia-reperfusion injury, gene regulation, obliterative bronchiolitis, rejection reaction, T cell, and stem cell.

Table 5. Top 11 Key Words in the Frequency Ranking

Ranking	Frequency	Centrality	Year	Keyword
1	57	0.40	1993	Lung transplantation
2	28	0.76	2003	Ischemia-reperfusion injury
3	20	0.78	2007	Gene regulation
4	15	0.48	2007	Obliterative bronchiolitis
5	13	0.20	1993	Rejection reaction
6	11	0.12	2007	T cell
7	8	0.32	2012	Stem cell
8	7	0.18	2003	Immune tolerance
9	6	0.08	2009	Donor lung preservation
10	6	0.08	2008	Lung injury
11	6	0.22	2013	Pulmonary fibrosis

Key word clustering analysis. Using CiteSpace, we performed key word clustering for lung transplant research projects based on a co-occurrence network. Figure 3 shows the clustering results, with Q and S values of 0.5597 and 0.8857, respectively, indicating significant clustering and strong credibility. The 6 main research clusters identified were ischemia-reperfusion injury, immune tolerance, obliterative bronchiolitis, stem cell, pulmonary fibrosis, and rejection reaction.

Research frontier analysis

We used the CiteSpace key word burst method to conduct cutting-edge research analysis using a 2-year time span. Figure 4 shows the top 12 key words according to the intensity of the key word burst. By combing the time distribution of these key words, we

Figure 2. Key Word Co-occurrence Map

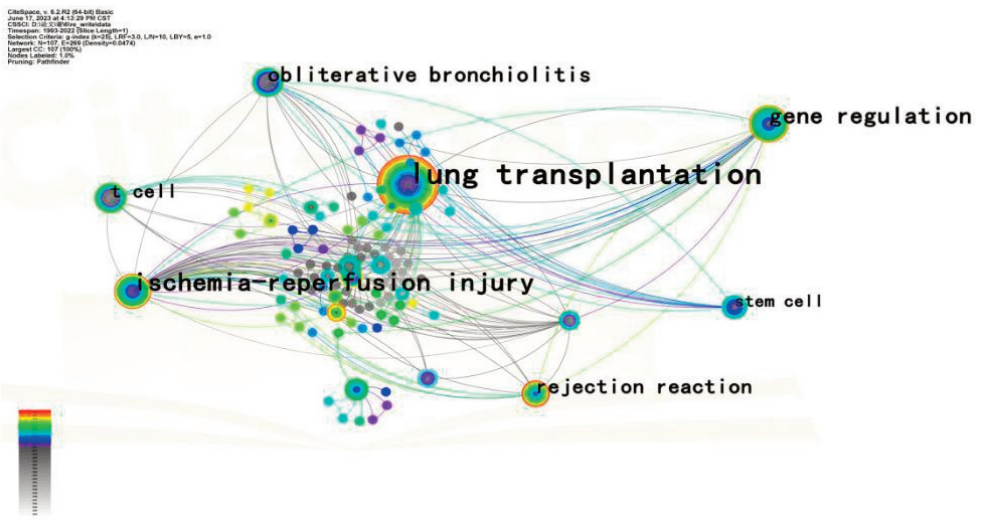


Figure 3. Key Word Clustering Timeline

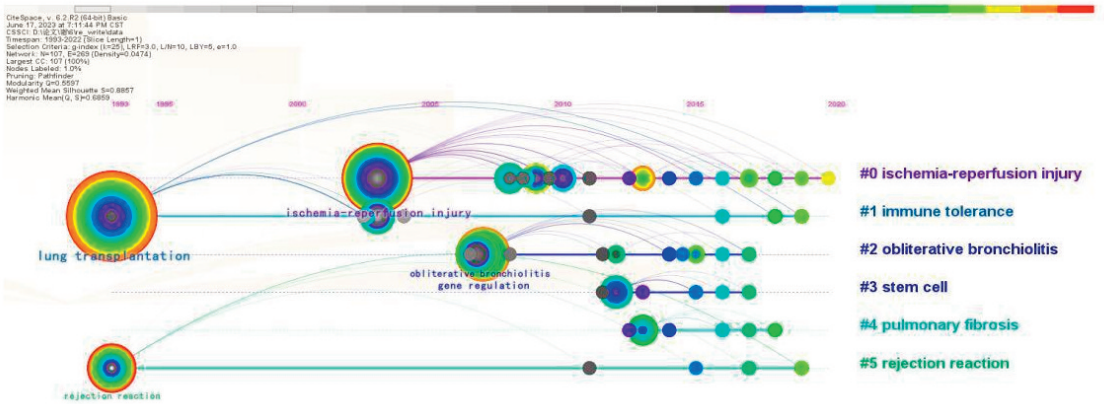
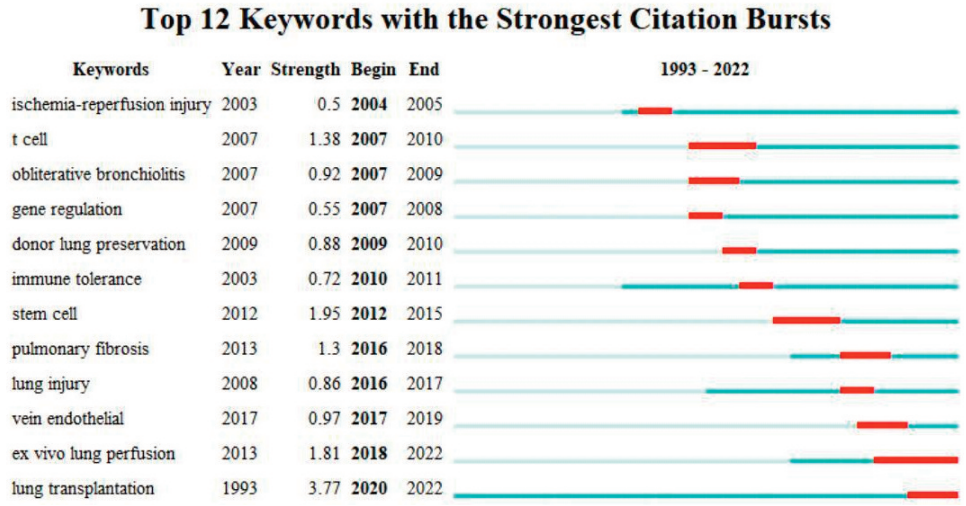


Figure 4. Top 12 Key Word Bursts



identified the research frontiers of lung transplant research in each period. Before 2004, there were almost no key word bursts. From 2004 to 2010, the main key word bursts were ischemia-reperfusion injury, T cell, obliterative bronchiolitis, gene regulation, and donor lung preservation. From 2010 to 2018, the main key word bursts were immune tolerance, stem cell, pulmonary fibrosis, and lung injury. The main key word bursts in the past 5 years were "vein endothelial" and "ex vivo lung perfusion."

Discussion

Analysis of overall funding quantity and amount

This study revealed that NSFC funded 262 projects related to lung transplantation, averaging 2.51 projects and ¥0.94 million annually from 1986 to 2022. Generally, lung transplant projects increased from 2006 to 2016, promoting the rapid development of lung transplant research. However, the overall funding and growth rate have decreased since 2016, with only 2 projects funded in 2022, which does not align with the field's development speed. This indicates a discrepancy between the advancement of scientific research and clinical technology in lung transplant. Furthermore, lung transplant research lags behind that of other solid-organ transplant research, such as liver, kidney, and heart.

This study revealed that the major projects funded were general and youth science foundation projects. Only 1 key project and 1 major research plan project were funded. Innovative research group projects, National Science Fund for Distinguished Young Scholars, and other high-level funding were lacking. This indicates a scarcity of high-level research achievements with international prominence in China and a limited number of research experts and teams at the forefront of the field. Therefore, enhancing the scientific research level in the field of lung transplant in China is essential for enhancing the development of lung transplant medical technology, which will be beneficial to patients who require lung transplant.

Analysis of the regional distribution of funded projects

Over the span of 37 years, NSFC has funded lung transplant projects in 20 provinces. However, the top 2 regions, Shanghai and Hubei, accounted for over half of the funded projects, with Shanghai accounting for over one-third, reflecting a significant regional imbalance. Moreover, only 30 institutions nationwide received funded projects, with Tongji University alone securing 22.58% of the funded projects. Additionally, the top 7 institutions are traditional medical schools, including 3 in Shanghai and 2 in Hubei. This indicates a significant disparity in competitive strength among institutions in the field of lung transplantation.

The distribution of project-funded institutions showed a clustering trend, mainly located in the eastern coastal and central developed provinces, with fewer institutions in the western region. This disparity is mainly attributed to the larger population base, better scientific research foundation, and greater economic strength of the eastern region compared with the western region. However, this phenomenon

hampers the application and advancement of lung transplant medical technology, potentially leading to patient outflow from the western region and increasing the economic burden for such patients. Simultaneously, it hinders the development of medical institutions in the western regions. To address this situation, urgent measures should be taken to improve medical and scientific research in lung transplant in western China. This can be achieved through collaborative efforts between provinces, remote consultations, and support from counterpart institutions. Furthermore, because organ transplant qualification requires approval from the National Health Commission of the People's Republic of China, providing preferential treatment to the western region is recommended when the basic requirements for organ transplantation are met. This would encourage the implementation of lung transplant and other solid-organ transplant procedures in the western region, enabling patients to receive treatment locally, benefiting lung transplant patients, and reducing economic burden to patients.

Analysis of research hotspots

We identified 6 main research clusters: ischemia-reperfusion injury, immune tolerance, obliterative bronchiolitis, stem cell, pulmonary fibrosis, and rejection reaction.

Ischemia-reperfusion injury is an important cause of respiratory failure after lung transplant.[10] Effective management of ischemia-reperfusion injury is key to the success of lung transplant. Finding effective measures to prevent and treat lung ischemia-reperfusion injury is of great clinical significance to increase the success rate of lung transplant and the long-term survival rate after transplant. The NSFC-funded research on ischemia-reperfusion injury mainly focuses on inhibiting, alleviating, and regulating this injury in cases of lung transplant. Examples include "IRF-8/IFN-γ study on the effect of selective autophagy and its mechanism in pulmonary ischemia-reperfusion injury," "Effect and mechanism of maresin1 on inhibiting ferroptosis and alleviating early lung ischemia-reperfusion injury after lung transplantation," and "Effect and mechanism of extracorporeal lung perfusion combined with drug intervention on ischemia-reperfusion injury after lung transplantation in rats."

To avoid transplant rejection, recipients must undergo lifelong administration of immunosuppressive drugs. Although they are effective in ensuring short-term survival, nonspecific reactions and other adverse effects of immunosuppressives have significant negative effects on the long-term survival of the graft. Therefore, establishing and maintaining donor antigen-specific immune tolerance may be fundamental to solving organ transplant rejection.[11] The NSFC-funded research on immune tolerance includes "Effect and mechanism of sirolimus combined with sTNFRI IgGFc electrogenetically modified dendritic cells on immune tolerance induced by lung transplantation in mice" and "Experimental study on induction of immune tolerance in lung transplantation rats."

Obliterative bronchiolitis is the main factor affecting long-term survival after lung transplant.12 Its mechanism involves tissue fibrosis and immune rejection. Presently, there is no effective treatment plan. The NSFC-funded research on bronchiolitis obliterans includes "Mechanism of blocking Adam10/Notch1 signaling pathway of myeloid macrophages to slow down bronchiolitis obliterans associated with lung transplantation," "Role and mechanism of autophagy associated epithelial-mesenchymal transformation regulated by farnesyl diphosphate synthase in bronchiolitis obliterans," and "Cellular and molecular regulation mechanism and intervention of bronchiolitis obliterans after IL-17 mediated lung transplantation."

Enhancing the "quality" and "quantity" of bone marrow mesenchymal stem cells is crucial for effectively treating lung ischemia-reperfusion injury, and recent advancements have shown promising progress in this regard.[13] The NSFC-funded research on stem cells includes "Experimental study on the treatment of lung ischemia reperfusion injury with HGF modified mesenchymal stem cell transplantation based on ischemic post-adaptation" and "Study on the specific differentiation and regulation mechanism of human adult lung stem cells in the Chung-guyok of acellular lung scaffold."

Pulmonary fibrosis is a respiratory disease that forms scars in the lung tissue, causing serious respiratory problems in patients. Currently, lung transplant is the only effective treatment for pulmonary fibrosis.[14] Research on the etiology of pulmonary fibrosis and antifibrosis drugs has been challenging worldwide. The NSFC-funded research on pulmonary fibrosis includes "Mechanism of AQP5 regulating the imbalance of immunogenic and

neurogenic CGRP to induce pulmonary fibrosis," "Effects of fine particles of air pollution on idiopathic pulmonary fibrosis and its molecular mechanism," and "Collagen receptor DDR2 synergizes with TGF-β: study on the molecular mechanism of the pathway promoting pulmonary fibrosis."

Transplant rejection reaction is an important problem in organ transplant and remains a constant focus in the context of lung transplant. The NSFC-funded research on rejection reaction includes "Effect and its mechanism research of glutamate transaminase 1 (GOT1) - shRNA on acute rejection of lung transplantation in rats," "Role and mechanism of CD8 memory T cell in immune rejection of lung transplantation," and "Tim-3/galectin-9 signaling pathway mediates acute rejection after lung transplantation and its mechanism."

Analysis of research frontiers

This study revealed that the main key word bursts from 2010 to 2018 were immune tolerance, stem cell, pulmonary fibrosis, and lung injury. However, in the past 5 years, the main key word bursts are now "vein endothelial" and "ex vivo lung perfusion" This indicates a change in the lung transplant research focus in China. Previously, the emphasis was on immune rejection and lung injury after lung transplant, and efforts were made to suppress and improve these aspects. Recent research has increasingly focused on pre-lung transplant, such as donor lung preservation, maintenance, and tissue research, to address the root cause by eliminating signals that induce inflammation and injury.

The study titled "Regulation and mechanism of vein endothelial on inflammation and injury during the preservation of donor lung in lung transplantation" conducted research at the molecular, cellular, whole animal model, and clinical sample levels and aimed to clarify the regulatory role of vein endothelial cells on inflammation and injury during cold ischemia preservation of donor lungs, providing a theoretical basis for the clinical development of strategies for preserving donor lungs, increasing the availability of donor lungs, and thus improving the long-term survival of grafts and recipients after lung transplant. The recent development of EVLP technology has expanded the utilization rate of donor lungs; however, its mechanism of alleviating reperfusion injury after transplant remains unclear. The study "Effect and mechanism of extracorporeal lung perfusion (EVLP) combined with drug intervention on ischemia-reperfusion injury after lung transplantation in rats" reported that combining EVLP with pharmacological interventions can improve donor lung compliance, peak airway pressure, pulmonary vascular resistance, and oxygenation capacity.

In general, China still has some ground to cover regarding lung transplant research. For example, few studies exist in China on the application of 3-dimensional printing technology in lung and xenogeneic lung transplant.[15-17] For many years, the world has used data registration queues to improve the availability of donor lungs with the maintenance of donor information, for the improvement of surgical methods, and for optimization of organ allocation strategies. There is increasing evidence for the clinical application of EVLP in lung transplant centers in Europe and the United States.[18]

With the growing aging population, the policies of organ transplant in various countries have been modified to remove previous restrictions on age, and the prognosis of elderly lung transplant recipients is now being investigated and analyzed.[19] However, the lung transplant centers in the United States have been more active in promoting the development of infant lung transplant technology and actively exploring perioperative extracorporeal lung assistance technology.[20] At present, research is lacking in the field of basic research and drug therapy related to lung transplant in China, especially with regard to microecology, organ function, and immune regulation, which have received more attention in other countries in recent years. Compared with studies in China, foreign studies have found that lung transplant recipients tend to have chronic graft failure and pulmonary colonization flora after lung transplant.[21]

The primary limitation and delimitation of our study are that the scope was limited to only NSFC-funded projects in the field of lung transplant research in China. This was primarily because of limited funding provided by other organizations for lung transplant research in China.

Conclusions

We identified the NSFC-funded projects in the field of lung transplant research from 1986 to 2022 and described their distribution characteristics in terms of number, funding amount, and project category.

CiteSpace co-occurrence analysis, cluster analysis, and other visual means were used to show research hotspots and frontiers. Overall, NSFC-funded projects have significantly contributed to the advancement of clinical application and basic research in lung transplantation. However, compared with other developed countries and research for other solid-organ transplants, there remain several noteworthy issues and areas of improvement in lung transplant research in China.

References

1. Li JH, Xu X, Wang YF, et al. Chinese expert consensus on organ protection of transplantation (2022 edition). *Hepatobiliary Pancreat Dis Int*. 2022;21(6):516-526. doi:10.1016/j.hbpd.2022.10.010
2. Rucker AJ, Nellis JR, Klapper JA, Hartwig MG. Lung retransplantation in the modern era. *J Thorac Dis*. 2021;13(11):6587-6593. doi:10.21037/jtd-2021-25
3. Chambers DC, Yusen RD, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-fourth Adult Lung And Heart-Lung Transplantation Report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant*. 2017;36(10):1047-1059. doi:10.1016/j.healun.2017.07.016
4. Chambers DC, Cherikh WS, Goldfarb SB, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-fifth adult lung and heart-lung transplant report-2018; focus theme: multiorgan transplantation. *J Heart Lung Transplant*. 2018;37(10):1169-1183. doi:10.1016/j.healun.2018.07.020
5. Chambers DC, Cherikh WS, Harhay MO, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult lung and heart-lung transplantation Report-2019; focus theme: donor and recipient size match. *J Heart Lung Transplant*. 2019;38(10):1042-1055. doi:10.1016/j.healun.2019.08.001
6. Chambers DC, Perch M, Zuckermann A, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-eighth adult lung transplantation report - 2021; focus on recipient characteristics. *J Heart Lung Transplant*. 2021;40(10):1060-1072. doi:10.1016/j.healun.2021.07.021
7. Chambers DC, Zuckermann A, Cherikh WS, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: 37th adult lung transplantation report - 2020; focus on deceased donor characteristics. *J Heart Lung Transplant*. 2020;39(10):1016-1027. doi:10.1016/j.healun.2020.07.009
8. Yu R, Zhu MJ, Wang XL, Zhao QF, Wang JR. Analysis of the National Natural Science Foundation of China (NSFC) for cardiovascular diseases related projects in integrated traditional Chinese and western medicine from 2000 to 2019. *Chinese J Integr Med Cardiocerebrovasc Dis*. 2023;21:248-252.
9. Chen C. Science mapping: a systematic review of the literature. *J Data Inf Sci*. 2017;2(2):1-40. doi:10.1515/jdis-2017-0006
10. Shah RJ, Diamond JM. Primary graft dysfunction (PGD) following lung transplantation. *Semin Respir Crit Care Med*. 2018;39(2):148-154. doi:10.1055/s-0037-1615797
11. 2017 American Transplant Congress Abstracts. *Am J Transplant*. 2017;17 Suppl 3:5-815. doi:10.1111/ajt.14304
12. Yusen RD, Edwards LB, Dipchand AI, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-third adult lung and heart–lung transplant report—2016; focus theme: primary diagnostic indications for transplant. *The Journal of Heart and Lung Transplantation*. 2016;35(10):1170-1184.
13. Rosca AM, Tutuianu R, Titorencu ID. Mesenchymal stromal cells derived exosomes as tools for chronic wound healing therapy. *Rom J Morphol Embryol*. 2018;59(3):655-662.
14. Tzilas V, Tzouvelekis A, Ryu JH, Bouros D. 2022 update on clinical practice guidelines for idiopathic pulmonary fibrosis and progressive pulmonary fibrosis. *Lancet Respir Med*. 2022;10(8):729-731. doi:10.1016/S2213-2600(22)00223-5
15. Dabaghi M, Rochow N, Saraei N, et al. A pumpless microfluidic neonatal lung assist device for support of preterm neonates in respiratory distress. *Adv Sci (Weinh)*. 2020;7(21):2001860. doi:10.1002/advs.202001860
16. Kirk AD. When pigs fly. *Am J Transplant*. 2022;22(4):1007-1008. doi:10.1111/ajt.16963
17. Burdorf L, Laird CT, Harris DG, et al. Pig-to-baboon lung xenotransplantation: Extended survival with targeted genetic modifications and pharmacologic treatments. *Am J Transplant*. 2022;22(1):28-45. doi:10.1111/ajt.16809
18. Chen Y, Wei D, Wang Z, et al. Experimental study on the role of IL-10 in improving donor lung function after ex vivo lung perfusion in rats of cardiac death. *Organ Transplant*. 2021:421-421. doi:10.3969/j.issn.1674-7445.2021.04.008
19. Costello JP, Carvajal HG, Abarbanell AM, Eghtesady P, Nath DS. Surgical considerations in infant lung transplantation: challenges and opportunities. *Am J Transplant*. 2021;21(1):15-20. doi:10.1111/ajt.16282
20. Schweiger T, Hoetzenecker K. Is chronological age still a hard selection criterion for lung transplantation? *J Heart Lung Transplant*. 2021;40(2):99-100. doi:10.1016/j.healun.2020.11.011
21. Combs MP, Wheeler DS, Luth JE, et al. Lung microbiota predict chronic rejection in healthy lung transplant recipients: a prospective cohort study. *Lancet Respir Med*. 2021;9(6):601-612. doi:10.1016/S2213-2600(20)30405-7

Xenotransplantation of Microencapsulated Parathyroid Cells as a Potential Treatment for Autoimmune-Related Hypoparathyroidism

Beyza Goncu,[1,2] Emrah Yucesan,[3] Harun Basoglu,[4,5] Burcu Gul,[6] Erhan Aysan,[7,8] Yeliz Emine Ersoy[7]**

Abstract

Objectives: **Hypoparathyroidism occurs as a part of a complex autoimmune syndrome or iatrogenically after neck surgery. The disease presents many challenges, such as hypocalcemia, hyperphosphatemia, and low/undetectable parathormone levels. Allotransplantation of parathyroid tissue or cells has been reported as a promising option to overcome these effects. Transplantation of microencapsulated parathyroid tissue or cells offers an immune escape, which particularly restores the parathyroid function for autoimmune-related hypoparathyroidism. So far, clinical and in vivo studies have demonstrated limited graft survival and instability for the available biocompatible materials. In addition, the transplant site, proper local isolation, and biocompatibility of materials are directly related to survival rate.**

Materials and Methods: **A microencapsulated parathyroid xenotransplant model by using high guluronic acid-containing ultrapure alginate transplanted into rat omentum was tested in vivo for 1 year.**

Results: **After stability of empty microcapsules was ensured, parathyroid cells were microencapsulated and transplanted in rats, with results compared versus rats with naked (nonencapsulated) parathyroid cells (both groups followed for 64 weeks). Rats remained normocalcemic, and preinflammatory cytokine levels showed dramatic changes. Despite a delay posttransplant, parathormone levels increased significantly. All retrieved microencapsules elicited pericapsular fibrotic overgrowth; however, the fibrosis area was shown to be well tolerated.**

Conclusions: **The possible role of accumulation/cell infiltration of immune response remains to be elucidated. In conjunction with this, the use of nonencapsulated parathyroid cells was also positively correlated with survival rates. A similar evaluation using ultrapure alginate materials and omental transplantation may enable the future determination for the long-term effects of correction of parathormone insufficiency in patients with severe hypocalcemic responses and other endocrine diseases.**

Key words: *Hypocalcemia, Microencapsulation, Parathyroid allotransplantation, Parathormone insufficiency.*

Introduction

The most common causes of hypoparathyroidism occur as a complication during thyroidectomy, and this form may become permanent. Another cause of hypoparathyroidism can be a part of complex autoimmune syndrome.[1,2] The disease itself can be accompanied by hypocalcemia, hyperphosphatemia, and low or undetectable parathormone (PTH) levels.[3] People who are diagnosed with this condition experience numbness, tingling, cramping around feet and fingers, bone pain, and fatique, as well as have severely reduced quality of life. Patients with this condition must use calcium, active vitamin D analogs, and antiphosphorus supplementation throughout their life.[4]

From the [1]Experimental Research Center, the [2]Vocational School of Health Services, Department of Medical Services and Techniques, the [3]Faculty of Medicine, Department of Medical Biology, and the [4]Faculty of Medicine, Department of Biophysics, Bezmialem Vakif University, Istanbul; the [5]Faculty of Medicine, Department of Biophysics, Karadeniz Technical University, Trabzon, Turkey; the [6]Faculty of Medicine, Department of Pathology and the [7]Faculty of Medicine, Department of General Surgery, Bezmialem Vakif University, Istanbul; and the [8]Department of General Surgery, Yeditepe University, Istanbul, Turkey

Acknowledgements: *The authors thank D.V.M. Mert Celikten for his guidance on laboratory animal procedures and thank Onder Huseyinbas, Burcu Ozdemir, and Ebru Kanimdan for their technical assistance. The authors highly appreciate the efforts of Monica Ann Ozkan, MSN, RN, in English editing of this article. This study was supported by the Research Fund of Bezmialem Vakif University, project numbers of 9.2016/5 and 4.2018/11, respectively. The authors declare that there are no conflicts of interest.*

Author contributions: *B. Goncu, E. Aysan, and Y. E. Ersoy conceived and designed the experiments; B. Goncu, E. Yucesan, H. Basoglu, and B. Gul performed the experiments; B. Goncu and E. Yucesan analyzed the data; B. Goncu and E. Yucesan contributed reagents/materials/analysis tools; B. Goncu and E. Yucesan wrote the paper; and E. Aysan and Y. E. Ersoy performed critical revision.*

**Y. E. Ersoy and E. Aysan contributed equally to this work.*

Corresponding author: *Beyza Goncu, Adnan Menderes Bulvari, Vatan Caddesi, 34093, Fatih, Istanbul, Turkey*

Phone: *+90 212 523 22 88 / 1466* ***E-mail:*** *bsgoncu@gmail.com or bgoncu@bezmialem.edu.tr*

Experimental and Clinical Transplantation (2023) 11: 901-912

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2020.0403

Currently, parathyroid allotransplantation (PTX) is the only curative treatment that increases the level of PTH[5] and is an acceptable approach for patients with hypoparathyroidism.[4,5] Another therapy option is recombinant drugs, but these are expensive and are only suitable for a limited number of patients.[6]

Previously, several microencapsulated PTX techniques have been described, but 2 major concerns always remain: (1) the source material selection (eg, cell or tissue[7]) and (2) determination of the optimal biocompatible transplant material. Each approach comes with positive and negative outcomes. As already known, the main problem for transplantation is immune rejection; however, microencapsulation has been seen as a superior applicable method.[8-10] Microencapsulation may be performed with many different materials. Among natural biocompatible materials, alginate is particularly preferred.[11,12] Herein, we microencapsulated parathyroid cells (Ptc) by using α-L-guluronic acid-rich ultrapure alginate and performed xenotransplantation into rat omentum. Our aims were to demonstrate the stability of microencapsules and to evaluate the proinflammatory responses and graft survival.

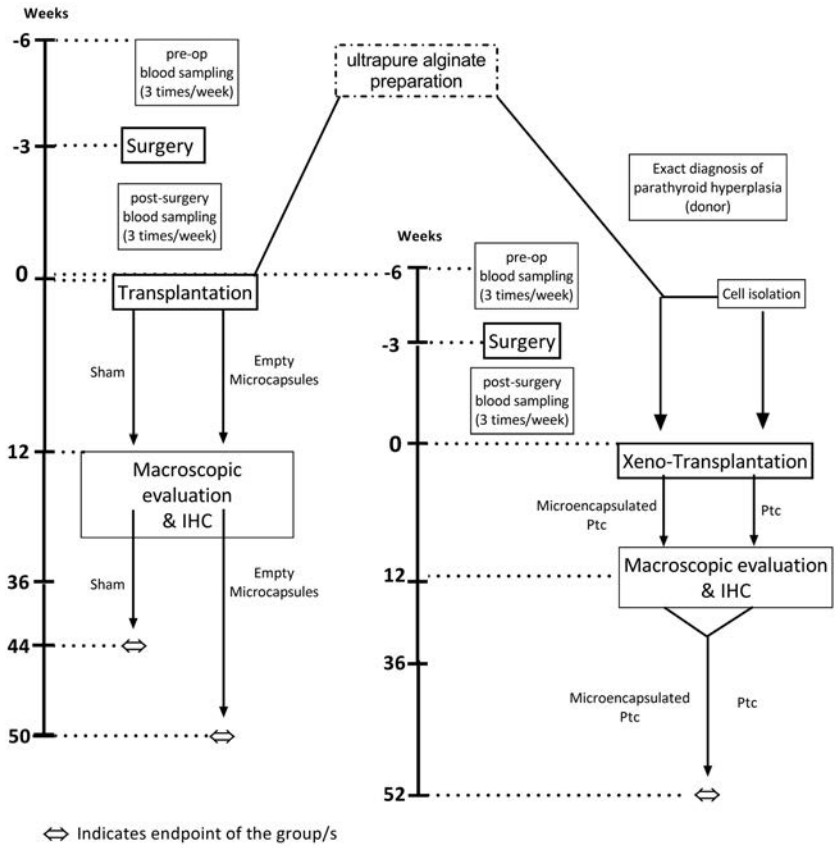
Materials and Methods

The animal study was approved by the Local Experimental Animals Ethics Committee (approval number: 2015/204). Xenograft preparation was performed after approval was received from the Local Human Ethics Committee (approval number: 71306642-050.01.04-19444). All protocols were in accordance with the ethical guidelines of the Helsinki Declaration, and written informed consent was obtained from the human donor. Methods and synopsis of the study are illustrated in Figure 1.

Animal experiments and parathyroidectomy procedure

Experiments were carried out on 32 female Wistar albino rats (12-15 weeks old) with an average weight of 240 g. Rats were housed and fed ad libitum and randomly divided into the following groups: empty

Figure 1. Methods and Synopsis of the Study



Abbreviations: IHC, immunohistochemistry; pre-op, preoperative; Ptc, parathyroid cells

microcapsules (n = 10), Ptc transplanted (n = 10), microencapsulated Ptc transplanted (n = 10), and sham (n = 2) (isotonic saline solution-injected group after laparotomy). Blood sampling was made from the jugular vein according to the methods of Parasuraman and colleagues.[13] Parathyroidectomy was performed on the 32 rats under sterile conditions.

Encapsulation of empty microcapsules

Ultrapure alginate was purchased from NovaMatrix. Low-viscosity (20-200 mPas), low-endotoxin (≤100 EU/g), and high guluronic acid content (≥60%) sodium alginate (Pronova UP LVG, NovaMatrix, Oslo, Norway) was prepared with ultrapure water with a 2% ratio (wt/vol), which was sterilized by ultraviolet exposure for 15 minutes as described previously.[14] Gelled alginate (0.5 mL) was mixed with isotonic saline solution at a 28% ratio. Capsulation was developed by calcium being used at a 300 mosM $CaCl_2$ solution and followed with a washing step for removal of the remaining $CaCl_2$. For the empty microcapsule group, isotonic saline solution (1 mL) containing empty microcapsules was transplanted into the omentum of each animal (n = 10).

Preparation of xenograft and microencapsulation

One male patient (32 years old) who had parathyroid hyperplasia due to chronic renal failure was enrolled into the present study. Half of each of the resected glands was delivered to the pathology laboratory for an exact diagnosis. The remaining parts of the glands were snap-frozen and stored at −80 °C; samples underwent the rapid thaw method before use. Parathyroid cell isolation and viability determination were assessed as previously described.[15] Before the transplant process, Ptc were cultivated with McCoy's 5A (modified) medium and placed in an incubator at 37 °C with 5% CO_2 in a humidified atmosphere. After overnight cultivation, cells were suspended in isotonic saline solution, and then viability was assessed with a Muse Cell Analyzer (Merck Millipore). For the group that underwent transplant with Ptc only, isotonic saline solution containing 50×10^6 Ptc were transplanted into the omentum of each animal (n = 10). Afterward, the remaining cell pellets were resuspended in 2% ultrapure alginate (preparation as described above).

Ultrapure alginate (0.5 mL, 2%) was mixed with a final volume of 150 μL isotonic saline solution that contained 50×10^6 Ptc in suspension prepared for each animal in the microencapsulated Ptc transplant group (n = 10). The alginate-cell suspension was placed into a 30-gauge needle and dropped into 300 mosM $CaCl_2$ solution. Spheroids were rapidly formed upon contact with the $CaCl_2$ solution. The resulting microencapsules were counted and collected into tubes containing 1 mL of isotonic saline solution.

Transplantation into the omentum

All transplant procedures were performed under sterile conditions for each experimental group (microencapsulated Ptc transplant group, empty microcapsule transplant group, and nonencapsulated Ptc transplant group). For animals receiving microencapsulated Ptc, approximately 28 to 33 microencapsulated Ptcs were transplanted into each animal's omentum. At week 12 and 52, rats in the microencapsulated Ptc and empty microcapsule transplant groups underwent omentectomy for histopathological evaluation. Other organs were controlled macroscopically, and removal was performed only when adherence of microcapsules occurred to other organs (except omentum). Only the Ptc transplant group was evaluated macroscopically.

Sandwich enzyme-linked immunosorbent assay

The following protocol was adjusted and optimized based on the work from Aydin.[16] The following solutions were used: coating buffer (0.01 M sodium carbonate buffer, pH 9.6), blocking buffer (1% [wt/vol] bovine serum albumin in 1× phosphate-buffered saline [PBS] with 0.05% Tween 20), 1× PBS (pH 7.4), washing buffer of PBS-T (PBS containing 0.05% [vol/vol] Tween 20), antibody dilution buffer (PBS containing 1% [wt/vol] bovine serum albumin and 0.05% [vol/vol) Tween 20), 2 M hydrogen peroxide as a stop solution (at 1:1 vol), and substrate solution. The substrate solution was prepared by combining 2 mL of solution/microplate of 1 mg 3,3',5,5'-tetramethyl benzidine (TMB) in dimethyl sulfoxide with 9 mL of 0.1 M citrate phosphate buffer (pH 5.0) containing 1 μL of 30% hydrogen peroxide. All chemicals were purchased from Merck except TMB (from Sigma). All primary and secondary antibodies were purchased from Abcam.

Anti-rat PTH polyclonal antibody was used as a coating and a detection antibody. Colorimetric detection was performed with anti-rat immunoglobulin G antibody. Anti-human PTH monoclonal antibody was used as a coating antibody. Anti-

human PTH antibody of human PTH (hu-PTH) was used as a detection antibody; for colorimetric detection, anti-rabbit immunoglobulin G antibody was used. Standard preparation was performed with recombinant hu-PTH protein with a 1 to 500 pg/mL dilution range.

For the PTH sandwich enzyme-linked immunosorbent assay (ELISA), undetachable, high-binding 96-well microplates (Nest Biotechnology) were coated with antibody, which was diluted in a coating buffer (100 μL/well) and subsequently incubated at 4 °C overnight (up to 18 h). Wells were then aspirated and washed 2 times with PBS-T (250 μL/well) and blocked with blocking buffer (200 μL/well) for 1 hour at room temperature. After the blocking step, wells were aspirated and 100 μL of serially diluted standards and serum samples were added in duplicate. Microplates were incubated at 4 °C overnight (up to 12 h) and then placed at room temperature for further processing (around 10-20 min). Samples were aspirated and washed 3 times with PBS-T (250 μL/well). Detection antibody (100 μL) was added to each well and incubated for 2 hours at room temperature, which was then followed with aspiration and washing steps. Afterward, horseradish peroxidase-labeled colorimetric detection antibody (100 μL) was added to the wells, and microplates were incubated for another 1 hour at room temperature. As a final step, microplates were washed 4 times, 100 μL of TMB substrate solution were added to each well, and the samples were allowed to react with the labeled antibody at room temperature for 15 minutes in the dark. The reaction was stopped by adding stop solution, and the absorbance was measured at 450 nm with a microplate reader (Bio-Rad).

Serum calcium, interleukin 17α, tumor necrosis factor α, and interleukin 6 enzyme-linked immunosorbent assays

All serum calcium levels were measured with the Chemistry Analyzer IDEXX VetTest (IDEXX Laboratories). Interleukin 17α (IL-17α), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) levels were quantified using commercial ELISA kits according to the manufacturer's instructions (BioLegend).

Immunohistochemical testing

Collected tissues of omentectomy and partial pancreatectomy, which contained the microcapsules (for both the microencapsuled Ptc and the empty microcapsule transplant groups) and freely floating microcapsules from rat peritoneal cavity, were collected at week 12 and at weeks 50 to 52 (end of the experiment) after the transplant procedure for each group. Frozen tissue sections were then stained with hematoxylin and eosin. In addition, 2 sections of each animal were scored with the use of an image analysis system (Nikon Instruments). Sections were graded by using a grading scale: grade 0 indicated no fibrosis (no fibroblast and/or collagen fibers), grade 1 indicated low-level fibrosis (low number of fibroblast and/or collagen fibers), grade 2 indicated moderate-level fibrosis (moderate fibroblast and/or collagen fibers), and grade 3 indicated severe fibrosis (high number of fibroblast and/or collagen fibers). Each grading represented the average value of 3 capsules per section.

Scanning electron microscopy

Before transplant, microencapsulated Ptcs were visualized by field emission gun scanning electron microscopy using a QUANTA 450 field emission gun (FEI). Scanning electron microscopy was performed at the Science and Technology Center at Bulent Ecevit University.

Statistical analyses

All data collected during the study period (64 weeks in total) were compared, with statistical comparisons made with unpaired Mann-Whitney *t* tests. For time-interval groups, we used a 2-way analysis of variance or comparable mixed-effects analysis followed by a Tukey post hoc test (when there was a missing value) or Sidak's or Dunnett's multiple-comparisons test. In addition, ELISA data were calculated by using the sigmoidal 4-parameter logistic model. All data were visualized with the use of GraphPad Prism software version 8.3. $P < .05$ was considered statistically significant.

Results

All recipient animals (n = 32) were euthanized, with data derived from the 4 groups: microencapsulated Ptc transplant group, Ptc transplant group, empty microcapsule transplant group, and sham group.

Sham group outcomes

The sham group only received injection of isotonic saline solution after laparotomy and were followed for 44 weeks. The sham group exhibited alterations

in food intake and weight loss, which led to early use of euthanasia before the other groups.

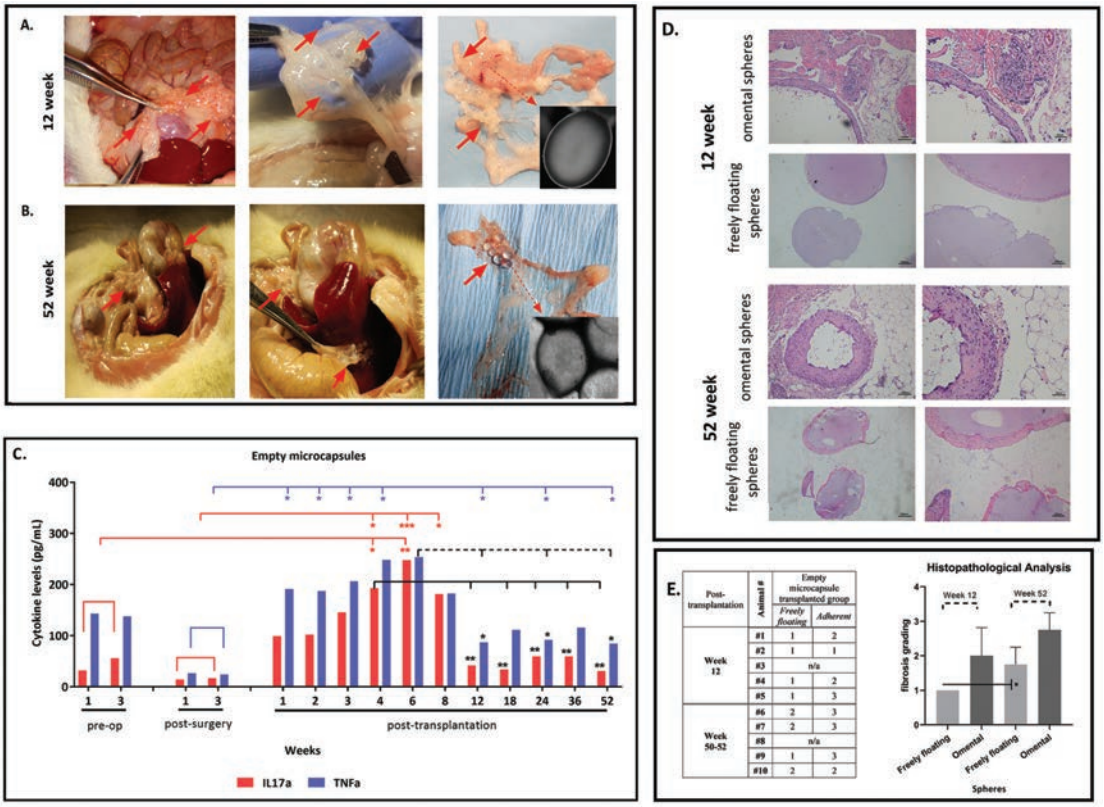
Empty microcapsule transplant group outcomes

At week 1 posttransplant, 1 animal in the empty microcapsule transplant group did not recover after surgery. After transplant, 4 animals in this group were killed and omentectomy and partial pancreatectomy were performed for macroscopic and immunohistochemistry evaluations at week 12 posttransplant (Figure 2A). One animal died at week 28 posttransplant; immediate autopsy showed peritoneal adhesion. Four empty microcapsules had adhered to the liver, and immunohistochemistry was performed (Figure 3). Liver sections showed that neutrophil and leukocyte-rich inflammation was present in the portal area and around the hepatic veins. At week 52, the remaining animals (n = 4) were killed (Figure 2B). Furthermore, omental spheres were enveloped by fibrotic cells with an increasing rate. Despite these multicellular fibrotic layers, freely floating empty microcapsules showed less surrounding cell envelopment even at 1 year after transplant (Figure 2D). Immunohistochemistry evaluation and grading of omental and partial pancreas specimens were performed for both tissues containing omental spheres and the freely floating spheres at week 12 and 52, respectively. When time intervals were compared between groups, fibrosis rate only increased significantly from week 12 to 52 in the freely floating group ($P = .0286$) but not in the group with omental adhered spheres ($P = .3714$) (Figure 2E).

Microencapsulated parathyroid cell transplant group

Before surgery, Ptc were isolated and cultured. Results for cell viability and PTH release level were 94.3% and 1270 pg/mL, respectively. Analyses of scanning electron microscopy images showed that the

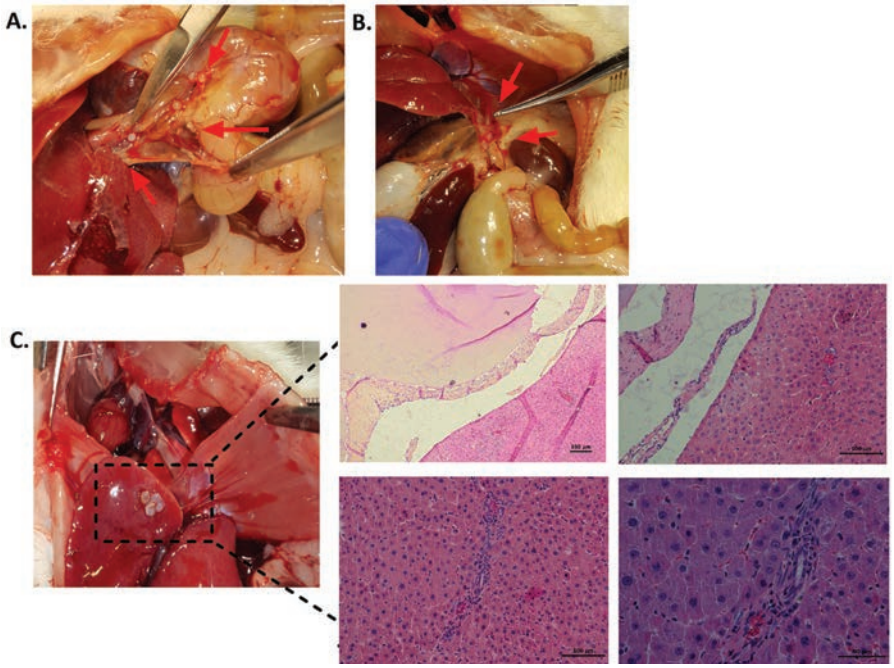
Figure 2. Representative Surgical Images of Empty Microcapsule Transplant Group, Cytokine Levels in Serum Samples, and Stained Sections of Spheres



Abbreviations: IL17a, interleukin 17α; pre-op, preoperative; TNFa, tumor necrosis factor α

(A and B) Comparision of 2% ultrapure alginate adhesion for omental organs between week 12 and 52. Insets show explanted capsule (red dashed arrows). **(C)** Cytokine levels for the empty microcapsule transplant group throughout 52 weeks. IL-17α levels significantly increased at weeks 4 to 8. TNF-α levels increased significantly posttransplant. During the posttransplant period, IL-17α levels dramatically decreased at weeks 12 to 52 compared with that shown at posttransplant week 4 (black straight lines). A similar decline in TNF-α levels at weeks 12, 24, and 52 occurred (black dashed lines). Data are shown as means ± SD. $^{*}P < .05$, $^{**}P < .005$, $^{***}P < .0001$. **(D)** Hematoxylin and eosin-stained sphere histology. **(E)** Histopathological fibrosis grading of the collected microcapsules from the peritoneal cavity and omental sections. Fibrosis significantly increased posttransplant for freely floating microcapsules ($P = .0286$).

Figure 3. Adhered Empty Microcapsule Immunohistochemistry Results



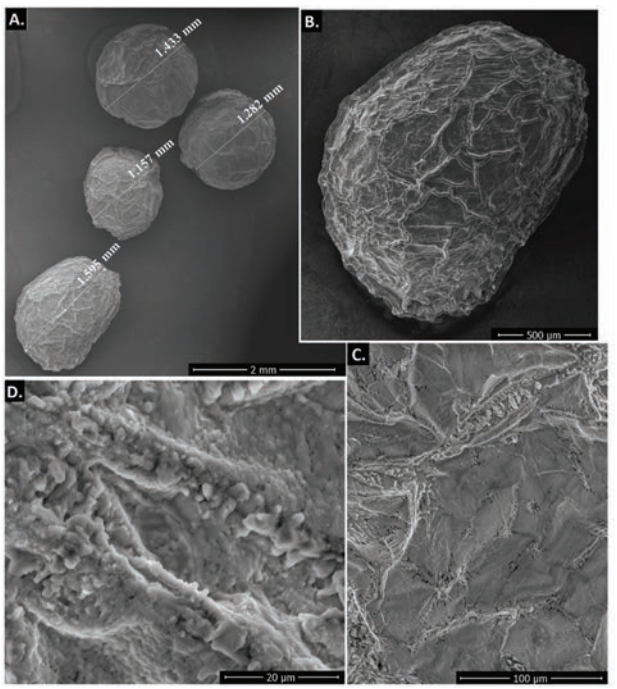
(A and B) One animal underwent macroscopical evaluation. During the autopsy, intraperitoneal adhesion was observed (red arrows). **(C)** Hematoxylin and eosin-stained sections of the liver showed adhered empty microcapsules (black dashed lines) from the empty microcapsule transplant group at week 28 posttransplant. Immunohistochemistry-stained sections of liver sections showed neutrophil and leukocyte-rich inflammation in the portal area and around the hepatic veins.

microencapsulated Ptc sphere diameter ranged from 1.28 to 1.59 mm (Figure 4). After transplant, half of the animals were killed and evaluated macroscopically at week 12 posttransplant. We did not detect any unexpected adherence inside the peritoneal cavity, and the morphology of recovered microencapsulated Ptc spheres remained stable. At week 52 (endpoint of the study), the remaining animals were killed. During extraction from the omentum, two-thirds of the transplanted microcapsules were freely floating inside the peritoneal cavity. In the microencapsulated Ptc transplant group, fibrotic cellular overgrowth on the capsule surface of the freely floating and omental spheres was demonstrated, which was surrounded with a varied range of fibrotic cells (data presented for week 52). Retrieved spheres were graded by immunohistochemistry fibrosis grading, with fibrosis rate not found to be significant (Figure 5). As shown in the histological data in Figure 5, the Ptc stained faintly, and freely floating spheres contained less Ptc.

Parathyroid cell transplant group

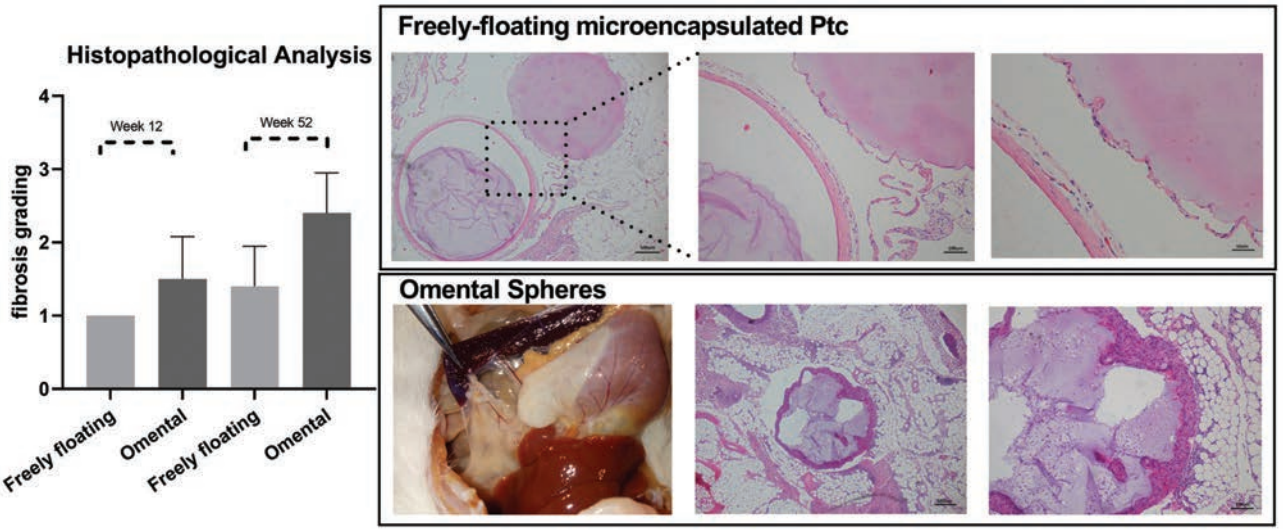
One animal failed to recover at week 1 posttransplant. At 12 and 52 weeks after xenotransplant, animals were

Figure 4. Scanning Electron Microscopy Images Illustrating Microencapsulated Parathyroid Cells With Ultrapure Alginate Before Transplantation



(A) Magnification ×50, 5.00 kV, 2 mm of working distance. Lines demonstrate the perimeter of each microencapsule section circumscribed area. **(B)** Magnification ×130, 5.00 kV, 500 µm of working distance. **(C)** Magnification ×1000, 13 kV, 100 µm of working distance. **(D)** Magnification ×4000, 13 kV, 20 µm of working distance.

Figure 5. Histopathological Analysis of Spheres and Stained Sections of Microencapsulated Parathyroid Cell Transplant Group After Transplant



Abbreviations: Ptc, parathyroid cells

Graph shows histopathological fibrosis analysis. Changes in fibrosis were not statistically significant ($P > .05$). Data are shown as means ± SD. Freely floating sphere and omental sphere magnifications are shown as ×10 and ×4 and ×4 and ×10, respectively.

killed and macroscopically evaluated. There were no peritoneal adhesions inside the cavity.

Rat parathormone levels

Rat PTH levels were determined with the use of sandwich ELISA. The results for sham and empty microcapsule transplant group performed as first group, and the microencapsulated Ptc transplant and Ptc-only transplant groups were evaluated as second group. Assay sensitivity and threshold levels were accepted based on nontransplant (sham) group data of each preoperative, postsurgery, and posttransplant time interval separately. Rat PTH levels were 19.84 and 20.65 pg/mL at preoperative week 1 and week 3, respectively. After surgery, levels dramatically decreased for all groups as expected. During posttransplant, these levels remained below 6.29 pg/mL, which is lower than the average threshold level (7.4 pg/mL) (Figure 6A).

Human parathormone levels

Human PTH levels were evaluated for the sham and empty microcapsule transplant group, for the Ptc-only transplant group, and for the microencapsulated Ptc transplant group. Assay sensitivity and threshold levels were accepted based on the empty microcapsule transplant group data of each preoperative, postsurgery, and posttransplant time interval separately.

The preoperative hu-PTH levels were below 2.1 pg/mL for each group; during postsurgery, the hu-PTH levels remained below 3.46 pg/mL. During the posttransplant period, the microencapsulated Ptc transplant group showed functionality after week 3. Subsequently, the hu-PTH level started to decrease at week 18, with the same reduction continuing through to the endpoint of the experiment (week 52); thereafter, hu-PTH levels remained above the accepted threshold level.

Only the Ptc-only transplant group showed a significant increase starting from week 1 post-transplant; this level remained higher than in the other groups even at week 52 (Figure 6B).

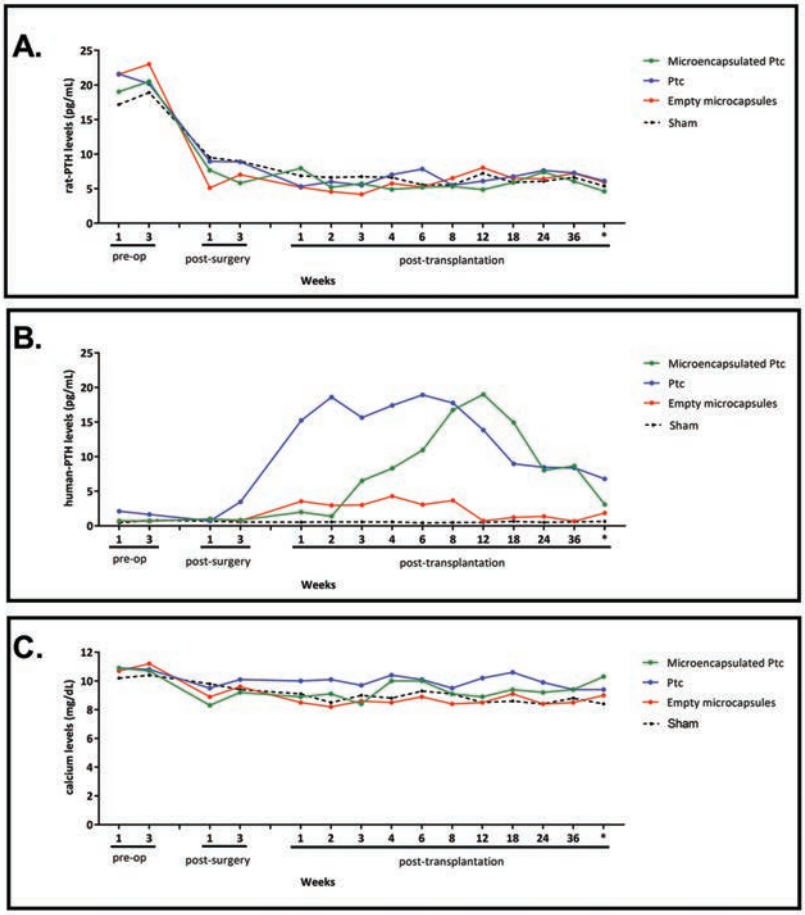
Calcium levels

Before surgery, all animals were normocalcemic, with average calcium level of 9.1 to 10.7 mg/dL; levels were elevated with an average of 0.5 to 0.7 mg/dL throughout all experimental processes (Figure 6C). With regard to physiological function of calcium levels, rats in the Ptc transplant and microencapsulated Ptc transplant groups lost weight; this was not found to be significant because results were not within the exclusion criteria and there were no changes in the food intake.

Cytokine levels

For cytokine evaluation, IL-17α and TNF-α levels were measured in the empty microcapsule transplant group throughout 56 weeks. We found IL-17α levels to be significantly increased at weeks 4 to 6 and at

Figure 6. Serum Parathormone and Calcium Levels Over Time



Abbreviations: pre-op, preoperative; Ptc, parathyroid cells; PTH, parathormone
(A) Rat PTH levels **(B)** Human PTH levels. **(C)** Calcium levels. *Indicates 44-week follow-up period for sham group and 50- to 52-week follow-up period for microencapsulated Ptc, Ptc, and empty microcapsule groups.

weeks 6 to 8 compared with the preoperative and postsurgery levels, respectively. After transplant, IL-17α levels were dramatically decreased at week 12 through 52 compared with posttransplant week 4. Moreover, TNF-α levels were increased significantly posttransplant compared with postsurgery levels. A similar decline at 12, 24, and 52 weeks occurred compared with posttransplant week 6. Cytokine levels are shown in Figure 2C.

For the Ptc-only and microencapsulated Ptc transplant groups, IL-17α, TNF-α, and IL-6 levels were measured in each animal throughout the 64-week study period and compared with levels in the nontransplant group (sham).

In the sham group posttransplant, IL-6 levels were significantly increased compared with our examination of TNF-α levels, particularly at week 18 (P = .0054). After this time point, the animals had reduced food and water intake and displayed some pain-like movements. At week 36, the animals started to lose weight; at week 44, the animals were euthanized (Figure 7A).

In the microencapsulated Ptc transplant group, during the posttransplant period fromweek 1 to week 6, IL-17α levels were relatively high. At week 6 posttransplant, TNF-α levels reached a maximum level, showing a significant difference (P = .0131). Afterward, TNF-α levels gradually decreased and remained below the average range. In addition, IL-6 levels reached their highest level at week 4, although IL-6 levels were relatively stable throughout the 58-week period (Figure 7B).

In the Ptc-only transplant group, no significant differences were detected for TNF-α, IL-17α, and IL-6 throughout the 58-week period. Interestingly, at posttransplant week 8, IL-17α changes were statistically significant compared with IL-6 changes (P = .033) (Figure 7C).

We also analyzed cytokines separately at week 18 posttransplant, with IL-6 levels being statistically significant between the microencapsulated Ptc and Ptc-only transplant groups ($P = .0016$) (Figure 7D).

Discussion

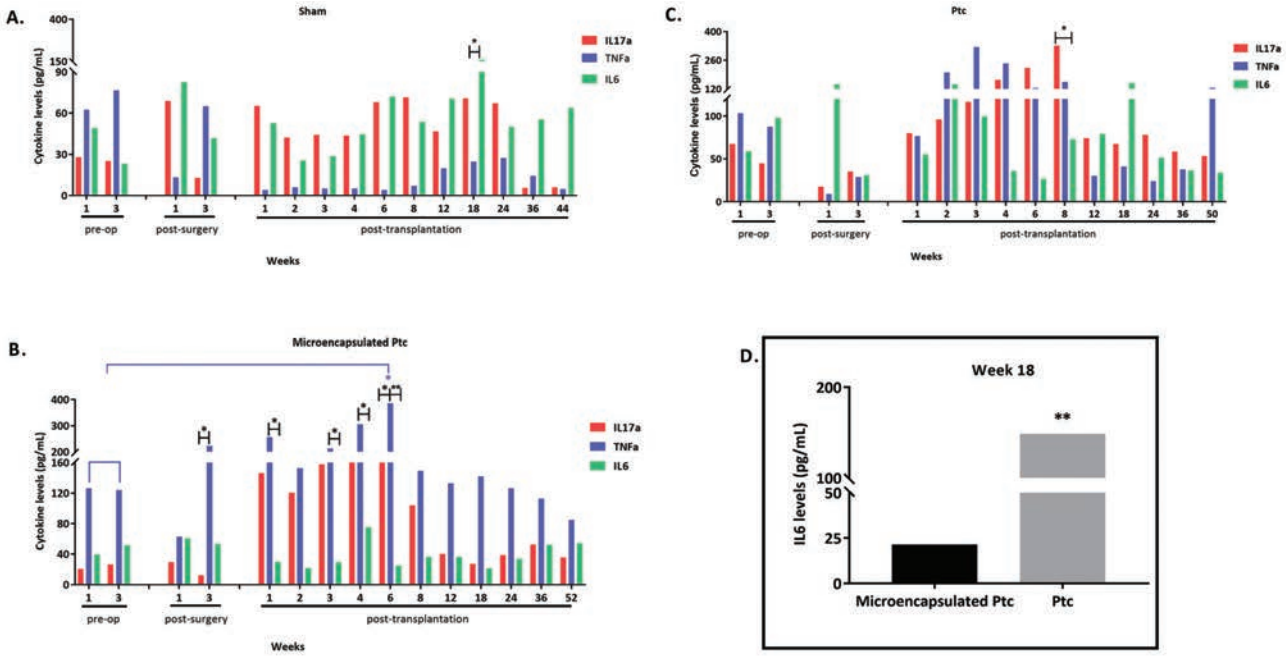
Treatment strategies for autoimmune disorders have so far made slow progress. Ongoing and future studies into investigations of promising treatment options are needed as there are yet no specified therapies.[17]

Hypoparathyroidism is mostly iatrogenic and is rarely congenital (and may also be caused by an autoimmune processes.[1,2,6,18] The management of hypoparathyroidism relies upon symptomatic treatment.[4] Parathyroid allotransplantation may be an alternative therapeutical.[4,5] In a study of the largest number of reported cases, which included 316 allo- and redo-PTX that were performed between 1996 and 2017, varying degrees of success were reported.[5] The necessity of immunoisolation requires more definitive choices, such as allotransplantation by biocompatible materials to avoid immune response.

Graft rejection represents a major drawback in transplantation. To overcome rejection, studies have described different methods and a variety of in vitro and in vivo experiments for PTX.[9,14,19-26] Xenotransplantation triggers an inflammatory pathway, and this leads to a limitation of graft survival.[27] The immunological barriers of allotransplantation and xenotransplantation differ with regard to the transplanted grafts from either cells or organs. However, grafts consisting of cells are exposed more directly to the recipient's immune system.[27,28] Currently, immunoisolation with the use of polymeric materials is one of the contributions to the field of transplantation.[28,29]

Another challenge for PTX is determining the most favorable transplant site. With xenografting of parathyroid tissue/cells, several in vivo routes have been used, including intramuscular,[19,20,30] subcutaneous,[31] and intraperitoneal[32-34] injection. Notably, in a previous study from Nawrot and colleaugues, the peritoneal cavity served as a natural incubator for parathyroid xenotransplantation.[34] Another study reported more effective results when transplanted intraperitoneally.[35] In addition, in vivo

Figure 7. Cytokine Levels Over Time



Abbreviations: IL, interleukin; pre-op, preoperative; Ptc, parathyroid cells; TNFa, tumor necrosis factor α

(A) Cytokine levels in sham group (n = 2) over 53 weeks in total. IL-6 levels were significantly increased compared with TNF-α at week 18 ($P = .0054$) posttransplant. **(B)** Cytokine levels of microencapsulated Ptc transplant group. TNF-α levels were significantly increased at week 3 postsurgery ($P = .0185$) compared with IL-17α; the same increment occurred at week 6 compared with both IL-17α and the preoperative values ($P = .0189$, $P = .0131$, respectively). TNF-α levels were significantly higher than IL-6 levels during weeks 1, 3, 4, and 6 posttransplant ($P = .0122$ for week 1, $P = .0442$ for week 3, $P = .0101$ for week 4, and $P < .001$ for week 6). **(C)** Cytokine levels of Ptc transplant group. IL-17α levels were significantly higher than IL-6 levels at week 8 ($P = .0330$) posttransplant. **(D)** IL-6 levels were statistically significant at week 18 ($P = .0016$). *$P < .05$; **$P < .005$.

studies have reported longer survival rates for islets.[36,37] Therefore, in this study, we prefered use of the omentum to particularly aim for long-term graft function.

Encapsulated PTX has been described in only 7 case reports for 12 recipients.[7,8,21,23,38-40] Hasse and colleagues performed the first microencapsulated PTX for 2 recipients and reported 3-month graft survival.[21] Zimmerman and colleagues performed PTX for 1 recipient. After 3 months, the implantation site of the recipient showed no trace of parathyroid tissue particles or microcapsules.[8] In a later study, in which Khryshchanovich and colleagues transplanted macroencapsulated parathyroid tissue particles and reported 3-month follow-up data, the group demonstrated a nonspecific inflammatory response to the microcapsules.[40] The last case report on microencapsulated Ptc transplantation was performed by Yucesan and colleagues.[23]

To the best of our knowledge, this is the first and the longest follow-up in vivo study on encapsulated parathyroid cells. Several in vivo approaches have revealed that use of Ptc rather than tissue is useful[32] and feasible.[9,19,20,22,33,41] Encapsulation of the parathyroid gland by using semipermeable and biocompatible materials may promote longer survival rates. Hasse and colleagues evaluated different alginate materials and reviewed the results in less than 30 weeks.[9,19-22,41] Other studies have used different encapsulation materials, such as sodium alginate,[26] amitogenic barium alginate,[41,42] barium-alginate-poly-acrylicacid,[32] alginate-poly-lysine-alginate,[33] alginate-poly-lysine-alginate-co-MPEG, and polyvinylidine difluoride.[40] In addition, 1 study demonstrated that mannuronic acid residues stimulated cytokines 10 times higher than guluronic acid.[43] In contrast, another study reported that calcium cross-linked alginate promoted IL-1β secretion in vivo within the first 12 hours after subcutaneous injection.[44] Nontheless, there has been limited mention of the other features of alginate, such as the cross-linking degree, rigidity, or permeability of the alginate capsules in vivo.[12] Therefore, we prepared microencapsules with the ultrapure version for higher biocompatibility and to provide egg-box structure for successful pore integrity and vascularization process into the omentum. It is important to emphasize that the lack of studies is a true obstacle for progress in this field. With this insight, we should be able to demonstrate the methodological details to overcome the current lab-to-lab variations.

Two main goals were planned for this study. The first goal was to investigate the empty ultrapure alginate sphere stability and the adherence capability into the omental tissue and to follow the inflammatory status. We first optimized the in vitro Ptc isolation process and continued to determine the percentage of ultrapure alginate and the cell mass per 27 to 30 microspheres in vitro.[14] Later, we sought to investigate the adherence capability of omental tissue. During the sphere retrieval process, >65% of the spheres had adhered to the omentum, and the remaining capsules were freely floating inside the peritoneal cavity. The fibrosis grade was found to be significantly higher in the freely floating microspheres. The inflammatory response (TNF-α and IL-17α) also increased significantly for up to 6 weeks. We also compared xenotransplant graft longevity by determining PTH and preinflammatory cytokine (TNF-α, IL-17α, and IL-6) levels. The pulsatile secretion of PTH has shown intraindividual variability.[45] The basal PTH level from different patients with secondary hyperparathyroidism has its own secretory dynamics.[42] Therefore, we used 1 parathyroid hyperplasia donor for all processes and this was the main limitation in our study of 10 animals per group.

Transplant-associated stress targets inflammatory responses,[46] and previous studies have demonstrated that synergism of certain cytokines, such as TNF-α and IL-6, may inhibit allograft acceptance[47] or IL-6 promotes IL-17α-dependent rejection.[48] Therefore, cytokines were examined to anticipate the possible role of cytokine-mediated elimination/rejection. Both TNF-α and IL-17α levels for all groups were mostly positively correlated. The presence of IL-6 levels were significantly higher at week 18 posttransplant and at same time hu-PTH levels started to decrease but remained above the preoperative levels. We assumed that the inflammatory response did not hinder transport of nutrients or oxygenation even with the development of subsequent per capsular fibrosis. Of note, fibrosis was significantly altered if the spheres did not adhere to the omentum. The nature of the omental tissue could promote this outcome, and this remains to be elucidated.

Our findings concur with the main idea that encapsulation of xenografts provides immunoisolation. The observed delay in hu-PTH release of microencapsulated Ptc was possibly caused by the

physical barrier formed by microencapsules. Prolonged cytokine release possibly accumulated during the fibrotic cellular growth response to microencapsules. We considered that a main feature of ultrapure alginate is allowing free access to exit for only relatively small proteins, in this case hu-PTH (approximately 12 kDa). Based on the decrease in PTH levels, graft rejection occured after almost 1 year. It is possible that secreted cytokines from monocytes and/or T cells may result in the induction of breaking points, as demonstrated at week 18 for IL-6 and PTH levels.

Conclusions

Our study on the potential clinical use of microencapsulated Ptc transplantation to the omental tissue demonstrated that it is suitable for long-term graft survival. This method may similarly be used for a broad range of applications, such as cell-based protein therapies or other cellular transplant procedures. Importantly, clinical studies are required for the therapeutic benefits for hypoparathyroidism in the future.

References

1. Bello MO, Garla VV. Polyglandular autoimmune syndrome type I. In: *StatPearls;* 2021.
2. Dadu R, Rodgers TE, Trinh VA, et al. Calcium-sensing receptor autoantibody-mediated hypoparathyroidism associated with immune checkpoint inhibitor therapy: diagnosis and long-term follow-up. *J Immunother Cancer.* 2020;8(1):e000687. doi:10.1136/jitc-2020-000687
3. Bilezikian JP. Hypoparathyroidism. *J Clin Endocrinol Metab.* 2020;105(6):1722-1736. doi:10.1210/clinem/dgaa113
4. Yucesan E, Goncu B, Basoglu H, et al. Fresh tissue parathyroid allotransplantation with short-term immunosuppression: 1-year follow-up. *Clin Transplant.* 2017;31(11). doi:10.1111/ctr.13086
5. Barczynski M, Golkowski F, Nawrot I. Parathyroid transplantation in thyroid surgery. *Gland Surg.* 2017;6(5):530-536. doi:10.21037/gs.2017.06.07
6. Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers.* 2017;3:17055. doi:10.1038/nrdp.2017.55
7. Cabane P, Gac P, Amat J, et al. Allotransplant of microencapsulated parathyroid tissue in severe postsurgical hypoparathyroidism: a case report. *Transplant Proc.* 2009;41(9):3879-3883. doi:10.1016/j.transproceed.2009.06.211
8. Zimmermann U, Cramer H, Jork A, et al. Microencapsulation–based cell therapy. In: Rehm HJ, Reed G, eds. *Biotechnology*. Wiley; 2001:547-571.
9. Hasse C, Zielke A, Klock G, et al. Amitogenic alginates: key to first clinical application of microencapsulation technology. *World J Surg.* 1998;22(7):659-665. doi:10.1007/s002689900449
10. Hwang SR, Seo DH, Byun Y, Park JW. Preparation and in vivo evaluation of an orally available enteric-microencapsulated parathyroid hormone (1-34)-deoxycholic acid nanocomplex. *Int J Nanomedicine.* 2016;11:4231-4246. doi:10.2147/IJN.S110573
11. Paredes Juarez GA, Spasojevic M, Faas MM, de Vos P. Immunological and technical considerations in application of alginate-based microencapsulation systems. *Front Bioeng Biotechnol*. 2014;2:26. doi:10.3389/fbioe.2014.00026
12. Goncu B, Yucesan E. Microencapsulation for clinical applications and transplantation by using different alginates. In: Abu-Thabit N, ed. *Nano- and Micro-Encapsulation - Techniques and Applications.* IntechOpen Limited; 2020. https://www.intechopen.com/books/nano-and-microencapsulation- techniques-and-applications/microencapsulation-for-clinical-applications-and-transplantation-by-using-different-alginates. doi:10.5772/intechopen.92134
13. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother.* 2010;1(2):87-93. doi:10.4103/0976-500X.72350
14. Yücesan E, Başoğlu H, Göncü B, et al. In-vitro optimization of microencapsulated parathyroid cells. *Dicle Med J.* 2017;44(4):373-380. doi:10.5798/dicletip.362435
15. Goncu B, Yucesan E, Ozdemir B, et al. A new transport solution for parathyroid allotransplantation: effects on cell viability and calcium-sensing receptors. *Biopreserv Biobank.* 2018;16(4):278-284. doi:10.1089/bio.2018.0008
16. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides.* 2015;72:4-15. doi:10.1016/j.peptides.2015.04.012
17. Oftedal BE, Wolff ASB. New era of therapy for endocrine autoimmune disorders. *Scand J Immunol.* 2020;92(5):e12961. doi:10.1111/sji.12961
18. Al-Azem H, Khan AA. Hypoparathyroidism. *Best Pract Res Clin Endocrinol Metab.* 2012;26(4):517-522. doi:10.1016/j.beem.2012.01.004
19. Hasse C, Bohrer T, Barth P, et al. Parathyroid xenotransplantation without immunosuppression in experimental hypoparathyroidism: long-term in vivo function following microencapsulation with a clinically suitable alginate. *World J Surg.* 2000;24(11):1361-1366. doi:10.1007/s002680010225
20. Hasse C, Zielke A, Klock G, Schlosser A, Zimmermann U, Rothmund M. Isotransplantation of microencapsulated parathyroid tissue in rats. *Exp Clin Endocrinol Diabetes.* 1997;105(1):53-56. doi:10.1055/s-0029-1211727
21. Hasse C, Klock G, Schlosser A, Zimmermann U, Rothmund M. Parathyroid allotransplantation without immunosuppression. *Lancet.* 1997;350(9087):1296-1297. doi:10.1016/S0140-6736(05)62473-7
22. Hasse C, Schrezenmeir J, Stinner B, et al. Successful allotransplantation of microencapsulated parathyroids in rats. *World J Surg.* 1994;18(4):630-634. doi:10.1007/BF00353783
23. Yucesan E, Basoglu H, Goncu B, Akbas F, Ersoy YE, Aysan E. Microencapsulated parathyroid allotransplantation in the omental tissue. *Artif Organs.* 2019;43(10):1022-1027. doi:10.1111/aor.13475
24. Aysan E, Duzkoylu Y, Can I, Buyukpinarbasili N. Xenotransplantation of human cryopreserved parathyroid tissue isolated from parathyroid adenomas to normocalcemic rabbits. *Turk J Surg.* 2017;33(2):91-95. doi:10.5152/turkjsurg.2017.3427
25. Can I, Aysan E, Yucesan E, et al. Parathyroid allotransplantation in rabbits without cultivation. *Int J Clin Exp Med.* 2014;7(1):280-284
26. Picariello L, Benvenuti S, Recenti R, et al. Microencapsulation of human parathyroid cells: an "in vitro" study. *J Surg Res.* 2001;96(1):81-89. doi:10.1006/jsre.2000.6054
27. Cascalho M, Platt JL. The immunological barrier to xenotransplantation. *Immunity.* 2001;14(4):437-446. doi:10.1016/s1074-7613(01)00124-8
28. Wang H, Yang YG. Innate cellular immunity and xenotransplantation. *Curr Opin Organ Transplant.* 2012;17(2):162-167. doi:10.1097/MOT.0b013e328350910c
29. Mahou R, Passemard S, Carvello M, et al. Contribution of polymeric materials to progress in xenotransplantation of microencapsulated cells: a review. *Xenotransplantation.* 2016;23(3):179-201. doi:10.1111/xen.12240
30. Timm S, Otto C, Begrich D, et al. Short-term immunosuppression after rat parathyroid allotransplantation. *Microsurgery*. 2003;23(5):503-507. doi:10.1002/micr.10165

31. Smeds S, Trulsson L, Garovoy M, Gumbert M, Clark OH. Survival of human parathyroid tissue xenotransplanted in nude mice after 9 to 55 months' cryopreservation. *APMIS*. 1999;107(4):445-450. doi:10.1111/j.1699-0463.1999.tb01579.x
32. Gaumann A, Laudes M, Jacob B, et al. Xenotransplantation of parathyroids in rats using barium-alginate and polyacrylic acid multilayer microcapsules. *Exp Toxicol Pathol*. 2001;53(1):35-43. doi:10.1078/0940-2993-00160
33. Lin L, Song Y, Song C, Xu P, Song C. Successful xenotransplantation of microencapsulated newborn pig parathyroid cells in the treatment of hypoparathyroidism in rats. *Chin Med J (Engl)*. 2003;116(8):1161-1165
34. Nawrot I, Wozniewicz B, Szmidt J, Sladowski D, Zajac K, Chudzinski W. Xenotransplantation of human cultured parathyroid progenitor cells into mouse peritoneum does not induce rejection reaction. *Cent Eur J Immunol*. 2014;39(3):279-284. doi:10.5114/ceji.2014.45937
35. Fukuda S, Yabe SG, Nishida J, Takeda F, Nashiro K, Okochi H. The intraperitoneal space is more favorable than the subcutaneous one for transplanting alginate fiber containing iPS-derived islet-like cells. *Regen Ther*. 2019;11:65-72. doi:10.1016/j.reth.2019.05.003
36. Stice MJ, Dunn TB, Bellin MD, Skube ME, Beilman GJ. Omental pouch technique for combined site islet autotransplantation following total pancreatectomy. *Cell Transplant*. 2018;27(10):1561-1568. doi:10.1177/0963689718798627
37. Liljeback H, Espes D, Carlsson PO. Unsurpassed intrahepatic islet engraftment - the quest for new sites for beta cell replacement. *Cell Med*. 2019;11:2155179019857662. doi:10.1177/2155179019857662
38. Tibell A, Rafael E, Wennberg L, et al. Survival of macroencapsulated allogeneic parathyroid tissue one year after transplantation in nonimmunosuppressed humans. *Cell Transplant*. 2001;10(7):591-599.
39. Ulrich F, Klupp J, Thürmer F, et al. Allotransplantation of encapsulated human parathyroid tissue in patients with permanent hypoparathyroidism. *Transplantation*. 2004;78(2):79.
40. Khryshchanovich V, Ghoussein Y. Allotransplantation of macroencapsulated parathyroid cells as a treatment of severe postsurgical hypoparathyroidism: case report. *Ann Saudi Med*. 2016;36(2):143-147. doi:10.5144/0256-4947.2016.21.3.1130
41. Bohrer T, Stelter K, Steler I, Dietrich C, Sitter H, Hasse C. Microencapsulation of allogeneic parathyroid tissue succeeds by delaying immunization of the host, not by protecting the graft against the host's activated immune system. *Transplantationsmedizin*. 2006;18(1):36.
42. Moskalenko V, Ulrichs K, Kerscher A, et al. Preoperative evaluation of microencapsulated human parathyroid tissue aids selection of the optimal bioartificial graft for human parathyroid allotransplantation. *Transpl Int*. 2007;20(8):688-696. doi:10.1111/j.1432-2277.2007.00496.x
43. Otterlei M, Ostgaard K, Skjak-Braek G, Smidsrod O, Soon-Shiong P, Espevik T. Induction of cytokine production from human monocytes stimulated with alginate. *J Immunother*. 1991;10(4):286-291. doi:10.1097/00002371-199108000-00007
44. Chan G, Mooney DJ. Ca(2+) released from calcium alginate gels can promote inflammatory responses in vitro and in vivo. *Acta Biomater*. 2013;9(12):9281-9291. doi:10.1016/j.actbio.2013.08.002
45. Chiavistelli S, Giustina A, Mazziotti G. Parathyroid hormone pulsatility: physiological and clinical aspects. *Bone Res*. 2015;3:14049. doi:10.1038/boneres.2014.49
46. Paredes-Juarez GA, de Haan BJ, Faas MM, de Vos P. The role of pathogen-associated molecular patterns in inflammatory responses against alginate based microcapsules. *J Control Release*. 2013;172(3):983-992. doi:10.1016/j.jconrel.2013.09.009
47. Shen H, Goldstein DR. IL-6 and TNF-alpha synergistically inhibit allograft acceptance. *J Am Soc Nephrol*. 2009;20(5):1032-1040. doi:10.1681/ASN.2008070778
48. Varelias A, Gartlan KH, Kreijveld E, et al. Lung parenchyma-derived IL-6 promotes IL-17A-dependent acute lung injury after allogeneic stem cell transplantation. *Blood*. 2015;125(15):2435-2444. doi:10.1182/blood-2014-07-590232

Monkeypox in a Kidney Transplant Recipient: Case Report and Literature Review

Fakhriya Alalawi,[1] Fatima Alhadari,[2] Hani Tobia,[1] Amna Alhadari[1]

Abstract

Monkeypox (mpox) infection is usually a self-limiting disease; however, kidney transplant recipients may be at a higher risk of serious complications, due to their immunosuppressed status. Nevertheless, the reported mpox cases in transplant recipients are very few, thus data on the clinical course and prognosis details of mpox in transplant recipients are scarce. Indeed, only 2 cases of mpox in kidney transplant recipients have been reported: one patient achieved good clinical recovery, and the other patient experienced a disseminated form of the disease with urinary and gastrointestinal complications. Yet, both patients recovered fully with no allograft involvement. Here, we report a case and images of a 46-year-old male patient, with a history of posttraumatic splenectomy in 1999 and a living related kidney transplant in 2010, who presented to us with fever, sore throat, and skin rash. After thorough examination, a throat swab sent for mpox DNA polymerase chain reaction was positive; similarly, a cutaneous swab taken from a skin lesion was positive for mpox by DNA polymerase chain reaction, although he had no history of recent travel or contact with mpox cases. Our patient received supportive care and made a good clinical recovery with no disease sequelae. In this report, we describe the patient's clinical course and outcome, as well as photographic illustrations of skin lesion progression. With the present outbreak of mpox cases, clinicians should consider mpox in differential diagnoses of skin rash in immunosuppressed patients. Early identification of the infection, through viral detection by DNA polymerase chain reaction from samples taken from the skin rash, is necessary to facilitate a prompt diagnosis.

Key words: *Kidney transplantation, Mpox virus, Skin rash*

Introduction

Monkeypox (mpox) is a zoonosis caused by the monkeypox virus, an orthopox virus similar to smallpox, and causes a disease similar to smallpox but less severe.[1] Although smallpox was eradicated in 1980, mpox continues to occur in people living in tropical rainforests of central and west Africa.[1] After decades of quiescence, mpox has re-emerged as an outbreak that began in May 2022.[2] The World Health Organization (WHO) declared the mpox outbreak to be an international health emergency on July 23, 2022, after over 16 000 cases were reported from 75 countries, including 5 deaths.[3] Moreover, the WHO has recommended a new preferred term "mpox" as a synonym for this disease.[1]

Transplant recipients may acquire mpox infection through direct contact with skin-to-skin cutaneous lesions, sexual transmission, or respiratory droplets by exposure to infected individuals.[4,5] In the literature, only a few cases of mpox infection in immunocompromised patients have been reported to date.

Case Report

Informed consent was obtained from the patient for his images and other clinical information to be reported in the journal.

Here, we report the case of a 46-year-old male patient from the United Arab Emirates who had a history of posttraumatic splenectomy in 1999. He underwent a living related kidney transplant in 2010, in Germany, and the donor was his brother. His native kidney disease was focal segmental glomerulosclerosis. After transplant, he had been

From the [1]Department of Nephrology, Dubai Hospital, Dubai Academic Health Corporation; and the [2]Department of Dermatology, Rashid Hospital, Dubai Academic Health Corporation, Dubai, United Arab Emirates

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Fakhriya Alalawi, Academic Department of Medicine at DMC, Department of Nephrology, Dubai Hospital, Dubai Academic Health Corporation, Dubai, United Arab Emirates*

E-mail: *fjalalawi08@yahoo.co.uk*

Experimental and Clinical Transplantation (2023) 11: 913-916

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0222

maintained on tacrolimus and mycophenolate. Steroids were withdrawn rapidly after the transplant. Additionally, he developed posttransplant diabetes mellitus and was maintained on the combination of glucagon-like peptide 1 plus pioglitazone and sodium-glucose cotransporter-2 inhibitors (empagliflozin and metformin). He had excellent allograft function with a creatinine level of 0.7 to 0.8 mg/dL and an estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration method) of 110.5 mL/min/1.73 m^2, with a minimum proteinuria protein-to-creatinine ratio of 299 mg/g.

He was admitted in May 2023 with a history of fever, severe sore throat, and diarrhea of 4 days duration. He reported no history of recent travel; however, he had contact with his son, who had severe diarrheal illness but with no skin rashes.

Upon presentation, he was ill in appearance, febrile with a temperature of 38 °C, blood pressure 119/74, pulse 114 beats/min, respiration 18 breaths/min, height 170 cm, weight 87.8 kg, and saturation of blood oxygen 99%.

Physical examination, apart from pharyngeal swelling, was unremarkable. Furthermore, an incidental finding of maculopapular skin rash was noted across the scalp, shoulders, and arms. According to the patient, the rash appeared 2 weeks earlier, and he had remained otherwise asymptomatic. The rash appeared first on the scrotum and then spread to the entire body. The rash was well defined, multiple, and erythematous; some areas appeared with skin-tone papules and nodules with central umbilication; and some areas appeared with crusted lesions on an erythematous base. The rash was scattered across the body but was absent from the palms of the hands and soles of the feet. On genitalia, the papules showed coalescence and formed a plaque over the scrotum. No lesions were noted on the oral mucosa.

The laboratory findings are shown in Table 1, and the rash (at the neck) is shown in Figure 1. Chest radiography revealed accentuated vascular markings in both lung fields, with no definite air space opacities.

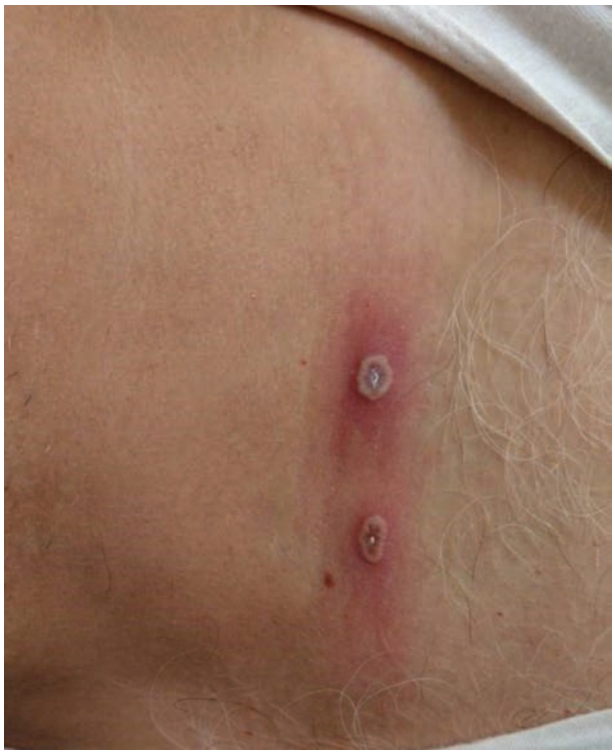
The patient was examined and assessed by the dermatologist, who proposed a differential diagnosis of varicella zoster virus, mpox, or herpetic lesions. The patient was started empirically on antibiotics for the high inflammatory markers and fever. Further investigations showed negative test results for cytomegalovirus immunoglobin M and no reactivity for rapid plasma reagin. The results for all of the following tests were negative: HIV1 and HIV2 antigen/antibody; herpes simplex virus 1 DNA polymerase chain reaction (PCR); blood herpes simplex virus 2 DNA PCR; blood varicella zoster virus DNA PCR; Cryptococcus neoformans antigen; Cryptococcus quantification antibodies; and blood Epstein-Barr virus DNA PCR. The gastrointestinal panel was positive for Sapovirus. A throat swab sent for mpox DNA polymerase chain reaction was positive; similarly, a cutaneous swab taken from a skin lesion (cutaneous) was positive for mpox by DNA polymerase chain reaction. On further inquiry, he had no history of recent travel or contact with mpox cases.

Table 1. Laboratory Findings Upon Presentation

Result	Value	Reference Range
FBC (CBC+DIFF)		
WBC, $\times10^3$ cells/μL	22.3 (H)	3.6-11.0
RBC, $\times10^6$ cells/μL	5.68 (H)	4.50-5.50
Blood hemoglobin, g/dL	15.0	13.0-17.0
Hematocrit, %	46.3	40.0-50.0
MCV, fL	81.5	77.0-95.0
MCH, pg	26.4 (L)	27.0-32.0
MCHC, g/dL	32.3	31.5-34.5
RDW, %	15.2 (H)	11.5-14.0
Platelets, $\times10^3$ cells/μL	465 (H)	150-410
MPV, fL	8.6	7.4-10.4
Neutrophil (absolute), $\times10^3$ cells/μL	14.80 (H)	2.00-7.00
Lymphocytes (absolute), $\times10^3$ cells/μL	3.40 (H)	1.00-3.00
Monocytes (absolute), $\times10^3$ cells/μL	2.30 (H)	0.20-1.00
Eosinophils (absolute), $\times10^3$ cells/μL	1.70 (H)	0.00-0.50
Basophils (absolute), $\times10^3$ cells/μL	0.10	
Neutrophils, %	66.4	
Lymphocytes, %	15.1	
Creatinine, mg/dL	0.83	0.70-1.20
eGFR, mL/min/1.73 m^2	109.3	>60
Venous CRP, mg/L	119.5 (H)	<5.0
Urea electrolytes		
Sodium, mmol/L	135 (L)	136-145
Potassium, mmol/L	4.0	3.3-4.8
Chloride, mmol/L	96 (L)	98-108
Bicarbonate, mmol/L	19.4 (L)	20-28
Urea, mg/dL	23	12-40
Anion gap, mmol/L	20 (H)	6-14
PCT, ng/mL	0.14 (H)	<0.05
Random glucose, mg/dL	92	65-140
Lactic acid, mmol/L	1.6	0.5-2.2
Liver function test		
Total bilirubin, mg/dL	0.3	0-1.0
ALP, U/L	97	40-129
ALT, U/L	16	0-41
Total protein, g/dL	8.4	6.6-8.7
Albumin, g/dL	3.4	3.4-4.8
Globulin, g/dL	5.0 (H)	2.8-3.4

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; CBC+DIFF, complete blood count with differential; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration method); FBC, full blood count; with a differential count; H, higher than reference range; L, lower than reference range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration (ratio of MCH to MCV); MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, procalcitonin; RBC, red blood cells; RDW, red cell distribution width; WBC, white blood cells

Figure 1. Umbilicated Papular Rash at the Neck, Upon Presentation



The infectious disease unit was consulted, but there was no active treatment for mpox, so he was continued on antibiotics. We ceased his mycophenolate while he was an inpatient but continued his treatment with tacrolimus. The patient made a remarkable recovery; his inflammatory markers trended down, and he had normal allograft functions throughout. He was discharged from the hospital and transitioned to home isolation. Figure 2 and Figure 3 show the course progression of the rash, which faded over the subsequent 3 weeks:

Figure 2. Mpox Rash After 8 Days of Presentation

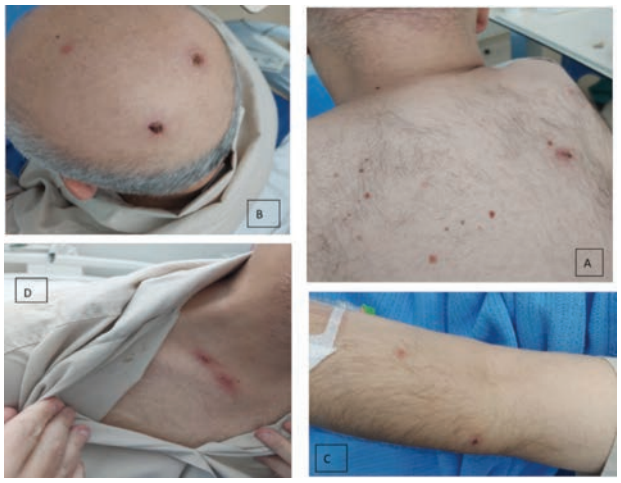
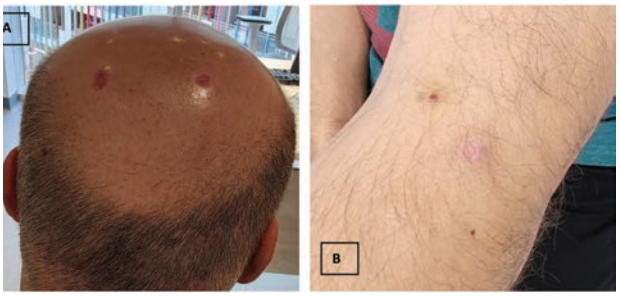


Figure 3. Healed Rash Over the Scalp and Left Arm at Follow-Up Visit, 35 days After Presentation



Discussion

Infection with mpox is usually self-limiting; however, it can be serious in immunocompromised individuals. The incubation period is estimated to be 2 to 20 days, with most cases appearing at 9.1 days.[3,6,7] The disease classically lasts for 2 to 4 weeks.[8] The clinical syndrome is characterized by fever with other prodromal symptoms, such as malaise, myalgias, and headache, together with lymphadenopathy and rash. Fever can occur before or after the appearance of the rash. Respiratory symptoms such as sore throat (as in our patient), nasal congestion, pharyngitis, or cough can occur.[5]

Skin rashes are characteristically described as firm or rubbery, well-circumscribed, and deep-seated, which often develop umbilication (resembling a dot on the top of the lesion). Skin rashes can be located on the face, hands, feet, chest, mouth, and genital and anorectal areas. The rash develops simultaneously on various sites and can be confined to only a few lesions or a single lesion or can be disseminated across many sites on the body (as in our patient).[6] The lesions progress through 4 stages (macular, papular, vesicular, and pustular) and then forms scabs that resolve during a course of 2 to 3 weeks.[9-11] Lesions confined only to the genital area can be confused with other sexually transmitted infections.[11] Whenever possible, samples should be taken directly from the skin rash, crusts, fluid, or biopsy if necessary. Viral DNA PCR is the preferred method for detection of the mpox virus.[1,12]

The immunosuppressed status of kidney transplant recipients could represent a higher risk to develop subsequent complications after infection. Complications of mpox can include encephalitis, sight-threatening keratitis, pneumonitis, secondary bacterial infections, and death; these potential complications are based on reported observations of severe complications in immunocompromised

HIV-infected patients.[4,5,8] Yet the reported transplant cases are very few, thus data of the clinical course and prognosis of mpox in transplant recipients are scarce. Indeed, only 2 cases of mpox in kidney transplant recipients have been reported in the literature: one patient achieved a good recovery,[13] and the other case was a disseminated form of the disease with urinary and gastrointestinal complications, in the form of urine retention, proctitis, and secondary bowel obstruction.[14] Yet both patients recovered fully with no allograft involvement.

Treatment of mpox in renal transplant recipients is mainly supportive to relieve symptoms, manage complications, and prevent long-term sequelae.[2-4] There is presently no standardized approach to manage immunocompromised patients with mpox in terms of reducing immunosuppression reduction, and no specific protocol has been established. We reduced the immunosuppressants in our patient by temporary cessation of mycophenolate therapy, based on previous observational research of COVID-19 infection management. Even before the skin rash was fully resolved in our patient, mycophenolate was restarted by the time the patient was discharged. Antiviral medications can be used to reduce illness duration and symptom severity; nevertheless, there is no antiviral therapy approved by the United States Food and Drug Administration for the mpox tirus.[6] A few investigational antiviral medications drugs are available, under the guidance of infectious disease specialists, such as Tecovirimat, an antiviral drug that was developed for smallpox.[4,8] There is, however, limited availability of tecovirimat. Additionally, reduction of tacrolimus and sirolimus levels are suggested, because these are weak inducers of cytochrome P450 3A and 2B6 and contraindicated in patients with glomerular filtration rate of <30 mL/min.[8] There have also been reports of mpox cases treated with cidofovir and brincidofovir.[5] However, the effectiveness of these medications is not well established in renal transplant patients, and further research is necessary.[13] No antiviral therapy was started here since infectious disease specialists recommended none. The vaccinia immunoglobulin intravenous (human) is available under expanded access protocol from the Center for Disease Control for treatment of mpox cases. Nonetheless, this treatment has not been studied in kidney transplant recipients.[4,8,15]

Given the limited evidence for the therapeutic efficacy of these investigational medications, transplant infectious disease specialists together with the primary transplant provider, should be involved in the patient's treatment decisions.

Conclusions

With the present outbreak of mpox cases, clinicians should consider mpox in the differential diagnoses of skin rash in immunosuppressed patients. Early identification of the infection, through viral DNA PCR detection in the skin rash, is necessary to facilitate a prompt diagnosis.

References

1. World Health Organization. Mpox (monkeypox). Accessed December 7, 2023. https://www.who.int/news-room/fact-sheets/detail/monkeypox
2. Hraib M, Jouni S, Albitar MM, Alaidi S, Alshehabi Z. The outbreak of monkeypox 2022: an overview. *Ann Med Surg (Lond).* 2022;79:104069. doi:10.1016/j.amsu.2022.104069
3. Centers for Disease Control and Prevention. Mpox. Accessed December 7, 2023. https://www.cdc.gov/poxvirus/monkeypox/symptoms/index.html
4. Al Jurdi A, Kotton CN. Monkeypox in transplant recipients: no breaks between outbreaks. *Transplantation.* 2022;106(11):e512-e513. doi:10.1097/TP.0000000000004337
5. Adler H, Gould S, Hine P, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect Dis.* 2022;22(8):1153-1162. doi:10.1016/S1473-3099(22)00228-6
6. Centers for Disease Control and Prevention. Key characteristics for identifying Mpox. Accessed December 7, 2023. https://www.cdc.gov/poxvirus/monkeypox/clinicians/clinical-recognition.html
7. Guzzetta G, Mammone A, Ferraro F, et al. Early estimates of monkeypox incubation period, generation time, and reproduction number, Italy, May-June 2022. *Emerg Infect Dis.* 2022;28(10):2078-2081. doi:10.3201/eid2810.221126
8. American Society of Transplantation. Monkeypox FAQS for transplant community. August 4, 2022. Accessed July 14, 2023. https://www.myast.org/monkeypox-faqs-transplant-community#3
9. Farahat RA, Sah R, El-Sakka AA, et al. Human monkeypox disease (MPX). *Infez Med*. 2022;30(3):372-391. doi:10.53854/liim-3003-6
10. McCollum AM, Damon IK. Human monkeypox. *Clin Infect Dis.* 2014;58(2):260-267. doi:10.1093/cid/cit703
11. Ortiz-Martinez Y, Rodriguez-Morales AJ, Franco-Paredes C, et al. Monkeypox: a description of the clinical progression of skin lesions: a case report from Colorado, USA. *Ther Adv Infect Dis.* 2022;9:20499361221117726. doi:10.1177/20499361221117726
12. Boan P. Monkeypox a concern for donor-derived infection? *Transplantation.* 2023;107(2):303-304. doi:10.1097/TP.0000000000004445
13. Heis F, Heis M, Khan M, Ashok M. Monkeypox in a renal transplant recipient: is it a hazard for immunocompromised patients? *Eur J Case Rep Intern Med.* 2023;10(6):003857. doi:10.12890/2023_003857
14. Attieh RM, Brumble L, Elwasila SM, Wadei HM. Disseminated monkeypox infection in a kidney transplant recipient: a case report. *Transplant Proc.* 2023;55(3):667-671. doi:10.1016/j.transproceed.2023.02.031
15. NHS Blood and Transplant. INF1640/1. Monkeypox and organ transplantation. Rapid assessment. Accessed July 15, 2023. https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/26782/inf16401.pdf

Short Tandem Repeat Analysis in a Living Related Donor Adult Renal Transplant Recipient with Rare Natural Chimerism

Xueqin Meng,[1,2] Bing Yang,[3] Dongya Wu,[1,2] Tingting Pan,[1,2] Feng Zhang,[1,2] Haiyang Xie,[1,2] Wenqin Xie,[4] Xinhua Chen,[1,2] Shusen Zheng[1,2]

Abstract

After renal transplant, immunosuppression therapy is used to reduce the risk of rejection. Here, we describe the case of an adult living related donor renal transplant recipient with rare natural chimerism, as discovered by short tandem repeat sequence analysis. In our process of matching transplant patients, we perform human leukocyte antigen testing and short tandem repeat chimerism testing to decide posto-perative immunosuppression strategy for transplant patients. We analyzed the short tandem repeat chimerism status before renal transplant and determined that this patient represented a rare case of natural chimerism. Assessment of organ recipient chimerism can inform physicians regarding a dosage reduction of immunosuppressive agents. Short tandem repeat sequence analysis provides substantial information regarding existing polymor-phisms and can identify chimerism, if present, and thereby guide immunosuppression strategies after renal transplant, which may improve the long-term immunosup-pression-free survival of renal transplant recipients.

Key words: *Chimera status, Kidney transplantation*

From the [1]Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery and the [2]NHC Key Laboratory of Combined Multi-organ Transplantation, The First Affiliated Hospital; the [3]Department of Breast Surgery, The Second Affiliated Hospital; and the [4]Kidney Disease Centre, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Acknowledgements: *This work was supported by grants from the Zhejiang Provincial Natural Science Foundation of China (LZ21H180001) and the Jinan Provincial Laboratory Research Project of Micro Ecological Biomedicine (JNL-2022002A). Other than described, the authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Xinhua Chen, NHC Key Laboratory of Combined Multi-organ Transplantation, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*

E-mail: *Xinhua_chen@zju.edu.cn*

Experimental and Clinical Transplantation (2023) 11: 917-920

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0104

Introduction

In kidney transplant studies, research has focused on microchimerism in order to induce the post-transplant immune tolerance.[1] Microchimerism assessment can be used as a diagnostic method for various diseases.[2-4] We suggest that microchimerism assessment can be used as a reference biomarker to monitor the status of the organ recipient's immune tolerance after kidney transplant. Here, we report our application of this method for a renal transplant patient. We performed blood type and human leukocyte antigen (HLA) tests before transplant. In this process, the dispersed chimerism was inadver-tently discovered by short tandem repeat (STR) sequence analysis, which is generally used in genetic disease detection and forensic medicine. Here, we report the STR sequence analysis in a living related donor adult renal transplant recipient with rare natural chimerism.

Materials and Methods

This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The patient provided written informed consent.

General history

A 23-year old male patient had been previously treated for uremia and had received hemodialysis for 3 months. He was admitted to our hospital in November 2021 with complaint of itching and fatigue. At that time, creatinine was 1700 μmol/L, and renal biopsy indicated immunoglobin A nephro-pathy (grade V, Oxford classification: M1E0S1T2C2). The patient was diagnosed with uremia and underwent hemodialysis 3 times per week. To determine eligibility for renal transplant, the patient underwent

chromosomal and HLA high-resolution typing tests. The patient and his parents collected the whole blood and throat swab samples.

Human leukocyte antigen typing

Whole blood and throat swab samples from the patient were sent for assessment of ABO blood group genotype, chromosome karyotype, chromosomal microdeletions, and microrepeated variations for STR, as detected by a blood DNA purification kit (Maxwell 16, Promega) and a buccal swab DNA purification kit (Maxwell 16 LEV, Promega). Sequence-specific oligonucleotide probes (LABType SSO HLA DNA typing trays; One Lambda) were used for the DNA analyses. The ABO blood group genotyping kit for human erythrocytes was from Xiaoping.

Genomic microdeletion and microduplication detection

Genomic microdeletion and microduplication detection procedures were performed according to the high-throughput ligation probe amplification technique by Shanghai Tianhao Biotechnology (Shanghai, China). The assay provides 1602 probes covering more than 200 core regions and a certain range of regions on both sides of the core regions. The basic principle of this technique is the use of the ligase-linked reaction with high specificity to cross-link the target region, then by introduction of nonspecific sequences of different lengths at the end of the ligation probe and by ligase addition reaction, different-length ligation products corresponding to the sites are obtained. The polymerase chain reaction products are then amplified by universal primers labeled with fluorescent capillary electrophoresis, and the products are separated by electrophoresis. Finally, the peaks of each site are obtained by analysis of the electrophoretic patterns.

Results

The HLA subtypes of the patient's family are shown in Table 1. The patient inherited 2 genes from his father and 1 from his mother. The results showed the HLA A locus of the patient (Figure 1). The patient was identified to have a standard ABO blood type (A01 type) (Figure 2). The patient was identified to have a standard chromosome karyotype without sequence variation (Figure 3). Figure 4 shows that STR loci D7S820, G2S0002, and D18S51 all have 3 peaks, which represent 3 alleles.

Table 1. Human Leukocyte Antigens in the Patient and Parents

HLA	Father	Patient	Mother
A*	02:01, 02:06	33:03, 02:06, 02:01	11:01, 33:03
B*	15:11, 40:06	44:03, 40:06, 15:11	44:03, 54:01
C*	03:03, 08:01	14:03, 08:01, 03:03	01:02, 14:03
DRB1*	09:01, 14:05	09:01, 13:02, 14:05	08:03, 13:02
DQB1*	03:03, 05:03:01G	03:03, 06:04, 05:03:01G	06:01, 06:04
DPB1*	02:02, 05:01:01G	02:02, 05:01:01G, 04:01	02:02, 04:01

Abbreviations: HLA, human leukocyte antigen

Figure 1. Human Leukocyte Antigen A Locus of Patient

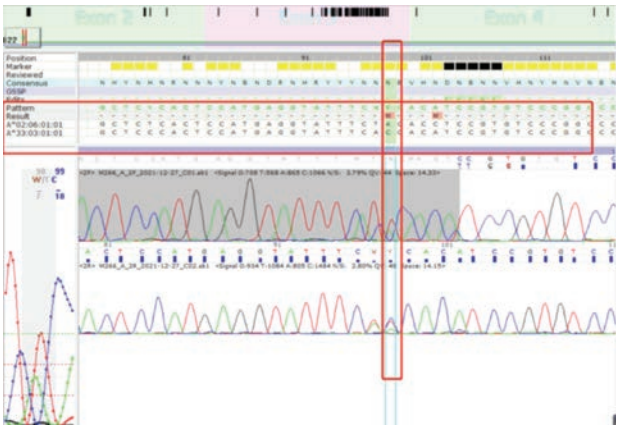


Figure 2. Results Showed A01 Type, Without Sequence Variation

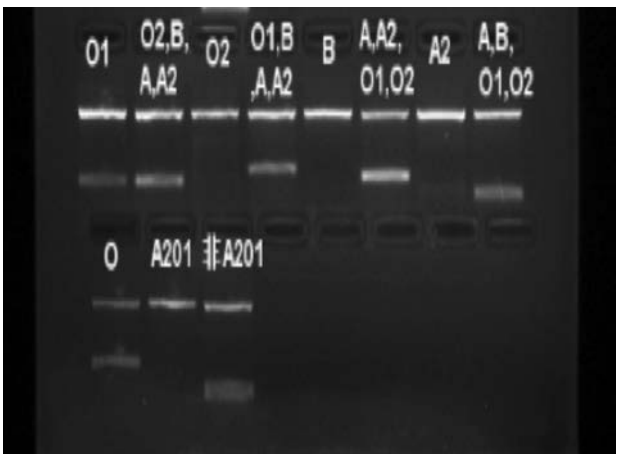


Figure 3. Results Showed A01 Type, Without Sequence Variation

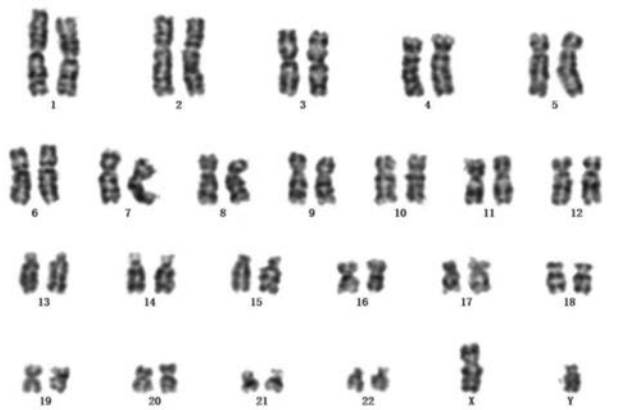


Figure 4. Results of Chromosomal Microdeletions and Microrepeated Sequence

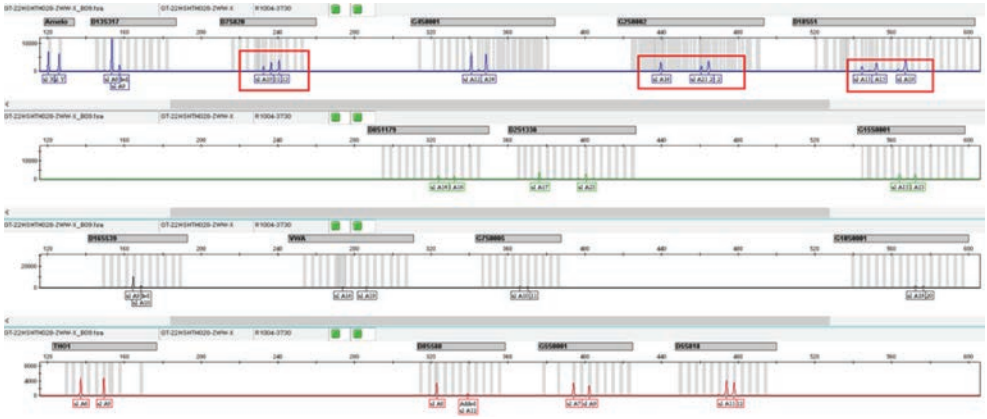


Figure 5 shows the number of copies detected by each probe on all chromosomes. The probes on the autosome show 2 copies, and the probes on the sex chromosome show 1 copy. If there are 3 copies or 1 copy of multiple probes in a row, then this indicates that there is duplication or absence in the area where the probe is located (the experimental results showed no duplication or absence).

With regard to STR chimerism detection, the STR peak plot showed 2 sequence variants inherited from the father and 1 standard (invariant) sequence inherited from the mother (Figure 6). Moreover, the peak map of STR showed that the peak map area of

Figure 5. Number of Copies Detected by Each Probe on All Chromosomes

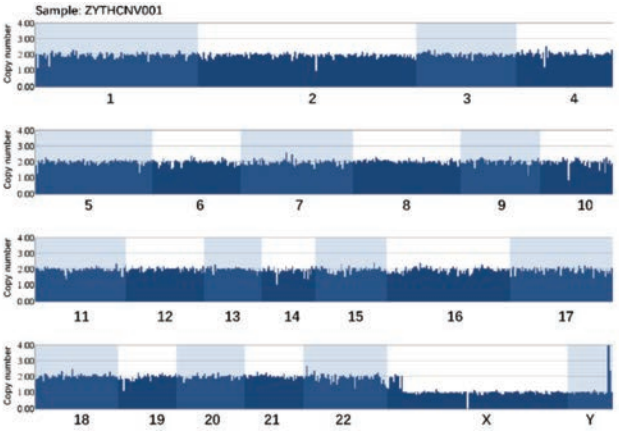
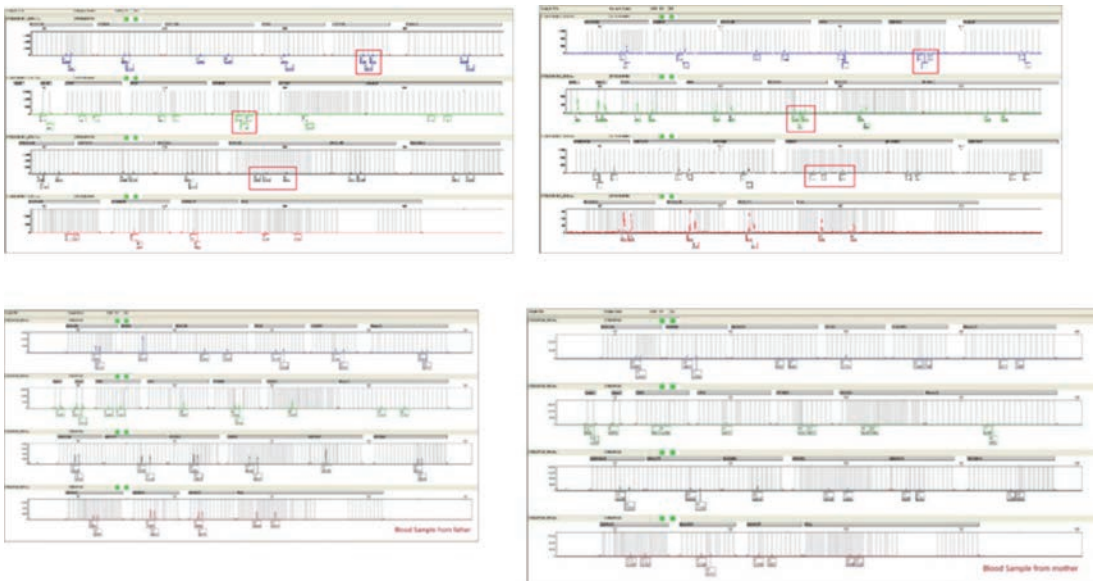


Figure 6. Triallelic Patterns at Loci D7S820, CSF1PO, and D18S51 Genotypes



There are 4 data sets, including the patient's blood sample (top left), the patient's swab sample (top right), his father (bottom left), and his mother (bottom right).

the 2 sequence variants inherited from the father was basically the same as that of the mother. The 3 isogene peaks were observed at loci D7S820, CSF1PO, and D18S51.

After transplant, the induction immunosuppression therapies included tacrolimus (2 mg twice daily), mycophenolate mofetil (0.5 g twice daily), and prednisone (5 mg/d); then mycophenolate mofetil was prescribed as the maintenance immunosuppression therapy. The patient recovered from the transplant within 3 days. The laboratory results returned to baseline after a week. The follow-up laboratory reports showed healthy function of the kidney (Figure 7).

Discussion

Our patient underwent HLA-matched living related kidney transplant; however, further follow-up was required to determine whether the patient was able to continue to tolerate kidney transplant in the long-term after cessation of the immunosuppressive agents. Here, we report a rare case of natural chimerism.

There have been previously reported cases for which donor origin was from the transplanted organs.[5,6] However, our case is quite different in that our living related kidney transplant recipient expressed rare natural chimerism..

The father provided the donor kidney for his son. Both of the father's 2 haploids were passed to the son. The HLA test results showed that the son who received the transplanted kidney and his donor father were fully compatible at 6 sites, which represents a rare case of natural chimerism. We observed D7S820, G2S0002, and D18S51 by detection of chromosome microdeletion and microrepeated sequence variation, and we observed D7S820, CSF1PO, and D18S51 by STR chimerism detection; all had 3 allelic peaks, and 2 of the loci (D7S820 and D18S51) were mutually verified.

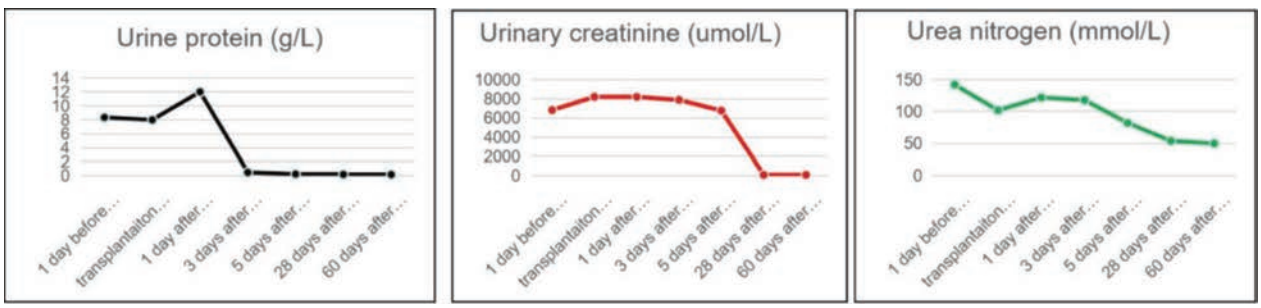
We followed up this patient after kidney transplant for 2 years, and we expected to reduce immunosuppressive agents in the later stage. We targeted this patient for further long-term follow-up observation.

The preoperative detection of this patient suggests that biomarker monitoring can guide operant immune tolerance and facilitate a better understanding of the immune status of grafts during the process of immunosuppression reduction. Short tandem repeat sequence analysis provides substantial information regarding existing polymorphisms and can identify chimerism, if present, and thereby guide immunosuppression strategies after renal transplant, which may improve the long-term immunosuppression-free survival of renal transplant recipients.

References

1. Fan J, Zhang AP, Zheng ZZ, et al. A case of type 1 triallelic patterns at D5S818, D18S51, D6S1043, and FGA demonstrated by short tandem repeat analysis. *Int J Clin Pract*. 2022;2022:8600125. doi:10.1155/2022/8600125
2. Muller E, Barday Z. HIV-positive kidney donor selection for HIV-positive transplant recipients. *J Am Soc Nephrol.* 2018;29(4):1090-1095. doi:10.1681/ASN.2017080853
3. Lo Monte AI, Palumbo VD, Damiano G, et al. Double endocrine neoplasia in a renal transplant recipient: case report and review of the literature. *Transplant Proc.* 2011;43(4):1201-1205. doi:10.1016/j.transproceed.2011.02.040
4. Detry O, De Roover A, de Leval L, et al. Transmission of an undiagnosed sarcoma to recipients of kidney and liver grafts procured in a non-heart beating donor. *Liver Transpl.* 2005;11:696-699. doi:10.1002/lt.20457
5. Foltys D, Linkermann A, Heumann A, et al. Organ recipients suffering from undifferentiated neuroendocrine small-cell carcinoma of donor origin: a case report. *Transplant Proc.* 2009;41(6):2639-2642. doi:10.1016/j.transproceed.2009.06.026
6. Baehner R, Magrane G, Balassanian R, et al. Donor origin of neuroendocrine carcinoma in 2 transplant patients determined by molecular cytogenetics. *Hum Pathol.* 2000;31(11):1425-1429. doi:10.1016/s0046-8177(00)80015-5

Figure 7. Preoperative and Postoperative Laboratory Results



Pulse Methylprednisolone-Induced Sinus Bradycardia: A Case Report

Ozlem Beyler, Cengiz Demir

Abstract

Corticosteroids have a wide range of uses. The most common adverse side effects of high-dose pulse steroids are hyperglycemia, gastrointestinal intolerance, and psychiatric symptoms. Cardiac arrhythmias have been reported in patients who receive high-dose steroid therapy. Bradycardia is a rare adverse side effect of pulse steroid therapy. We present the case of a 57-year-old male patient who developed symptomatic sinus bradycardia after he received pulse methylprednisolone therapy as treatment for graft-versus-host disease. The patient's pulse steroid therapy was discontinued, and the dose of methylprednisolone was reduced to 100 mg/day. He was treated conservatively and with close observation; the patient's heart rate increased to 68 beats/min after 1 day, and then to 78 beats/min. The diagnosis of methylprednisolone-induced bradycardia was made after exclusion of other common etiologies of sinus bradycardia. This case report demonstrates the importance of careful cardiovascular monitoring in patients who receive high-dose methylprednisolone because of dose-related cardiovascular risks.

Key words: *Cardiovascular risk, Graft-versus-host disease, High-dose steroid therapy, Pulse therapy*

Introduction

Corticosteroids have a wide range of uses due to the anti-inflammatory and immune modulatory properties of these agents. Because of these properties, corticosteroids are used in the treatment of various hematologic conditions, as well as the treatment of many diseases. Acute graft-versus-host disease (aGVHD) is a complication of allogeneic hematopoietic stem cell transplant that occurs early after transplant.

The skin, gastrointestinal tract, and liver are the main target organs in patients with aGVHD. For the treatment of aGVHD at grade ≥2, treatment with systemic glucocorticoids (eg, methylprednisolone) has been widely adopted. Treatment is usually started with 2 mg/kg/d methylprednisolone in divided doses.[1] Studies of high-dose intravenous methylprednisolone (eg, 10-20 mg/kg/d) have shown a high response rate.[2,3] Our patient had extensive skin and gastrointestinal and liver involvement, so we planned a regimen of high-dose methylprednisolone for 3 days followed by methylprednisolone at a dose of 2 mg/kg. The most common adverse side effects of high-dose pulse steroids are hyperglycemia, gastrointestinal intolerance, minor infections, and psychiatric symptoms. Overall, cardiac arrhythmias have been reported in 1% to 82% of patients given high-dose corticosteroid therapy.[4,5]

Bradycardia is a rare side effect of pulse steroid therapy and is usually asymptomatic. In this case report, we present an episode of severe asymp-tomatic sinus bradycardia detected during routine follow-up of vital functions, which developed 3 days after pulse steroid therapy in a 57-year-old male patient with aGVHD.

Case Report

Bone marrow biopsy was performed in a 57-year-old male patient due to pancytopenia in July 2021. In the patient's bone marrow, cellularity was 80%, myeloid/erythroid ratio was 15/1, and blast ratio was 15%. The patient was diagnosed with myelodysplastic syndrome and was given 4 cycles of hypomethylation therapy. However, allogeneic stem cell transplant was performed from an unrelated

From the Gazi Yasargil Training and Research Hospital, Diyarbakir, Turkey

Acknowledgements: *The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

Corresponding author: *Ozlem Beyler, Gazi Yasargil Training and Research Hospital, Department of Hematology, Diyarbakir, Turkey*
E-mail: *drozlembeyler@gmail.com*

Experimental and Clinical Transplantation (2023) 11: 921-924

Copyright © Başkent University 2023
Printed in Turkey. All Rights Reserved.

DOI: 10.6002/ect.2023.0095

9/10 matched male donor in June 2022, to treat the increased need for transfusion and high risk of complications according to the International Prognostic Scoring System in this patient who did not respond to the treatment.

Echocardiogram results were unremarkable, and contractility and valve function were within reference limits, both after the diagnosis of myelodysplastic syndrome and before the allogeneic stem cell transplant. In September 2022, chimerism was 100%. After taper of the cyclosporine dose was initiated, widespread erythema and skin dryness was observed on the head, neck, trunk, and extremities of the patient. The patient's cyclosporine dose was increased, and he received steroid therapy for a short time. Skin lesions regressed with treatment, but desquamation and postinflammatory hyperpigmentation remained present in some areas.

In January 2023, thrombocytopenia was detected. Bone marrow biopsy showed increased myeloid series, 70% cellularity, and sufficient multilobular megakaryocytes. The patient's cyclosporine dose was reduced. In the control physical examination, there was widespread erythema, dry skin, and hyperpigmentation. Liver was palpable in deep inspiration, and the Traube space was open. A skin biopsy was taken from the right scapula. In the complete blood count, white blood cell count was 9.81×10^9 cells/L, neutrophils 7.97×10^9 cells/L, hemoglobin 9.6 g/dL, hematocrit 28.1%, and platelet count 26×10^9 cells/L. The platelet count in the peripheral smear was consistent with the results from whole blood.

The patient, who also had an increase in liver and cholestasis enzymes (Table 1), was hospitalized with a prediagnosis of liver and skin GVHD. His biopsy was reported as skin GVHD. Mycophenolate mofetil (2 doses of 1000 mg) and intravenous methylprednisolone (1 g/d) were planned for 3 days. On day 3 of treatment, heart rate was 55 beats/min, and sinus bradycardia was observed on elec-trocardiography in the routine follow-up of vital functions. We observed that the patient was asymptomatic at this time.

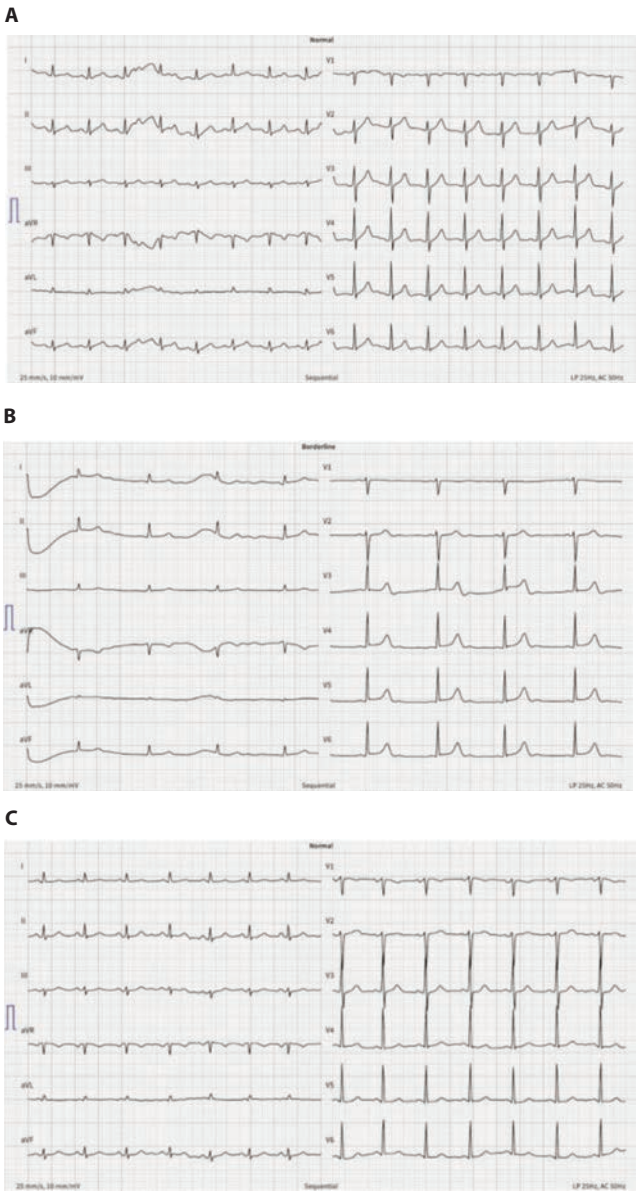
Figure 1 shows the electrocardiography results before the pulse steroid treatment, during the bradycardia, and after the dose reduction of the steroid treatment. Troponin T and creatinine kinase were within reference limits. The dose of methylprednisolone was reduced to 100 mg/d, and 1 day later the patient's heart rate increased to 68 beats/min, and then subsequently to 78 beats/min.

Table 1. Laboratory Results of Case Patient

	Pretreatment Baseline	Posttreatment Day 1	Posttreatment Day 2	Posttreatment Day 3
Urea, mg/dL	40	38	37	28
Creatinine, mg/dL	0.7	0.56	0.52	0.59
ALT, U/L	76	102	125	161
AST, U/L	106	109	85	91
GGT, U/L	162	221	260	316
ALP, U/L	443	479	403	342
Total bilirubin, mg/dL	3	3.4	2.2	2.7
Direct bilirubin, mg/dL	2.8	1.3	1.6	1.9
LDH, U/L	367	290	274	308

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase

Figure 1. Electrocardiography Results



A, Electrocardiography before the pulse steroid treatment (heart rate, 95 beats/min). **B,** Electrocardiography during the bradycardia (heart rate, 50 beats/min). **C,** Electrocardiography after the dose reduction of the steroid treatment (heart rate, 84 beats/min).

Due to the absence of bradycardia among the adverse side effects of other drugs and because the heart rate recovered after corticosteroid dose reduction, bradycardia was associated with pulse steroid therapy.

Discussion

Corticosteroids are widely used for many pathologic conditions in clinical practice. High-dose corticosteroid therapy (known as pulse corticosteroid therapy) is used for the treatment of hematologic, ophthalmic, neurologic, nephrologic, dermatologic, and rheumatologic diseases, as well as some neoplastic diseases.[6] Although the adverse side effects of chronic corticosteroid use are well known (eg, increased appetite, hyperglycemia, hypertension, depression, osteoporosis, and Cushing syndrome), awareness remains low regarding the adverse side effects that occur with short-term high doses of corticosteroid.

In recent years, rhythm changes due to corticosteroid treatments have been reported, mostly in adults. These rhythm changes can be seen as tachyarrhythmias or bradyarrhythmias.[7] These arrhythmias include atrial fibrillation, sinus tachycardia or bradycardia, premature atrial contractions, and premature ventricular contractions.[8] Although arrhythmias are usually asymptomatic and mitigated with simple treatment, symptoms such as palpitations, chest pain, unconsciousness, and cardiac arrest may occur in some patients.[9]

Steroids are known to cause arrhythmias, but the mechanism of action remains controversial. Animal studies have shown that high-dose methylprednisolone has various effects on the cardiovascular system, particularly blunting the chronotropic response to catecholamines through β1 receptor sensitivity.[10-12] These effects may result from (1) a direct effect on electron exchange in the myocardial cell membrane and (2) changes in the sensitivity of the sinoatrial node to catecholamines.

Many factors may contribute to the development of bradycardia after pulse corticosteroid therapy. These predisposing factors include rapid intravenous infusion of corticosteroids (typically less than 30 minutes) and underlying heart disease or kidney disease.[13,14] Electrolytes, especially potassium, should be monitored prior to treatment, and any deficiencies should be corrected. In patients without comorbidities, corticosteroids are less likely to cause bradycardia, but such a complication was seen in our patient, despite the absence of a renal or cardiac comorbid disease. In our case, bradycardia was detected 3 days after initiation of pulse corticosteroid therapy.

In the literature, sinus bradycardia has generally been reported between 1 and 7 days after the initiation of pulse corticosteroid therapy.[15,16] Late onset may make it difficult to consider corticosteroids as the cause of bradycardia. Cardiac arrhythmias at varying rates have been reported in patients receiving high-dose corticosteroids.[4,5] These undesirable effects are usually associated with the intravenous route of administration. As in our case, bradycardia is usually associated with high-dose intravenous corticosteroid administration, but some cases of bradycardia have been reported after low-dose intravenous and oral corticosteroid therapy.[7,8,16-18] As in our case, methylprednisolone or prednisone has been reported as the cause of steroid-related bradycardia.[7,8,15,16] However, intravenous dexamet-hasone has also been identified as a causative agent.[5,19]

Corticosteroid-induced bradycardia is generally well tolerated in the absence of underlying heart or kidney disease or electrolyte disturbances.[7,14,16] Dizziness and chest pressure/pain are often reported as symptoms.[8,20] Our patient was asymptomatic and had a medical history of normal cardiac functions; no sudden cardiac pathology was detected in the re-evaluation with echocardiography after the incidental detection of bradycardia. Generally, bradycardia resolves within 3 to 10 days after corticosteroid cessation or dose reduction.[16,19,21] In accordance with the literature, bradycardia completely resolved without any other intervention after the steroid dose was reduced in our patient.

Conclusion

Steroids are frequently used in the treatment of many diseases. Our report provides evidence to suggest that pulse steroid therapy may be complicated by adverse side effects that are different from the effects observed in long-term corticosteroid therapy. Patients with an underlying heart or kidney disease who receive high-dose steroids are at particularly high risk to develop bradycardia, although it is rare. These patients should be followed closely in terms of

electrolyte disturbance and heart rate. Cardiac complications should be considered in patients with chest pressure, pain, or shortness of breath, and symptomatic patients should seek immediate medical attention.

References

1. Weisdorf DJ, Snover DC, Haake R, et al. Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. *Blood.* 1990;76(3):624-629.
2. Shulman HM, Sharma P, Amos D, Fenster LF, McDonald GB. A coded histologic study of hepatic graft-versus-host disease after human bone marrow transplantation. *Hepatology*. 1988;8(3):463-470. doi:10.1002/hep.1840080305
3. Snover DC, Weisdorf SA, Ramsay NK, McGlave P, Kersey JH. Hepatic graft versus host disease: a study of the predictive value of liver biopsy in diagnosis. *Hepatology.* 1984;4(1):123-130. doi:10.1002/hep.1840040122
4. Klein-Gitelman MS, Pachman LM. Intravenous corticosteroids: adverse reactions are more variable than expected in children. *J Rheumatol.* 1998;25(10):1995-2002.
5. Miura M, Ohki H, Yoshiba S, et al. Adverse effects of methylprednisolone pulse therapy in refractory Kawasaki disease. *Arch Dis Child.* 2005;90(10):1096-1097. doi:10.1136/adc.2004.062299
6. West TW. Transverse myelitis: a review of the presentation, diagnosis, and initial management. *Discov Med.* 2013;16(88):167-177.
7. Akikusa JD, Feldman BM, Gross GJ, Silverman ED, Schneider R. Sinus bradycardia after intravenous pulse methylprednisolone. *Pediatrics.* 2007;119(3):e778-e782. doi:10.1542/peds.2006-0029
8. Taylor MR, Gaco D. Symptomatic sinus bradycardia after a treatment course of high-dose oral prednisone. *J Emerg Med.* 2013;45(3):e55-e58. doi:10.1016/j.jemermed.2013.04.020
9. Moses RE, McCormick A, Nickey W. Fatal arrhythmia after pulse methylprednisolone therapy. *Ann Intern Med*. 1981;95(6):781-782. doi:10.7326/0003-4819-95-6-781_3
10. Hall ED, Plaster M, Braughler JM. Acute cardiovascular response to a single large intravenous dose of methylprednisolone and its effects on the responses to norepinephrine and isoproterenol. *Proc Soc Exp Biol Med.* 1983;173(3):338-343. doi:10.3181/00379727-173-41653
11. Svorcik C, Bicikova L. Effect of drugs on the stimulation threshold of the human heart. *Cor Vasa.* 1978;20(3):184-195.
12. Fujimoto S, Kondoh H, Yamamoto Y, Hisanaga S, Tanaka K. Holter electrocardiogram monitoring in nephrotic patients during methylprednisolone pulse therapy. *Am J Nephrol.* 1990;10(3):231-236. doi:10.1159/000168087
13. Lucas KG, Howrie DL, Phebus CK. Cardiorespiratory decompensation following methylprednisolone administration. *Pediatr Hematol Oncol.* 1993;10(3):249-255. doi:10.3109/08880019309029492
14. Hsu DT. Steroids and bradycardia: how slow can you go? *J Pediatr Hematol Oncol.* 2008;30(2):119-120. doi:10.1097/MPH.0b013e31815f8906
15. Tvede N, Nielsen LP, Andersen V. Bradycardia after high-dose intravenous methylprednisolone therapy. *Scand J Rheumatol.* 1986;15(3):302-304. doi:10.3109/03009748609092597
16. Al Shibli A, Al Attrach I, Hamdan MA. Bradycardia following oral corticosteroid use: case report and literature review. *Arab J Nephrol Transplant.* 2012;5(1):47-49.
17. Beyan E, Urun Y, Uzuner A. Bradycardia due to methylprednisolone therapy. *J Clin Rheumatol.* 2004;10(4):230. doi:10.1097/01.rhu.0000135562.11724.60
18. Hasan AQ, Al-Khazraji A. Corticosteroids-induced bradycardia: a case report and literature review. *J Med Sci Clin Res.* 2016;4(7):1566-1569. doi:10.18535/jmscr/v4i7.64
19. Ohlsson A, Heyman E. Dexamethasone-induced bradycardia. *Lancet.* 1988;2(8619):1074. doi:10.1016/s0140-6736(88)90088-8
20. Vasheghani-Farahani A, Sahraian MA, Darabi L, Aghsaie A, Minagar A. Incidence of various cardiac arrhythmias and conduction disturbances due to high dose intravenous methylprednisolone in patients with multiple sclerosis. *J Neurol Sci.* 2011;309(1-2):75-78. doi:10.1016/j.jns.2011.07.018
21. Pudil R, Hrncir Z. Severe bradycardia after a methylprednisolone "minipulse" treatment. *Arch Intern Med.* 2001;161(14):1778-1779. doi:10.1001/archinte.161.14.1778-a

Index to Volume 21 Issue 11

Author Index

Financial Disclosure, Copyright Transfer, and Ethical Conduct Form

Title of Work : .....

.....

Manuscript Number : ....

Names of Authors : ....

This form must be completed and signed by every author of the above-referenced work (the "Work") and returned to the Experimental and Clinical Transplantation (The Journal) office at the time of manuscript submission. The Journal will not publish any Work or make any commitment or agreement to publish any Work until The Journal receives a completed questionnaire signed by all authors of the Work.

Conflict of Interest and Financial Disclosure

If the manuscript is accepted for publication, disclosure by an author of a conflict of interest will be published along with the manuscript. It will not be disclosed to reviewers of the manuscript and will have no bearing on acceptance or rejection of the manuscript, which will be evaluated on its scientific merit.

Question 1: Did you receive any outside funding or grants in support of your research for or preparation of the Work?

Yes, I/we received, in any one year, grants or outside funding from the following person(s) or entity(ies):

.....

If yes, you must indicate the amount here: In excess of $10,000 Less than $10,000

No.

Question 2: Have you, or a member of your immediate family, received from any commercial entity any payments or any pecuniary, in kind, or other professional or personal benefits including stock, honoraria, or royalties (collectively, "Benefits") or any commitment or agreement to provide such Benefits that were related in any way to the subject of the Work or the research that you conducted in connection with the Work?

Yes, I/we received, in any one year, payments or other Benefits or a commitment or agreement to provide such Benefits from the following commercial entity(ies).

.....
.....

If yes, you must indicate the amount here: In excess of $10,000 Less than $10,000

No.

Question 3: In addition to or instead of any Benefits that you may have received or anticipate receiving in connection with the Work, has any commercial entity paid or directed or agreed to pay or direct any Benefits to any research fund, foundation, department, division, center, clinical practice, or other charitable or nonprofit organization with which you or a member of your immediate family are affiliated or associated in any way?

Yes, such a fund, foundation, department, division, center, clinical practice, or other charitable or nonprofit organization has received, in any one year, payments of other Benefits or a commitment or agreement to provide such Benefits from the following person(s) or entity(ies).

.....

If yes, you must indicate the amount here: In excess of $10,000 Less than $10,000

No.

Copyright Transfer

Upon acceptance of the manuscript, all copyright ownership for the article is transferred to The Journal. Except for personal use, no part of the accepted paper may be published in any other language or in any book, periodical or similar written material, and no part may be stored without the written permission of the publisher. This agreement to transfer copyright authorizes Baskent University to protect the copyright material for the author(s) without removing author proprietary rights. It covers the exclusive rights to reproduce and distribute the article n print and electronic form.

We, the undersigned co-authors of this article, have contributed significantly to and share responsibility for the submitted manuscript entitled:

We confirm that this manuscript submitted to The Journal is original, and has not been submitted to another publication for simultaneous or previous consideration.

We further attest that we have indicated all sources of financial support for this study in "Acknowledgements" on the title page, and have herein disclosed all financial and/or other relationships that could be construed as conflict(s) of interest.

All authors must sign this statement stating that they have read and approve this manuscript. If any portion(s) of the manuscript has (have) been published elsewhere, this should be acknowledged on the title page, and the manuscript must be accompanied by appropriate written permission to include this part.

Ethical Conduct

We, the undersigned, attest that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in The Helsinki Declaration as well as the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. We, the authors, therefore declare that:

- All human studies undertaken as part of the research from which this manuscript was derived are in compliance with the regulations outlined in the **The Helsinki Declaration** of The World Medical Association.
- None of the authors or their institutions have been involved in commercial transactions or other unethical practices in obtaining donor organs as outlined in **The Declaration of Istanbul on Organ Trafficking and Transplant Tourism.**
- None of the authors or their institutions have been involved in obtaining or transplanting organs from executed prisoners or other donors where there is a risk that an autonomous consent for donation has not been given.
- All persons involved in trials gave their informed consent prior to their inclusion in the study.

Author's signature : .....

Name (please print) : ..... Date: .....

Author's signature : .....

Name (please print) : ..... Date: .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

Author's signature : .....

Name (please print) : ..... **Date:** .....

INSTRUCTIONS TO AUTHORS

In preparing manuscripts, authors should follow the specific instructions listed below (these are also available online at: www.ectrx.org). **Manuscripts that do not adhere to these guidelines will be returned to the author without consideration.**

MANUSCRIPT SUBMISSION

Experimental and Clinical Transplantation accepts original articles, case reports, reviews, and letters to the editor. Manuscripts must be submitted via Manuscript Central at

http://mc.manuscriptcentral.com/ect.

- Manuscripts that have been submitted for publication elsewhere will not be accepted.
- The Editor has the right to make editorial corrections with the knowledge and approval of the author.

A mandatory **Financial Disclosure, Copyright Transfer, and Ethical Conduct Form** is to be completed by the submitting author. The form can be downloaded from Manuscript Central during the online submission process. The manuscript will be assigned for peer review and editorial evaluation ONLY after this form has been submitted.

Experimental and Clinical Transplantation is a member of COPE (Committee on Publication Ethics) and requires all authors to adhere to the principles outlined in their International Standards for Authors:*

- The research being reported should have been conducted in an ethical and responsible manner and should comply with all relevant legislation.
- Researchers should present their results clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.
- Researchers should strive to describe their methods clearly and unambiguously so that their findings can be confirmed by others.
- Researchers should adhere to publication requirements that submitted work is original, is not plagiarized, and has not been published elsewhere.
- Authors should take collective responsibility for submitted and published work.
- The authorship of research publications should accurately reflect individuals' contributions to the work and its reporting.
- Funding sources and relevant conflicts of interest should be disclosed.

*Wager E, Kleinert S. (2011) Responsible research publication: international standards for authors. A position statement developed at the 2nd World Conference on Research Integrity, Singapore, July 22-24, 2010. Chapter 50 in: Mayer T & Steneck N, eds. Promoting Research Integrity in a Global Environment. Singapore: Imperial College Press / World Scientific Publishing; 2011:309-16.

TEXT REQUIREMENTS

There is no page limit for original articles, but authors are urged to be as concise as possible. For case reports, the text (excluding Figures, Tables, Legends, and References) should be a succinct description of no more than 1500 words, and should include no more than 3 Figures that emphasize elements of the case, including methodology used. Only generic names of drugs should be used throughout the text. Drugs, instruments, or equipment should be identified by the manufacturer's name in parentheses following the item, and procedures should be described in sufficient detail to allow other investigators to reproduce the results. When describing pharmaceutical interventions, include the dosage regimen in parentheses after the drug name.

Abbreviations

Except for expressing units of measurement, the use of abbreviations is discouraged. To introduce an abbreviation, on first mention in the text the full term should be spelled out, followed by the abbreviation in parentheses. **Abbreviations must not be used in the Abstract or the manuscript title**.

AAWP: Avoid Abbreviations Whenever Possible. The added length of spelling out words is more than compensated for by the increased readability when words are spelled out. Idiosyncratic abbreviations make text particularly difficult to read. Abbreviations widely used within a narrow discipline can make a manuscript uninterpretable to the interested reader from outside that discipline.

Do not create new or unusual abbreviations. For example, if your paper refers to the paw pressure test, just call it the paw pressure test throughout the paper, not the PPT.

Write as you speak. You would call an electrocardiogram an ECG, so it is acceptable to abbreviate it as ECG. However, spell out words if there is any possible ambiguity.

For additional information on proper use of medical abbreviations, refer to the Council of Biology Editors Style Manual, Fifth Edition (Bethesda, MD: Council of Biology Editors, 1983).

Units of Measurement

All measurements must be listed in **SI units**. Conventional units may be shown in parentheses after the SI units. Temperatures must be expressed in degrees Celsius (°C).

FORMAT REQUIREMENTS

As a guide, authors should follow the requirements set forth in the American Medical Association Manual of Style, 10th Edition.

Text Style: Manuscripts must be typed on A4 paper in double-spaced format, using 12-point Times New Roman font, with ample margins at the top, bottom, and sides of each page. Type the text flush left. Enter only 1 space between words and between sentences. For line breaks within a paragraph, use the automatic wrap-around feature of your word processor. **Do not place a line break (ie, do not press the "Enter" button) at the end of any sentence within a paragraph**. Ensure that the hyphenation option is switched off. Leave 1 blank line (press "Enter" twice) between heading and text. Begin each new paragraph with a tab indent. Number pages consecutively, with the Title Page as page 1. Type the page number in the upper right-hand corner of each page. Papers must be written in English, and authors are urged to aim for clarity, brevity, and accuracy of information and language. Authors whose first language is not English should have their papers checked for linguistic accuracy by a native English speaker.

Title Page

This page of the manuscript should contain a concise and informative title comprising no more than 200 characters. List the authors' (10 maximum) full names and affiliations. If the authors are affiliated with more than 1 institution, indicate the appropriate information using a superscripted Arabic numeral to match each author's name with that of the author's department and institution. State any name disclaimers that apply. Indicate the name, mailing address, telephone, fax number, and e-mail address of the corresponding author. Grants and/or financial support for the work should be noted in a statement on this page.

Abstract

A structured Abstract is required for original articles only. The Abstract cannot exceed 300 words, and should include 4 sections with the following subheadings:

1. Objectives

2. Materials and Methods

3. Results

4. Conclusions

Nonstandard abbreviations, references, and footnotes should not be used. Three-to-five key words that do not appear on the Title Page should be included on the Abstract page. It is recommended that authors consult the Medical Subject Headings section of the National Library of Medicine (http://www.nlm.nih.gov/mesh/MBrowser.html).

Main Text

The text of original articles should follow the Abstract and include the following sections: Introduction, Materials and Methods, Results, Discussion, References, Tables, and Figure Legends. Figures, themselves, must be uploaded as separate files.

The Introduction should appear without a heading. It should be concise and present relevant background information.

The Materials and Methods section should contain a statement indicating that all protocols, experimental studies, and clinical trials involving human subjects were approved by the ethics committee of the institution before the study began, and that the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. It also must be stated that written informed consent was obtained from patients or their guardians. Individual patients should be identified by number rather than by initials. For animal studies, it must be stated that, before the study, all protocols were approved by the institution's animal welfare regulatory committee, and/or all protocols were in conformity with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health 86-23, revised in 1985.

Authors are responsible for any false statements, and for failure to fulfill the above-mentioned requirements.

References

The reference style for Experimental and Clinical Transplantation is that set forth in the American Medical Association Manual of Style, 10th Edition. In-text citations should use Arabic superscripted numerals outside periods and commas, and inside colons and semicolons. They should be numbered consecutively throughout the text. The same system applies for references that are cited in a Table or Figure. Journal names (as listed in the January issue of Index Medicus for each year) and unpublished works and/or data should not be used as references, although written communications may be noted as such in the text. References cited as "in-press" must have been accepted for publication at the time the manuscript is submitted; they must not be in preparation or awaiting acceptance. Full pagination must be included. Authors are responsible for the accuracy of the references, and must verify them against the original document. Experimental and Clinical Transplantation limits the amount of references in Reviews to 75, in Original Articles to 50, and in Case Reports to 15. **Authors who do not follow this style will have their manuscripts returned to them unread.** The following styles are required:

Journal Articles: The names of all authors should be given unless there are more than 6, in which case the names of the first 3 authors are used, followed by "et al." Rainer S, Thomas D, Tokarz D, et al. Myofibrillogenesis regulator 1 gene mutations cause paroxysmal dystonic choreoathetosis. Arch Neurol. 2004;61(7):1025-1029.

Book Chapters: Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

Entire Books: Venables WN, Ripley BD. Modern Applied Statistics With S. 4th ed. New York, NY: Springer Publishing Co; 2003.

TABLES

Tables should be self-explanatory and should supplement, not duplicate, the text. They should be placed after the Reference section. Do not submit Tables as photographs. Each Table should appear on a separate page, and be accompanied by a brief double-spaced Table legend. The text within the Table should also be double-spaced. Each Table must be cited in consecutive numerical order in the text, and should be identified with an Arabic numeral following the word "Table." Place a horizontal line below the legend, below the column headings, and at the end of the Table. Do not use vertical lines. To place text beneath a column heading, use a tab(s), not a space(s). Place explanatory matter in footnotes, not in the Table legend. Use appropriate symbols **(eg, *, †, ‡, §, ||, ¶, #, **, ††, ‡‡)** to designate footnotes. The footnotes for each Table should contain expanded versions of all abbreviations used in the Table contents, and should identify statistical measures of variation, such as standard deviation and standard error of the mean. If dates pertaining to other published sources (copyrighted material used) are noted in the Table, the author must obtain written permission from the publisher and the original author(s), and acknowledge this fully in a footnote or in an addition to the legend. Below each Table, provide a key with the abbreviations used in that Table, followed by its meaning, for example: "ECG, electro-cardiogram; MMF, mycophenolate mofetil."

SUPPLEMENTAL MATERIAL

Supplemental materials, including appendices, supplemental tables and figures, and supplemental videos, are not allowed and are not published or linked to your article after publication. If the supplemental materials are only included for peer review purposes, please delete them before final submission, as well as their mention in the text. Supplemental tables and figures can be converted into regular tables and figures. However, please avoid supplemental materials, in general.

FIGURES

Number Figures as **Figure 1, Figure 2**, and refer to all of them in the text. Figures must be created with a computer program and submitted in their original formats. Legends and notes should be included in the article file and NOT in the Figure itself. Below each Figure, provide a key with the abbreviations used in that Figure, followed by its meaning, for example: "ECG, electrocardiogram; MMF, mycophenolate mofetil." Upload Figures separately from the text (Figure files should not be integrated with the text files).

Vector graphics exported from a drawing program should be stored in EPS format only.

Guidelines for Electronically Produced Figures/Illustrations

For production of the Figures/Illustrations of an accepted manuscript, the Baskent University production department requires electronically produced Figures to be saved in EPS (Encapsulated PostScript), PDF (Portable Document Format), or TIFF (Tag Image File Format) format. Most drawing programs (eg, Adobe Illustrator, Deneba Canvas, CorelDraw) have a save-as-EPS option in their Save dialog box. Other information about EPS can be found at the Adobe Web site.

Spreadsheet/Presentation Graphics: Most presentation programs (Excel, PowerPoint, and Freelance) produce data that cannot be stored in an EPS or TIFF format. Therefore, graphics produced by these programs cannot be used for reproduction.

Halftone Illustrations: Black and white and color Figures and Illustrations should be saved in EPS or TIFF format. Figures and Illustrations should be created using Adobe Photoshop whenever possible.

Scans: Scanned reproductions of black and white photographs should be provided as 300 ppi EPS or TIFF files. Scanned color Illustrations should be provided as EPS or TIFF files scanned at a minimum of 300 ppi with a 24-bit color depth.Line art should be provided as EPS or TIFF files at 600 ppi.

Figure Legends

The Figure legends should be typed in double-spaced format on a separate page following any Tables, with each Figure numbered and listed in the order it appears in the text. For Figures with multiple parts, each part must be identified by a letter following the Figure number (eg, 3A, 3B, 3C) and be clearly described in the legend. Any letter designations or arrows that appear in the Figure should be described fully. If any previously published Figure material is used, authors must obtain written permission from the publisher to reprint. The source of the original material must be cited in the reference list and in the Figure legend.

All permission releases must be submitted to the Editor at the time of manuscript submission.

GALLEY PROOFS

Proofs will be sent to the author (corresponding /first-named) by PDF for final proof reading and appropriate corrections and should be returned via Manuscript Central within 48 hours of receipt. Major changes such as deleting, shortening, amending sentences, Figures and Tables in the manuscript are not acceptable. Only minor corrections (eg, typesetting errors) will be considered without extra cost.

OFFPRINTS

Orders for offprints may be placed in the appropriate form accompanying the corrected proofs, or by contacting the Editorial Office

Middle East Society
for Organ Transplantation

MEMBERSHIP APPLICATION FORM

APPLICANT INFORMATION

LAST NAME

FIRST NAME

☐ MALE ☐ FEMALE DATE OF BIRTH / /

PLACE OF BIRTH

NATIONALITY

☐ MD ☐ PHD ☐ OTHER (SPECIFY)

PRESENT AFFILATION

MAILING ADDRESS

TEL FAX

E-MAIL

PRINCIPAL AREA(S) OF INTEREST

- ☐ KIDNEY TRANSPLANTATION
- ☐ LIVER TRANSPLANTATION
- ☐ HEART-LUNG TRANSPLANTATION
- ☐ VISCERAL TRANSPLANTATION
- ☐ BONE MARROW TRANSPLANTATION
- ☐ PEDIATRIC TRANSPLANTATION
- ☐ IMMUNOLOGY
- ☐ OTHER (PLEASE SPECIFY)

METHOD OF PAYMENT

☐ **CREDIT CARD**

☐ AMERICAN EXPRESS ☐ VISA ☐ MASTERCARD

CARD NUMBER

EXPIRES CVC NUMBER

AMOUNT $

NAME OF CARD HOLDER

SIGNATURE OF CARD HOLDER

DATE / /

☐ **WIRE TRANSFER**

- As a reference please state the name of the applicant when making the transfer.
- Please be aware that MESOT will not cover any charges incurred by the transfer and that all extra costs are the responsibility of the applicant.

ACCOUNT HOLDER	MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION
BANK NAME	DENIZBANK
BRANCH NAME/CODE	BASKENT KURUMSAL SUBE/3010
ACCOUNT NO	11356918
SWIFT NO	DENITRIS
IBAN NO	TR680013400001135691800001

MEMBERSHIP TYPE

☐ **REGULAR MEMBERS: $ 60 USD**
(Persons domicile in MESOT regions. Includes electronic access to ECT.)

☐ **ASSOCIATE MEMBERS: $ 50 USD**
(Persons domicile in countries other than the MESOT regions. Includes electronic access to ECT.)

☐ **CANDIDATE MEMBERS: $ 30 USD**
(Resident or trainees during the training period. Includes electronic access to ECT.)

☐ **SUPPORTING MEMBERS:**
(Please contact MESOT office for details on this category of membership.)

MEMBER SPONSORSHIP

FIRST SPONSOR

LAST NAME

FIRST NAME

SIGNATURE

MEMBER ID NUMBER

SECOND SPONSOR

LAST NAME

FIRST NAME

SIGNATURE

MEMBER ID NUMBER

Data Privacy
All information requested is strictly for the use of MESOT in compliance with international privacy protection legislation. By signing, I agree that MESOT may use this data to keep me fully informed of MESOT activities, and make my full contact details available to other MESOT members. By giving consent my mailing address may be made available to third parties deemed appropriate by the MESOT.

Please note:
- MESOT Membership is valid for the calendar year (January 1 -December 31).
- ECT issues will be sent to you via regular mail.
- Dues are payable in US dollars.

PLEASE MAIL OR FAX THE COMPLETED FORM TO

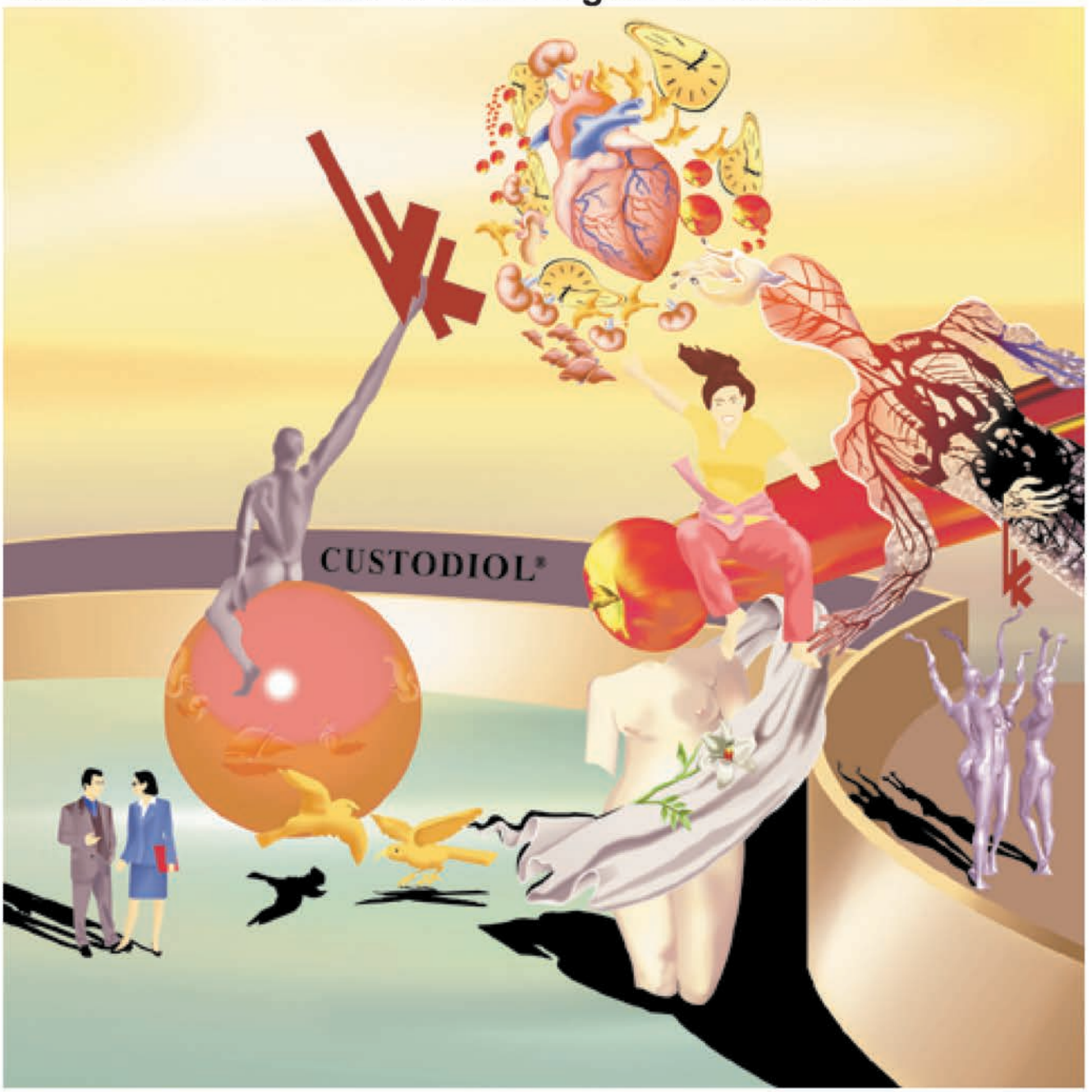
The Middle East Society for Organ Transplantation
Taskent Cad. No: 77, Bahcelievler 06490, Ankara - Turkey
Tel : +90 312 212 73 93 Fax : +90 312 215 08 35
Email: mesot@mesot-tx.org

YOU MAY ALSO APPLY FOR MEMBERSHIP ONLINE AT

www.mesot-tx.org

CUSTODIOL®

Your Solution for Multi-Organ-Protection



Composition: 1.000 ml of the solution contain: 0,8766 g sodium chloride (15,0 mmol), 0,6710 g potassium chloride (9,0 mmol), 0,8132 g magnesium chloride, 6 H_2O (4,0 mmol), 3,7733 g histidine hydrochloride monohydrate (18,0 mmol), 27,9289 histidine (180,0 mmol), 0,4085 g tryptophan (2,0 mmol), 5,4651 g mannitol (30,0 mmol), 0,0022 g calcium chloride, 2 H_2O (0,015 mmol), 0,1842 g potassium hydrogen 2-ketoglutarate (1,0 mmol). **Uses:** For cardioplegia during cardioplegic operations, organ protection during operations in bloodless organs (heart, kidney, liver), for preservation of organs for transplantation (heart, kidney, liver, lung, pancreas) and also for vein and artery transplants and for multiorgan protection. **Contraindications:** None known. **Dosage guidance, mode and duration of use:** Please see detailed instructions for use and specialist information. **Warning:** Custodiol® is not intended for intravenous or intra-arterial administration, but only for selective perfusion of the relevant organs, though also for surface cooling and preservation of the donor organ en route from donor to recipient. Custodiol® must therefore not be used for systemic infusion. **Presentation and pack sizes:** Bottles of 100 ml, 500 ml and 1.000 ml, bags of 5.000 ml. As at: 01/02.

Dr. F. Köhler Chemie GmbH D-64665 Alsbach-Hähnlein www.koehler-chemie.de

PAG

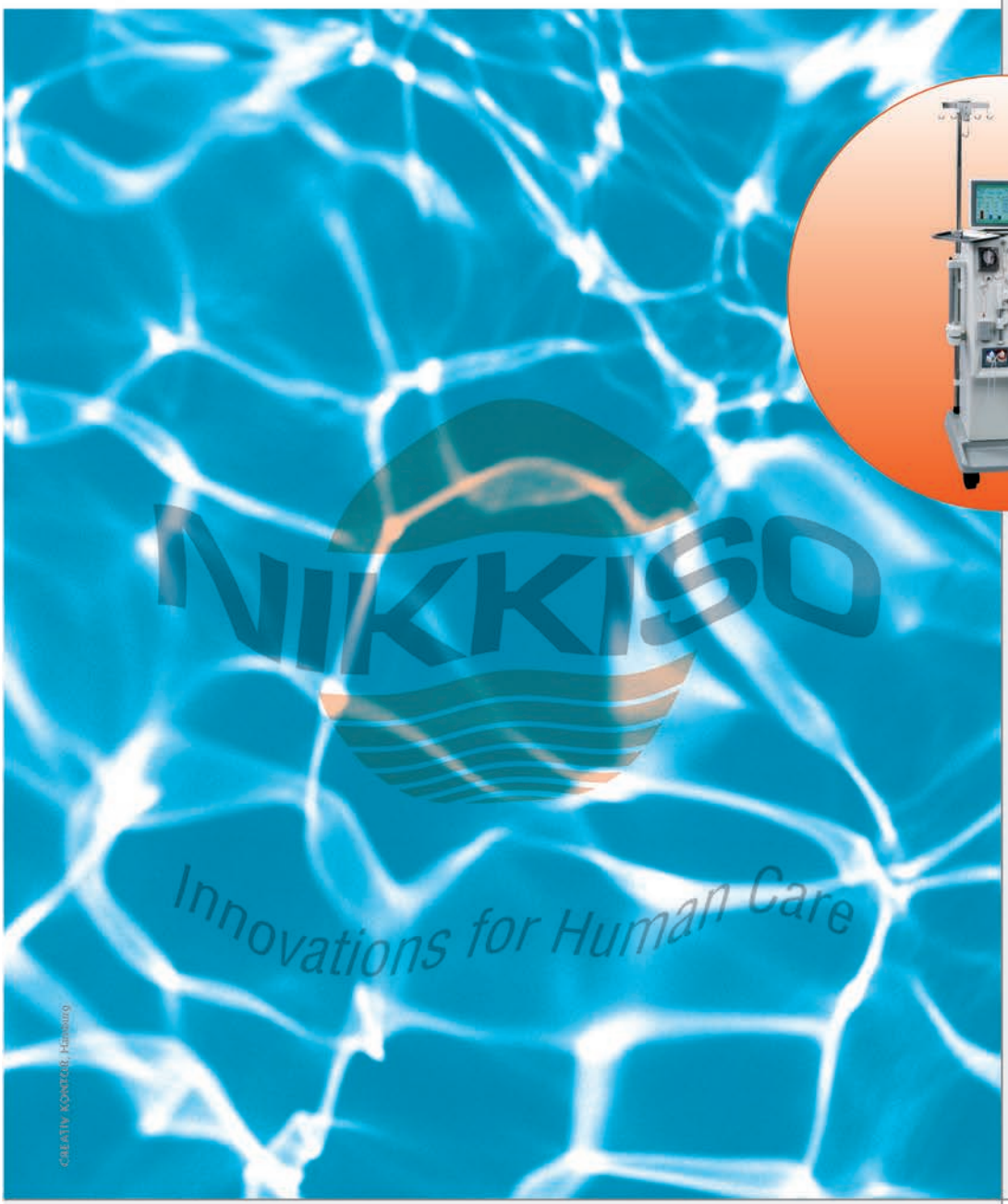
Local Distributor in Turkey: PAG LTD.
11. Sokak No: 40, 06490 Bahçelievler Ankara
Tel: +90 312 - 221 23 26 Fax: +90 312 - 212 49 67
E-mail: baskent@ada.net.tr internet: www.baskentholding.com.tr

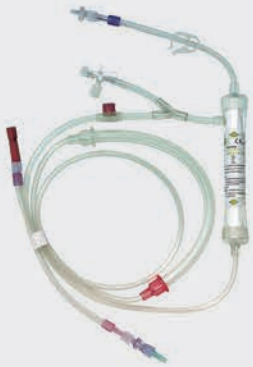
DR. F. KÖHLER CHEMIE

Arzneimittel

Nikkiso-Innovations for Human Care

FLY-series with PEPA®
(Polyester-Polymer-Alloy)





NIKKISO-Medical GmbH
Brödermannsweg 1 · D-22453 Hamburg · Telefon: +49-40-41 46 29-0 · Fax: +49-40-41 46 29 49

Local Distributor in Turkey:
VAKSAN A.S.
11. Sokak 40/6 · 06490 Bahcelievler · Ankara-TURKEY
Tel: + 90- 312 -2212326 · Fax: + 90- 312 -2120523 · e mail:baskent@ada.net.tr

WWW.NIKKISO-MEDICAL.COM

NIKKISO

Innovations for Human Care

NIKKISO Dialysis Systems

CE 0123

DBB-05

DBB-07

DBB-06

Nikkart®
Bicarbonate cartridges

PEPA® Dialyzers
FD-Series

Blood tubing lines
AV-Series

EU representative:
NIKKISO Europe GmbH
Desbrocksriede 1
30855 Langenhagen
Germany

Phone: +49 (0) 511 - 67 9999 - 0
Fax: +49 (0) 511 - 67 9999 - 11
E-Mail: info@nikkiso-europe.eu
Internet: www.nikkiso-europe.eu

Local Distributor in Turkey:
Vaksan A.S.
11. Sokak No. 40/12
06490 Bahcelievler-Ankara
Turkey

Phone: +90 - 312 - 212 15 14
+90 - 312 - 212 51 63
Fax: +90 - 312 - 212 05 23
E-Mail: baskent@ada.net.tr
Internet: www.baskentholding.com.tr

BAŞKENT UNIVERSITY
1993

GRADUATE PROGRAMS

PhD PROGRAMS

Institute of Educational Sciences
Department of Educational Sciences
Ph.D. on Education Administration

Department of Mathematics And Science Education
Ph.D.on Primary School Mathematics Education

Department of Turkish And Social Sciences Education
Ph.D. on Turkish Language Education
Institute of European Union And International Relations

Department of Political Science And International Relations
Ph.D. on Political Science (Based On A Bachelor'S Degree)
Ph.D. on Political Science (Based On A Master Degree)

Institute of Health Sciences
Department of Anatomy
Ph.D. on Anatomy And Clinical Anatomy

Department of Medical Biology
Ph.D. on Medical Biology

Department of Medical Genetics
Ph.D. on Medical Genetics

Department of Medical Microbiology
Ph.D. on Medical Microbiology

Department of Nursing
Ph.D. on Nursing

Department of Nutrition And Dietetics
Ph.D. on Nutrition And Dietetics

Department of Oral And Maxillofacial Surgery
Ph.D.on Oral And Maxillofacial Surgery

Department of Orthodontics
Ph.D. on Orthodontics

Department of Otorhinolaryngology
Ph.D. on Audiology

Department of Pediatric Dentistry
Ph.D. on Pediatric Dentistry

Department of Periodontology
Ph.D. on Periodontology

Department of Pharmacology
Ph.D. on Pharmacology

Department of Physiology
Ph.D. on Physiology

Department of Physiotherapy And Rehabilitation
Department of Prosthodontics
Ph.D. on Prosthodontics

Department of Public Health
Ph.D. on Public Health

Department of Treatment Of Dental Diseases
Ph.D. on Treatment of Dental Diseases And Endodontics
Institute of Science

Department of Biomedical Engineering
Ph.D on Biomedical Engineering (Based On A Bachelor'S Degree)
Ph.D.on Biomedical Engineering

Department of Computer Engineering
Ph.D.on Computer Engineering
Ph.D.on Computer Engineering (Based On A Bachelor'S Degree)

Department of Defence Technologies And Systems (Interdisciplinary)
Ph.D.on Defence Technologies And Systems
Ph.D.on Defence Technologies And Systems (Based On A Bachelor'S Degree)

Department of Electrical And Electronics Engineering
Ph.D.on Electrical And Electronics Engineering
Ph.D.on Electrical And Electronics Engineering (Based On A Bachelor'S Degree)

Department of Industrial Engineering
Ph.D.on Industrial Engineering
Ph.D.on Industrial Engineering (Based On A Bachelor'S Degree)

Department of Mechanical Engineering
Ph.D.on Mechanical Engineering
Ph.D.on Mechanical Engineering (Based On A Bachelor'S Degree)

Institute of Social Sciences
Department of Banking And Finance
Ph.D. on Banking And Finance

Department of Business Administration
Ph.D. on Accounting And Finance
Ph.D. on Business Administration
Ph.D. on Management And Organization

Department of Civil Law
Ph.D. on Civil Law

Department of Interior Architecture And Environmental Design
Ph.D. on Interior Architecture And Environmental Design

Department of Radio, Television And Cinema
Ph.D. on Radio, Television And Cinema

Department of Sociology
Ph.D on Sociology
Performing Arts Art Major

M.A PROGRAMS

Institute of Educational Sciences

Department of Computer And Instructional Technologies
Master Program of Computer And Instructional Technologies Education With Thesis
Department of Educational Sciences
Master Program of Curriculum And Instruction With Thesis
Master Program of Curriculum And Instruction Without Thesis
Master Program of Education Administration With Thesis
Master Program of Education Administration Without Thesis
Master Program of Guidance And Psychological Counselling With Thesis
Department of Foreign Languages
Master Program of English Language Teaching With Thesis
Department of Mathematics And Science Education
Master Program of Mathematics Education With Thesis
Master Program of Primary School Mathematics Education With Thesis
Department of Primary Education
Master in Pre-School Education With Thesis
Department of Turkish And Social Sciences Education
Master Program of Teaching Turkish As A Foreign Language With Thesis
Master Program of Turkish Language Education With Thesis

Institute of European Union And International Relations

Department of European Union
Master Program of European Union With Thesis
Master Program of European Union Without Thesis
Department of Political Science And International Relations
Master Program of International Relations With Thesis
Master Program of International Relations Without Thesis
Institute of Food, Agriculture And Livestock Development
Department of Sustainable Agriculture And Food Systems (Interdisciplinary)
Master Program of Sustainable Agriculture And Food Systems With Thesis

Institute of Health Sciences

Department of Anatomy
Master Program of Anatomy With Thesis
Department of Medical Biology
Master Program of Medical Biology With Thesis
Master Program of Stem Cell And Regenerative Medicine With Thesis
Master Program of Stem Cell And Regenerative Medicine Without Thesis
Department of Medical Genetics
Master Program of Medical Genetics With Thesis
Department of Medical Microbiology
Master Program of Medical Microbiology With Thesis
Department of Nursing
Master Program of Nursing With Thesis
Department of Nutrition And Dietetics
Master Program of Nutrition And Dietetics With Thesis
Department of Otorhinolaryngology
Master Program of Audiology And Speech-Voice Disorders With Thesis
Master Program of Audiology With Thesis
Department of Pharmacology
Master Program of Pharmacology With Thesis
Department of Physiology
Master Program of Physiology With Thesis
Department of Physiotherapy And Rehabilitation
Master Program of Physiotherapy And Rehabilitation With Thesis
Department of Public Health
Master Program of Public Health With Thesis
Master Program of Public Health Without Thesis
Department of Sport Sciences

Master Program of Exercise And Sportive Performance With Thesis
Master Program of Exercise And Sportive Performance Without Thesis
Institute of Science
Department of Architecture
Master Program of Architecture With Thesis
Department of Biomedical Engineering
Master Program of Biomedical Engineering With Thesis
Master Program of Biomedical Engineering Without Thesis
Department of Biotechnology (Interdisciplinary)
Master Program of Biotechnology With Thesis
Department of Computer Engineering
Master Program of Computer Engineering With Thesis
Master Program of Computer Engineering Without Thesis
Department of Defence Technologies And Systems (Interdisciplinary)
Master Program of Defence Electronics And Software With Thesis
Master Program of Defence Platforms With Thesis
Department of Electrical And Electronics Engineering
Master on Electrical And Electronics Engineering With Thesis
Department of Energy Engineering (Interdisciplinary)
Master Program of Energy Engineering With Thesis
Master Program of Energy Engineering Without Thesis
Department of Industrial Engineering
Master Program of Engineering And Technology Management With Thesis
Master Program of Engineering And Technology Management Without Thesis
Master Program of Industrial Engineering With Thesis
Department of Mechanical Engineering
Master Program of Mechanical Engineering With Thesis
Master Program of Mechanical Engineering Without Thesis
Department of Molecular Biology And Genetics
Master Program of Molecular Biology And Genetics With Thesis (English)
Department of Occupational Health And Safety (Interdisciplinary)
Master Program of Occupational Health And Safety With Thesis
Master Program of Occupational Health And Safety Without Thesis
Department of Quality Engineering (Interdisciplinary)
Master Program of Quality Engineering With Thesis
Master Program of Quality Engineering Without Thesis
Department of Statistics And Computer Science
Master Program of Information Technology And System Management With Thesis
Master Program of Information Technology And System Management Without Thesis

Institute of Social Sciences

Department of Accounting And Financial Management
Master Program of International Financial Reporting And Auditing With Thesis
Master Program of International Financial Reporting And Auditing Without Thesis
Department of American Culture And Literature
Master Program of American Culture And Literature With Thesis
Master Program of American Culture And Literature Without Thesis
Department of Art History And Museology
Master Program of Museology With Thesis
Master Program of Museology Without Thesis
Department of Banking And Finance
Master Program of Banking And Finance With Thesis
Master Program of Banking And Finance Without Thesis
Master Program of Capital Markets With Thesis
Master Program of Capital Markets Without Thesis
Department of Business Administration
Master Program of Accounting And Finance Without Thesis
Master Program of Accounting And Finance With Thesis
Master Program of Business Administration With Thesis
Master Program of Business Administration Without Thesis
Master Program of Marketing With Thesis
Master Program of Marketing Without Thesis
Program of Executive Mba Without Thesis
Department of Civil Law
Master Program of Civil Law With Thesis
Master Program of Medical Law Without Thesis
Department of Economics
Master in Economics Without Thesis
Master Program of Economics With Thesis
Department of Fashion And Textile Design
Master Program of Fashion Design With Thesis
Master Program of Fashion Design Without Thesis
Department of Financial Law
Master Program of Economy Law Without Thesis
Department of Gastronomy And Culinary Arts
Master Program of Gastronomy And Culinary Arts With Thesis
Master Program of Gastronomy And Culinary Arts Without Thesis
Department of Healthcare Management
Master Program of Healthcare Management With Thesis
Master Program of Healthcare Management Without Thesis
Department of Insurance And Risk Management
Master Program of Insurance And Risk Management With Thesis
Master Program of Insurance And Risk Management Without Thesis
Department of Interior Architecture And Environmental Design
Master Program of Interior Architecture And Environmental Design With Thesis
Master Program of Interior Architecture And Environmental Design Without Thesis
Department of International Trade
Master Program of International Trade And Marketing With Thesis
Master Program of International Trade And Marketing Without Thesis
Department of Management Information Systems
Master Program of Human Resources Management With Thesis
Master Program of Human Resources Management Without Thesis
Master Program of Management Information Systems With Thesis
Master Program of Management Information Systems Without Thesis
Department of Music And Performing Arts
Master Program of Composition With Thesis
Master Program of Musicology With Thesis
Master Program of Performance Without Thesis
Department of Psychology
Master Program of Clinical Psychology With Thesis
Master Program of Psychology With Thesis
Master Program of Social Psychology With Thesis
Department of Public Law
Master Program of Public Law With Thesis
Department of Public Relations And Publicity
Master Program of Public Relations And Publicity With Thesis
Master Program of Public Relations And Publicity Without Thesis
Department of Radio, Television And Cinema
Master Program of Radio, Television And Cinema With Thesis
Master Program of Radio, Television And Cinema Without Thesis
Department of Social Work
Master Program of Social Work With Thesis
Master Program of Social Work Without Thesis
Department of Sociology
Master Program of Sociology With Thesis
Master Program of Sociology Without Thesis
Department of Technology And Knowledge Management
Master Program of Technology And Knowledge Management With Thesis
Master Program of Technology And Knowledge Management Without Thesis
Department of Turkish Language And Literature
Master Program of Turkish Language And Literature With Thesis
Music Art Major
Master Program of Performance With Thesis
Performing Arts Art Major
Master Program of Performance With Thesis

DISTANCE EDUCATION

Institute of Educational Sciences
Department of Computer And Instructional Technologies
Master Program of Computer And Instructional Technologies Education Without Thesis

Department of Educational Sciences
Master Program of Education Administration

Institute of Science
Master Program of Informatics Systems Without Thesis

Institute of Social Sciences
Department of Banking And Finance
Master Program of Banking And Finance Without Thesis
Master Program of Capital Markets Without Thesis

Department of Business Administration
Master Program of Business Administration Without Thesis

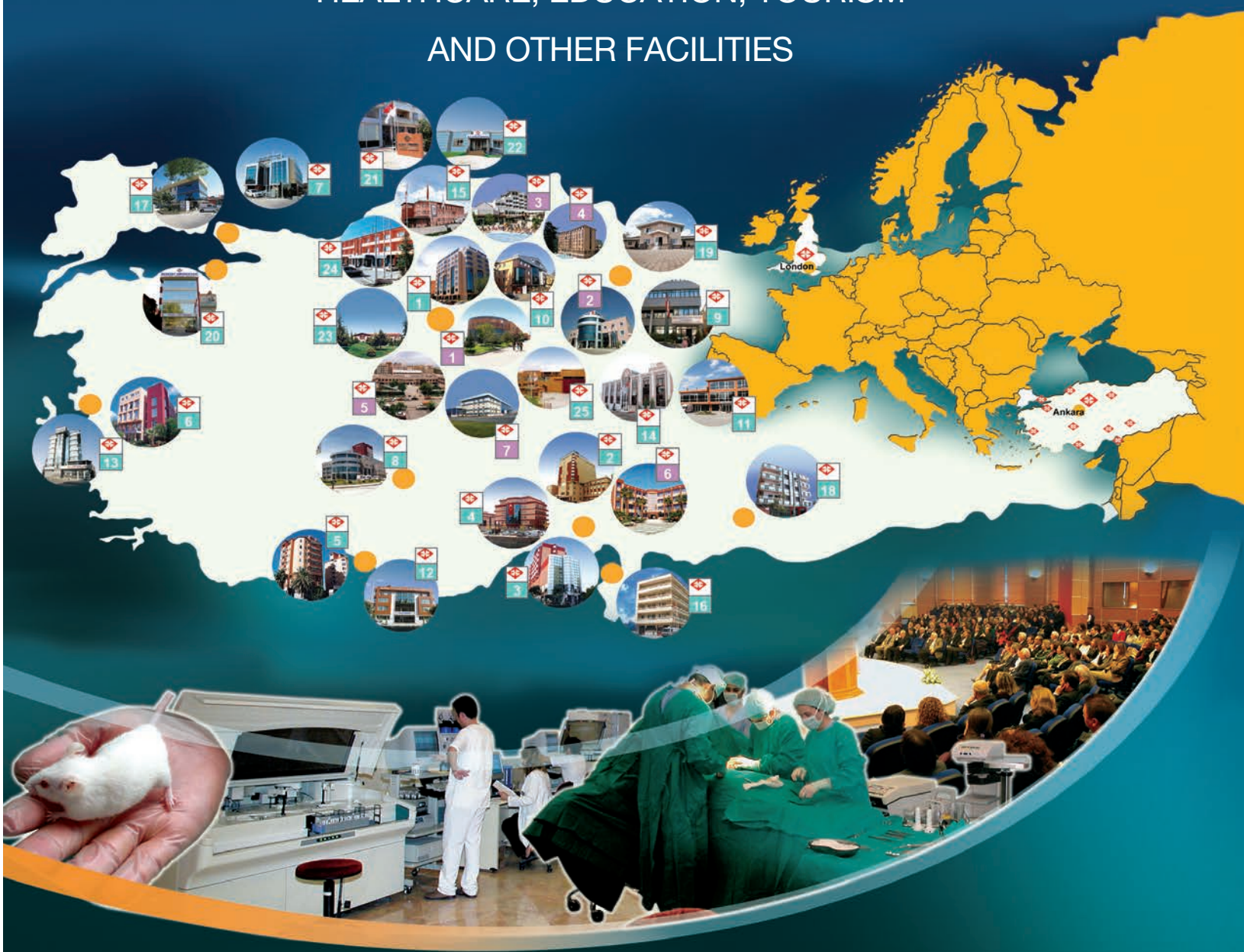
Department of Healthcare Management
Master Program of Healthcare Management Without Thesis

Department of Insurance And Risk Management
Master Program of Insurance And Risk Management Without Thesis

BAŞKENT UNIVERSITY

1993

HEALTHCARE, EDUCATION, TOURISM AND OTHER FACILITIES



1. BAŞKENT UNIVERSITY CAMPUS
2. CHANNEL B
3. PATALYA LAKESIDE HOTEL
4. PATALYA THERMAL RESORT HOTEL
5. ANKARA AYŞEABLA SCHOOLS
6. ADANA BAŞKENT PRIVATE SCHOOLS
7. AÇKAR DAIRY PRODUCTS

1. BAŞKENT UNIVERSITY ANKARA HOSPITAL
2. ADANA DR. TURGUT NOYAN YÜREĞİR TEACHING & MEDICAL RESEARCH CENTER
3. ADANA DR. TURGUT NOYAN SEYHAN TEACHING & MEDICAL RESEARCH CENTER
4. ADANA DR. TURGUT NOYAN KIŞLA TEACHING & MEDICAL RESEARCH CENTER
5. ALANYA TEACHING & MEDICAL RESEARCH CENTER
6. İZMİR ZÜBEYDE HANIM TEACHING & MEDICAL RESEARCH CENTER
7. İSTANBUL TEACHING & MEDICAL RESEARCH CENTER
8. KONYA TEACHING & MEDICAL RESEARCH CENTER
9. ANKARA MALTEPE WOMEN'S AND CHILDREN'S HEALTH CENTER
10. ANKARA HOSPITAL OUTPATIENT CLINICS
11. ANKARA ÜMİTKÖY OUTPATIENT CLINICS
12. ALANYA OBA OUTPATIENT CLINICS
13. İZMİR ÇİĞLİ OUTPATIENT CLINICS
14. ANKARA ÜMİTKÖY DIALYSIS CENTER
15. ANKARA YENİKENT DIALYSIS CENTER
16. ANKARA ÇİĞDEM DIALYSIS CENTER
17. İSKENDERUN DIALYSIS CENTER
18. İSTANBUL DIALYSIS CENTER
19. ŞANLIURFA DIALYSIS CENTER
20. TOKAT ZİLE DIALYSIS CENTER
21. YALOVA DIALYSIS CENTER
22. ZONGULDAK DIALYSIS CENTER
23. ZONGULDAK KARADENİZ EREĞLİ DIALYSIS CENTER
24. YAPRACIK PSYCHOSOCIAL REHABILITATION CENTER
25. AYAŞ PHYSICAL THERAPY & REHABILITATION CENTER
26. BASKENT GROUP HEALTH PRODUCTS TRADE and INDUSTRY INC.

www.baskent.edu.tr