| Volume: 23 | Issue: 8 | August 2025

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

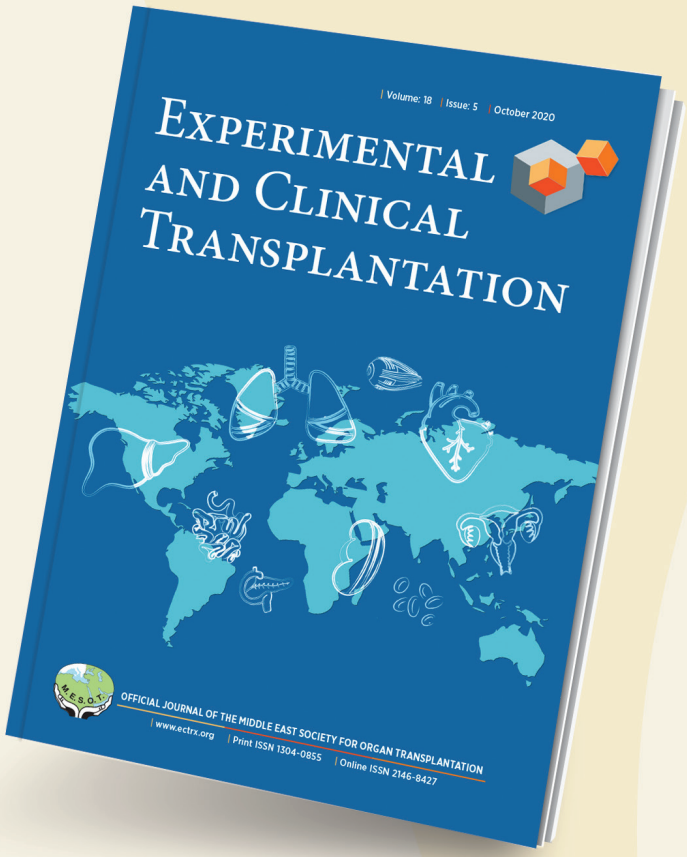
now 12 issues per year

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

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 28 August 2025

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.

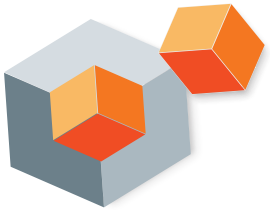
Experimental and Clinical Transplantation adheres to the ethical principles outlined by COPE (Committee on Publication Ethics).

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

19th CONGRESS OF
THE MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION
MESOT 2025

ANKARA TÜRKİYE NOVEMBER 4-5-6-7

KIZILCAHAMAM **PATALYA THERMAL RESORT HOTEL**

3 NOVEMBER 1975-2025 — 50TH Anniversary OF ORGAN TRANSPLANTATION IN TÜRKİYE

Workshop
November 4, 2025

1st MESOT Pre-Congress Workshop:
Brain and Circulatory Death, Life Donation and Transplantation 2025

WORKSHOP PRESIDENT: Prof. Mehmet Haberal
COURSE CHAIRS: Dr. Marcelo Cantarovich and Dr. Andrea Herrera-Gayol

INTERNATIONAL FACULTY

- Tayyar Altıkulaç · Ali Bardakoğlu · Mehmet Nuri Yılmaz
  Former Heads of Religious Affairs, Türkiye

- Mustafa Al Mousawi *Kuwait*
- Ali Al Obaidli *UAE*
- Brian Alvarez *Spain*
- Alp Aydınalp *Türkiye*
- Sibel Benli *Türkiye*
- Marcelo Cantarovich *Canada*
- Jeremy Chapman *Australia*
- Gabriela Chullo *Spain*
- Mohamed Salah Eldin Zaki *Egypt*
- Riyadh Fadhil *Qatar*
- John Fung *USA*
- Mohammad Ghnaimat *Jordan*
- Nadey Hakim *UK*
- Seyed Ali Malek-Hosseini *Iran*
- Marti Manyalich *Spain*
- Marwan Masri *Lebanon*
- Marco Sacchi *Italy*
- Bassam Saeed *Syria*
- Faissal A. M. Shaheen *KSA*
- Annika Tibell *Sweden*
- Stefan G. Tullius *USA*
- Andreas G. Tzakis *USA/Greece*
- Pınar Zeyneloğlu *Türkiye*

WORKSHOP WILL BE FOLLOWED BY THE **OPENING CEREMONY**

FOR PROGRAM DETAILS AND REGISTRATION: Please visit official Congress website at **www.mesot2025.com**

DATE: November 4, 2025 → **TIME:** 08:30-17:15

VENUE: **BASKENT UNIVERSITY CAMPUS,** İHSAN DOĞRAMACI MEETING HALL

19th CONGRESS OF

THE MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION

MESOT 2025

ANKARA TÜRKİYE NOVEMBER 4-5-6-7

www.mesot2025.com

3 NOVEMBER 1975-2025 — 50TH Anniversary OF ORGAN TRANSPLANTATION IN TÜRKİYE

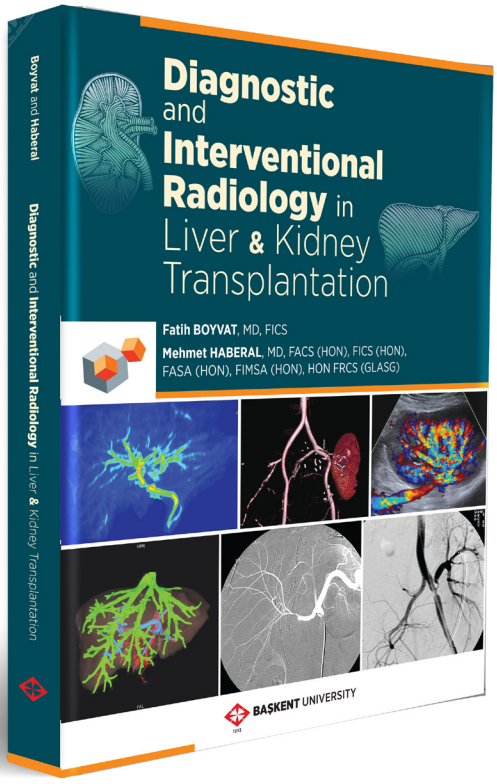
MESOT 2025 INTERNATIONAL FACULTY

- Mustafa Al-Mousawi *Kuwait*
- Mona AlRukhaimi *UAE*
- Hassan Argani *Iran*
- Antoine Barbari *Lebanon*
- Marcelo Cantarovich *Canada*
- Jeremy Chapman *Australia*
- Bernard Cohen *The Netherlands*
- Aziz El Matri *Tunisia*
- Ahmed Elsabbagh *USA*
- Riadh Fadhil *Qatar*
- Peter Friend *UK*
- John Fung *USA*
- Gabriel E. Gondolesi *Argentina*
- Nadey Hakim *UK*
- Andreas Herrera-Gayol *Canada*
- Refaat Kamel *Egypt*
- Seyed Ali Malek-Hosseini *Iran*
- Marti Manyalich *Spain*
- Marwan Masri *Lebanon*
- Muhammad M. Mohiuddin *USA*
- Nabil Mohsin *Oman*
- Anwar Naqvi *Pakistan*
- Axel Rahmel *Germany*
- Adibul H. Rizvi *Pakistan*
- Carolina Rumbo *Argentina*
- Bassam Saeed *Syria*
- Faissal A. M. Shaheen *KSA*
- Mohammed Faisal Shaheen *KSA*
- Nasser Simforoosh *Iran*
- Caner Süsal *Türkiye*
- Akın Tekin *USA*
- Annika Tibell *Sweden*
- George Tsoulfas *Greece*
- Stefan G. Tullius *USA*
- Andreas G. Tzakis *USA/Greece*
- Mohamed Salah Eldin Zaki *Egypt*

THE 19TH CONGRESS OF
ASIAN SOCIETY OF TRANSPLANTATION

26TH - 29TH NOV 2025, GRAND HYATT, KUWAIT

www.cast2025.org



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

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

International Center for Transplant Ethics

We are proud to announce the establishment of the International Center for Transplant Ethics under the aegis of the World Academy of Medical, Biomedical and Ethical Sciences at Başkent University

The center's mission is:

- to provide leadership in ethical activities and policy
- to promote ethical activities in transplantation
- to introduce ethically sound procurement policies and practice in order to prevent exploitation of individuals as organ providers based on human dignity and human rights.

AMERICAN COLLEGE OF SURGEONS

AMERICAN COLLEGE OF SURGEONS

Inspiring Quality: Highest Standards, Better Outcomes

I'm a general surgeon. Why should I join the ACS?

The College's top priority is providing value for its members. Become a member and take advantage of the following opportunities and resources for general surgeons.

Join the **LARGEST AND STRONGEST VOICE SUPPORTING GENERAL SURGEONS IN CONGRESS** and in front of important regulatory agencies by supporting the American College of Surgeons Professional Association Political Action Committee (ACSPA-*Surgeons*PAC).

Improve the quality of care at your hospital by joining an **ACS QUALITY PROGRAM** like the ACS National Surgical Quality Improvement Program® (ACS NSQIP®), the leading national program for a risk-adjusted, outcomes-based approach to improving surgical care.

Improve the care of the surgical patient by making sure your program is accredited by the **COMMISSION ON CANCER (CoC),** using education programs offered by Committee on Trauma (COT), and participating in the ACS clinical trials group in oncology.

Benefit from **OPPORTUNITIES FOR INCREASED COLLEGIALITY**, the ability to know and work with other surgeons and surgical specialties.

Use the **ACS SURGEON SPECIFIC REGISTRY (SSR)** to track your cases and outcomes in a convenient, easy-to-use way. This system allows ACS members to compare their outcomes with others in a confidential manner, supports Part 4 of Maintenance of Certification (MOC), and provides for electronic transmittal of cases to the American Board of Surgery for recertification.

Attend the **CLINICAL CONGRESS**, the College's premier annual meeting and the largest, most comprehensive surgeon-specific CME event in the world, and take advantage of the educational resources available. This five-day event offers high-quality educational programs designed to educate and train surgeons and improve the health and safety of surgical patients. Reduced registration is available for ACS members.

Benefit from **EDUCATION RESOURCES AND COURSES,** including the Surgical Education and Self-Assessment Program (SESAP), *Selected Readings in General Surgery (SRGS)*, and Fundamentals of Laparoscopic Surgery (FLS).

Apply for **SURGICAL RESEARCH OPPORTUNITIES AND TRAVEL SCHOLARSHIPS AND FELLOWSHIPS** available for Fellows, international surgeons, Associate Fellows, and Resident Members.

Access the **ACS CAREER CONNECTION** to find new opportunities in your surgical career. Use this free resource to post your resume and search for job opportunities in general surgery.

Attend the robust **ACS MEDICAL STUDENT PROGRAM,** an opportunity to meet College leaders and surgical educators at both the medical student and resident levels.

Participate in **OPERATION GIVING BACK,** the ACS volunteerism initiative and a comprehensive resource center, to find information and participate in volunteer opportunities.

Be included in the **FIND-A-SURGEON DATABASE** to help patients find you and share your profile and practice information with the public.

Become active in the **ACS AT A NATIONAL OR CHAPTER LEVEL** and gain the opportunity to work with a dedicated group of like-minded volunteers, especially on matters related to federal and state legislative advocacy.

Benefit from **LEADERSHIP TRAINING** and grow professionally by taking a leading role on College committees and in College Chapters.

Join the **ACS GENERAL COMMUNITY,** an exclusive online forum that allows members to share in online discussions, view online videos and full text articles, and share thoughts on difficult cases.

Participate in the **ACS TRANSITION TO PRACTICE PROGRAM**, which helps graduating residents transition into independent practice.

Promote **VOLUNTARY PHILANTHROPIC EFFORTS** by supporting the ACS Foundation.

The American College of Surgeons is the largest organization of surgeons in the world, uniquely positioned to lead the way in optimal patient care, surgical research, health policy, and continuing education and networking opportunities. It is the single strongest voice for surgeons in all specialties and, since its foundation in 1913, has been at the forefront of providing quality care for surgical patients and supporting surgeons wherever they practice.

BECOME AN ACS MEMBER TODAY!

Start taking advantage of the exclusive benefits available only to ACS members.

FACS indicates a commitment to quality and is well recognized by referring surgeons.

Scan the QR code or visit *www.facs.org/member-services/join* to fill out your membership application.

Prepared by the ACS Advisory Council for General Surgery

American College of Surgeons

Inspiring Quality:
Highest Standards, Better Outcomes

100+*years*

Experimental and Clinical Transplantation

Official Journal of the Middle East Society for Organ Transplantation

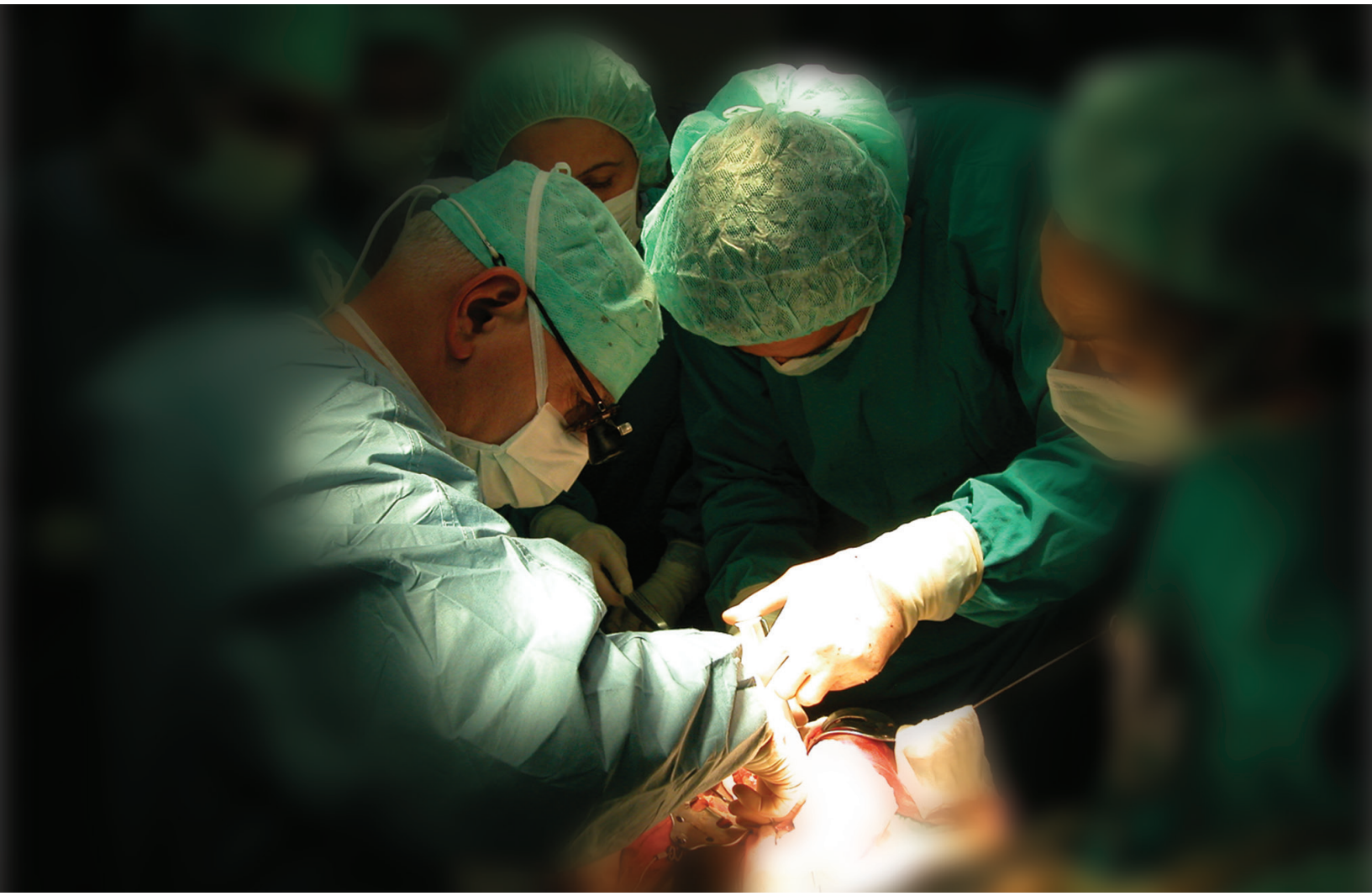
Volume: 23 Issue: 8 August 2025

Contents

BAŞKENT UNIVERSITY
FACULTY OF MEDICINE
DEPARTMENT OF GENERAL SURGERY

DIVISION OF
TRANSPLANTATION

Kidney & Liver Transplantation Observer & Fellowship Programs



www.baskent.edu.tr

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

Protection of the Endothelium and Endothelial Glycocalyx by Albumin and Sulodexide in Porcine Model of Kidney Transplant

Pavel Navratil,[1,2] Jiri Chalupnik,[2] David Rehak,[2] Diana Gorskaja,[3] Alena Ticha,[4] David Weishaupt,[5] Dana Cizkova,[5] Eva Cermakova,[6] Ales Bezrouk,[6] David Astapenko[2,7,8]

Abstract

Objectives: **Kidney transplant is a life-saving procedure for patients with end-stage renal disease. Success of kidney transplant is highly dependent on maintaining the integrity of the endothelium and its protective layer, the endothelial glycocalyx. Ischemia-reperfusion injury, a common challenge in kidney transplant, can disrupt the endothelial glycocalyx, leading to various post-transplant complications. We investigated the effects of albumin and sulodexide, 2 therapeutic agents, for protection of the endothelium and endothelial glycocalyx in a porcine model of kidney transplant.**

Materials and Methods: **Fourteen female piglets were prepared for kidney transplant simulation and randomly divided into 3 groups: a control group, an albumin-treated group, and a sulodexide-treated group. Various physiological parameters were monitored, and samples for serum and urine were collected at baseline and at multiple time points after reperfusion. Integrity of the endothelial glycocalyx was assessed from serum syndecan-1 levels and urinary glycosaminoglycan concentrations. Histology of the renal cortex allowed evaluation of tissue changes following the intervention.**

Results: **Statistically significant differences were observed in the sulodexide-treated group, where serum syndecan-1 levels were lower versus the control group at 5 minutes after reperfusion (*P* = .046), indicating a potential reduction in endothelial glycocalyx damage. Similarly, in the albumin-treated group, urinary glycosaminoglycan levels were significantly lower versus the control group at 5 minutes after reperfusion (*P* = .041), which may suggest a protective effect on the endothelial glycocalyx. However, these findings are preliminary, and no other significant differences were detected between the treatment groups and the control group at later time points. Histology of the renal cortex revealed that the changes were generally minor across all groups.**

Conclusions: **We suggest that albumin and sulodexide may offer beneficial effects in preserving endothelial function during kidney transplant. The potential for these agents to enhance graft survival and improve kidney transplant outcomes warrants further investigation.**

Key words: *End-stage renal disease, Ischemia-reperfusion injury, Kidney transplantation, Microcirculation*

Introduction

Compared with dialysis, kidney transplant (KT) is the preferred treatment for end-stage renal disease due to its potential to improve patient survival and quality of life.[1,2] The success of a transplant, however, is intricately linked to a myriad of physiological and pathophysiological processes, among which the integrity of the endothelium, as well as the associated endothelial glycocalyx (EG), plays a pivotal role. The EG, a dynamic and fragile layer lining the endothelium, is implicated in various renal pathologies, and its degradation can precipitate adverse outcomes after transplant.[3] It is thus impe-rative to understand and explore therapeutic strategies that could preserve or restore EG integrity during KT.

Ischemia-reperfusion injury (IRI) occurs when the blood supply to the organ is temporarily halted

From the [1]Department of Urology, University Hospital Hradec Kralove, Hradec Kralove, Czechia; the [2]Charles University, Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czechia; the [3]Department of Anesthesiology, Perioperative Medicine and Intensive Care, Bory Hospital Bratislava, Bratislava, Slovakia; the [4]Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Kralove, Hradec Kralove, Czechia; the [5]Department of Histology and Embryology, Faculty of Medicine in Hradec Kralove, Charles University, Hradec Kralove, Czechia; the [6]Department of Medical Biophysics, Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czechia; the [7]Department of Anesthesiology, Resuscitation, and Intensive Care Medicine, University Hospital Hradec Kralove, Hradec Kralove, Czechia; and the [8]Faculty of Health Studies, Technical University in Liberec, Liberec, Czechia

Acknowledgements: *This work was supported by MH CZ-DRO (UHHK, 00179906) and the Charles University in Prague COOPERATIO Program, research area SURG. 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: *Pavel Navratil, Department of Urology, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czechia*
Phone: *+420 495 833 378* ***E-mail:*** *pavel.navratil2@fnhk.cz*

Experimental and Clinical Transplantation (2025) 8: 509-516

DOI: 10.6002/ect.2024.0222

(ischemia) and subsequently restored (reperfusion), and IRI stands as a formidable challenge in the context of KT.[4] Reperfusion is essential to deliver oxygen to the deprived tissues; paradoxically, reperfusion can also instigate a cascade of inflammatory responses, oxidative stress, and cellular injury. This duality poses significant risks to the transplanted organ, with IRI being a predominant contributor to delayed graft function, acute rejection episodes, and even long-term graft loss.[5] Moreover, the endothelium and its protective EG layer are particularly susceptible to IRI, with disruptions in this layer serving as both a consequence and exacerbator of the injury.[6] Understanding the mechanisms of IRI and its effect on the endothelium and EG is crucial for development of strategies to mitigate the detrimental effects of IRI.

Albumin, a major plasma protein, is physiologically bound within EG; albumin protects against shedding and contributes to the maintenance of vascular integrity and healthy capillary permeability.[7] Furthermore, the capacity to modulate vascular permeability, maintain oncotic pressure, and scavenge reactive oxygen species positions albumin as a promising agent in the field of KT.[8]

Sulodexide is a refined blend of glycosaminoglycans consisting of dermatan sulfate and low-molecular-weight heparan sulfate. Sulodexide can adhere to endothelial cells and demonstrates both antithrombotic and profibrinolytic properties.[9] Given its structural resemblance to elements within EG, sulodexide is posited to supply precursors essential for EG restoration. Furthermore, contemporary research suggests that sulodexide not only aids in rejuvenation of endothelial function but also possesses anti-inflammatory capabilities that modulate the release of various cytokines and chemokines.[10,11]

This study delves into the protective effects of albumin and sulodexide on the endothelium and EG in a porcine model of KT, with corresponding comparisons to control conditions. By harnessing the potential of these agents, we aim to elucidate mechanisms that could optimize graft survival, enhance KT outcomes, and contribute to the longevity of the transplanted organ.

Materials and Methods

The experiment was carried out in the animal facility of the Faculty of Military Health in Hradec Kralove, the University of Defense in Brno, Czechia. The experimental protocol was approved by the Departmental Animal Protection Committee of the Ministry of Defense of Czechia (protocol No. 325312/2023-1457 from April 4, 2023). All treatments, handling, and care of experimental animals followed institutional guidelines and were performed in accordance with the Consensus Guidelines on Animal Ethics and Welfare established by the International Association of Veterinary Editors.

Animal preparation

Fourteen female piglets (*Sus scrofa f. domesticus*, sourced from Konarovice, Czechia) weighing 30.6 ± 3.1 kg received intramuscular premedication with azaperone (Stresnil 2 mg/kg; Sanochemia Pharmazeutika), atropine (Atropin Biotika 0.2 mg/kg; HBM Pharma), and ketamine (Calypsol 20 mg/kg; Gedeon Richter) 30 minutes before the protocol was started. After animals were shaved, we transported animals to the operating room. At this stage, there was an intact corneal reflex and motoric reaction against nociception to confirm an adequate level of anesthesia.

After arrival to the operating room, the animal was placed in the supine position, and a peripheral intravenous catheter (Vasofix Safety, 18 gauge; B. Braun Melsungen) was introduced through the ear vein, with sedation deepened into general anesthesia by propofol (1% propofol at 1 mg/kg body wt; Fresenius Kabi). The trachea was then intubated, and artificial ventilation was initiated and maintained with an anesthesia station machine (Cirrus Trans2/Vent 2; Datex) with the initial setup: 20 breaths/min, inspiration fraction of oxygen 0.40, and tidal volume 6 mL/kg body weight. The respiratory rate was changed to maintain the end-tidal concentration of carbon dioxide within the range of 4 to 5.7 kPa. General anesthesia was maintained with isoflurane (Forane 1.5%; AbbVie) in the fresh gas mixture. The intravenous fluid therapy was maintained by infusion of a balanced crystalloid solution (Plasma-Lyte; Baxter International) at room temperature with an infusion rate of 50 mL/h. Physiological functions including electrocardiogram, heart rate, and temperature were continuously monitored. For continuous blood pressure monitoring and blood sampling, an arterial catheter (Certofix Duo, 7 F, 200 mm; B. Braun Melsungen) was introduced through the common femoral artery by ultrasonography navigation.

Study groups

We randomly distributed the 14 pigs into 3 distinct groups: 5 pigs in study group 1 received albumin (20 g/animal, Alburex 20%; Behring), 5 pigs in study group 2 received sulodexide (600 IU/animal, Vessel Due F; AlfaSigma), and 4 pigs in the control group received a placebo treatment. The control group received an intravenous infusion of 100 mL of normal saline (Ringerfundin, B. Braun), administered in the same manner as the active substances. The group allocation of the animals was determined using the sealed envelope technique.

Procedure

After anesthesia induction, albumin (20 g in 100 mL; Alburex 20%; Behring) and sulodexide (600 IU diluted in 100 mL of normal saline; Vessel Due F; AlfaSigma) were administered intravenously. Laparotomy was performed with an incision from a point measured 1 cm caudal of the xiphoid to the pubis. Both kidneys were examined, and the kidney with preferable anatomy was chosen for the experiment, often due to gracile renal arteries or the presence of multiple renal veins. After intravenous administration of 1000 U of heparin (Heparin Leciva; Zentiva), the vessels of the contralateral kidney were clamped (nephrectomy), along with KT simulation of the ipsilateral kidney. The renal artery was secured with a tourniquet, subsequently incised, and then catheterized. Similarly, the vein was constricted medially with a tourniquet and incised. The ureter was severed distally to allow urine collection into designated tubes. Once secured, perfusion (500 mL for each kidney) via the renal artery was started with a preservation solution (Custodiol HTK; Essential Pharmaceuticals). After a thorough wash, the kidneys were cooled in situ with a preparation of chipped ice, which thereby simulated explant during multiorgan procurement and cold ischemia. After a 30-minute cold ischemia interval, the incisions on the artery and vein were closed with polypropylene sutures (Prolene 6-0; Ethicon) to reestablish the renal blood flow, which thereby simulated IRI.

Blood and urine samples were taken at baseline after midline incision and at postreperfusion minute 5, minute 30, minute 60, and minute 90. The pigs were euthanized by the end of the experiment with non-excitative intravenous euthanasia agent embutramide (T61; Intervet) with the dose according to the body weight and manufacturer's recommendation.

Biochemical analyses

We conducted baseline serum biochemical analyses, including sodium, potassium, chloride, urea, creatinine, and albumin, with a modular analyzer (Cobas 8000, Roche) and measured blood counts via an automated blood cell analyzer (model XN-10, Sysmex) and arterial venous blood gases with a blood-gas analyzer (Radiometer Medical ApS). Syndecan-1 is a biomarker of EG layer damage. To assess the integrity of the EG, serum syndecan-1 levels were measured at baseline and at predetermined time points up to 90 minutes after reperfusion using a porcine enzyme-linked immunosorbent assay kit (Woburn). We determined urinary glycosaminoglycan concentrations using the dimethyl methylene blue colorimetric assay, with measurements taken with a spectrophotometer (PharmaSpec model UV-1700; Shimadzu).

Histology and lectin histochemistry

Kidney samples were excised and immersed in 4% paraformaldehyde in phosphate-buffered saline for 24 hours at room temperature. After this fixation, the samples were either embedded in cryosection cutting compound (Tissue-Tek OCT; Sakura Finetek) and snap-frozen in liquid nitrogen-chilled isopentane (Sigma-Aldrich) or dehydrated and embedded in paraffin. Cryosections of 10-μm thickness were cut with a cryostat (Leica Biosystems), and paraffin sections were cut to a thickness of 5 μm. Every tenth slide in the series was stained with hematoxylin-eosin for histological examination of the tissue.

For lectin histochemical detection, thawed sections were incubated in a solution of *Lycopersicon esculentum* lectin conjugated with a high-intensity, photostable fluorescent tag (DyLight 594, 10 μg/ml; Vector Laboratories) for 30 minutes at room temperature. After thorough washing, we mounted the sections under coverslips by using fluorescence mounting medium (Dako) and examined with an epifluorescence microscope (Olympus model BX51) equipped with a digital camera (Olympus model DP71). To reveal autofluorescence, we also examined serial sections processed without incubation in the lectin solution.

For quantification of the lectin fluorescence signal in the renal glomeruli, we used imaging processing software (Fiji for ImageJ) for image analysis. Images of 548 glomeruli were converted to 8-bit gray scale. The glomeruli were always selected with the use of the polygon selection method. The mean gray value

(MGV) in the polygonal selection of the glomerulus on a scale from 0 to 255 was measured in each photo. The measurement results were tabulated and statistically processed.

Statistical analyses

We used NCSS statistical software (version 10; 2015 NCSS LLC) and data visualization software (Excel 2016; Microsoft) for data analyses. The normality of the data distribution was tested with the D'Agostino omnibus test. We presented data from normally distributed populations as mean values (with SD) and other data as median values (with first and third quartiles).

Parametric tests were used for data that followed a normal distribution. Specifically, a 1-way analysis of variance (ANOVA) was used to compare the means across different groups for assessment of statistically significant differences. We performed post hoc analysis by using the Tukey honestly significant difference test to identify the specific differing groups when the ANOVA results were significant. These parametric tests assume homogeneity of variances, which was confirmed using the Levene test. For comparisons involving only 2 groups, we used an independent samples *t* test to evaluate the difference in mean values, assuming equal variance.

To test differences among MGVs of different preparations of glomeruli with lectin detection, we used a nonparametric Kruskal-Wallis ANOVA, followed by post hoc Dunn tests with Bonferroni correction for multiple comparisons. Z values >3.8601 were considered statistically significant.

For the baseline characteristics at each time point, we reported median values with ranges (minimum-maximum). We conducted comparisons of outcomes across different time points between groups. We tested the null hypothesis of equivalence against the alternative that at least 2 groups differ. A nonparametric Kruskal-Wallis ANOVA was used followed by post hoc Dunn tests with the Bonferroni correction for multiple comparisons. $P < .05$ was considered statistically significant.

Results

Vital signs

Vital signs remained stable in all the animals throughout the experiment. The median mean arterial pressure ranged from 68 to 96 mm Hg. The lowest values were not below 50 mm Hg. None of the animals required any vasopressor. The median heart rate ranged from 68 to 121 beats/min. End-tidal carbon dioxide ranged from 4.9 to 6.6 mm Hg (Table 1).

Biochemical analyses

The results of the serum syndecan-1 measurements are presented in Table 2. Analyses of differences between the intervention groups versus the placebo showed the following variations. A significant difference was observed for the sulodexide group versus the placebo group at the first measurement after blood flow restoration (at 5 minutes; $P = .046$). At 30 minutes, the difference approached significance ($P = .059$; Figure 1). At 60 and 90 minutes, no difference was confirmed ($P = .373$ and $P = .770$, respectively). No significant differences were confirmed between the albumin groups and placebo groups at any measured time interval.

In addition to syndecan-1 serum levels, urinary glycosaminoglycan levels were measured (Table 3). For comparisons of individual groups versus the placebo group, a significant difference was found in the albumin group versus placebo group at the 5-minute mark ($P = .041$). Other outcomes in compa-risons of the albumin versus placebo groups and sulodexide versus placebo groups were not significant (Figure 2).

Table 1. Physiological Functions

Characteristic	Placebo Group (N = 4)	Albumin Group (N = 5)	Sulodexide Group (N = 5)
Blood pressure, mm Hg			
Systolic	90.3 ± 24.1	109.9 ± 19.6	100 ± 20.6
Diastolic	64.6 ± 16	67.7 ± 14.8	64.3 ± 15.9
Mean arterial	73.1 ± 18.4	81.7 ± 16.5	75.9 ± 16.7
Heart rate, beats/min	99.8 ± 12.5	96.4 ± 20	93.1 ± 12.6
SpO_2, %	99.9 ± 0.4	99.6 ± 0.9	99.8 ± 0.8
$EtCO_2$, kPa	4.7 ± 0.4	4.8 ± 0.5	4.8 ± 0.3
Respiratory rate, breaths/min	20 ± 1.8	21.2 ± 2.5	21.8 ± 2.9
Body temperature, °C	36 ± 1.1	36 ± 1.3	36.1 ± 1.2

Abbreviations: $EtCO_2$, end-tidal concentration of carbon dioxide; SpO_2, saturation of peripheral blood by oxygen

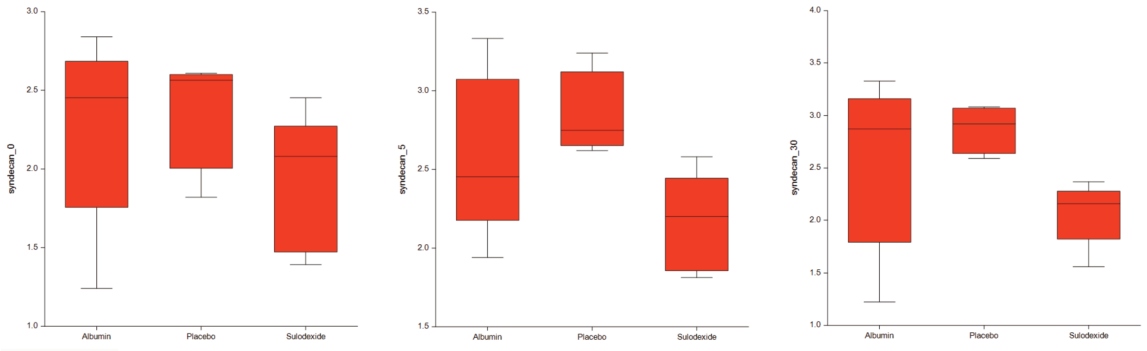
Results are shown as mean values ± SD. An isotonic electrolyte solution (Ringerfundin, B. Braun) was used as the placebo.

Table 2. Serum Syndecan-1 Levels Across Different Study Groups

Time After Reperfusion	Syndecan-1 Level, median (range), ng/mL		
	Placebo Group (N = 4)	Albumin Group (N = 5)	Sulodexide Group (N = 5)
Baseline	2.56 (1.82-2.61)	2.45 (1.24-2.84)	2.08 (1.39-2.45)
Minute 5	2.75 (2.62-3.24)	2.49 (1.94-3.33)	2.20 (1.81-2.58)
Minute 30	2.92 (2.59-3.08)	2.87 (1.22-3.30)	2.16 (1.56-2.37)
Minute 60	2.44 (2.14-3.40)	2.55 (1.76-3.34)	2.16 (1.54-3.01)
Minute 90	2.73 (1.42-3.05)	2.18 (2.09-3.40)	2.34 (1.94-2.95)

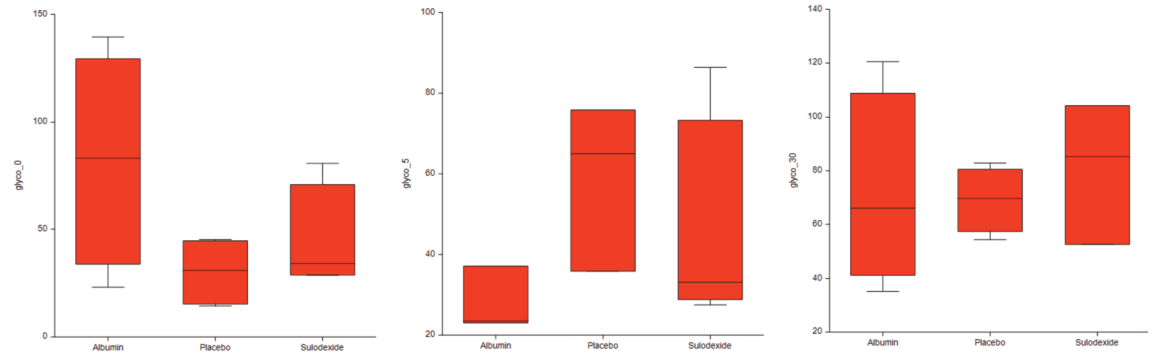
An isotonic electrolyte solution (Ringerfundin, B. Braun) was used as the placebo.

Figure 1. Syndecan-1 Serum Levels



Values for syndecan-1 (ng/mL) are shown at baseline (left), at 5 minutes after reperfusion (middle), and at 30 minutes after reperfusion (right).

Figure 2. Levels of Glycosaminoglycans in Urine



Values for glycosaminoglycan levels (ng/mL) are shown at baseline (left), at 5 minutes after reperfusion (middle), and at 30 minutes after reperfusion (right).

Table 3. Levels of Glycosaminoglycans in Urine Across Different Study Groups

Time After Reperfusion	Glycosaminoglycans in Urine, median (range), ng/mL		
	Placebo Group (N = 4)	Albumin Group (N = 5)	Sulodexide Group (N = 5)
Baseline	30.83 (14.47-45.30)	83.12 (23.00-139.39)	34.21 (28.86-80.58)
Minute 5	65.03 (35.82-75.90)	23.56 (23.01-70.80)	33.09 (27.46-86.44)
Minute 30	69.57 (54.39-82.87)	66.15 (35.21-120.66)	85.32 (52.55-104.16)
Minute 60	82.09 (81.86-96.30)	82.01 (67.43-95.58)	78.29 (58.18-110.68)
Minute 90	80.30 (76.90-83.71)	96.02 (42.34-96.58)	77.18 (39.78-86.66)

An isotonic electrolyte solution (Ringerfundin, B. Braun) was used as the placebo.

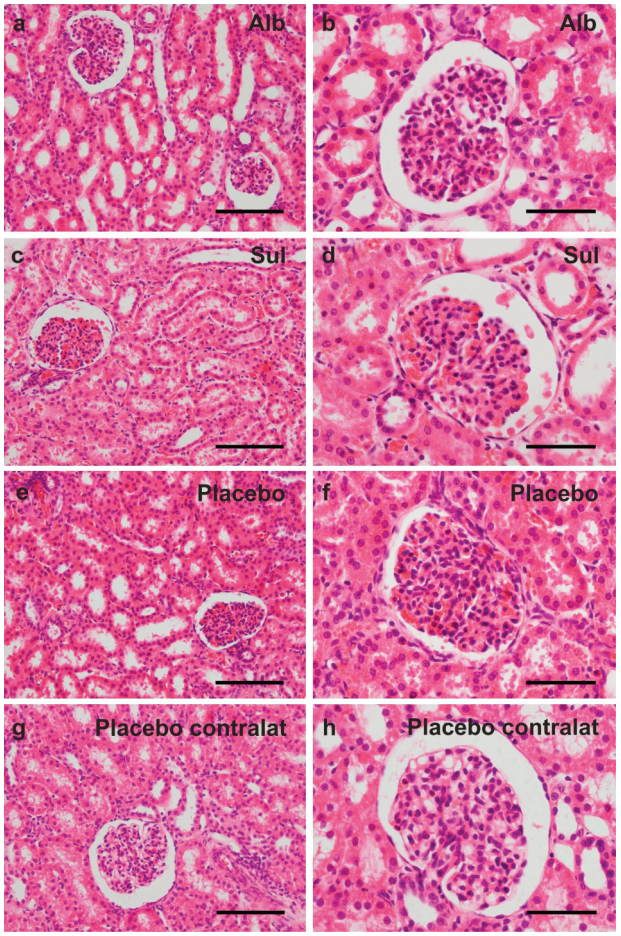
Histological examination of the renal cortex and lectin histochemistry

The histopathological changes in the renal cortex following 20 minutes of ischemia and subsequent 90 minutes after initiation of reperfusion were generally minor, although some signs were observed. The cytoplasm of the tubules in the renal cortex exhibited slightly increased eosinophilia after the administration of albumin (Figure 3a) and placebo in the operated kidney (Figure 3c), indicating a mildly higher degree of necrotic changes. Subtle dilation of the tubular lumen was also observed in these samples, in comparison with samples obtained after administration of sulodexide (Figure 3e) and placebo in the contralateral kidney (Figure 3g). In the renal corpuscles of the uninjured, contralateral kidney after placebo administration, the lumina of the glomerular fenestrated capillaries were well distinguishable and contained only sparse erythrocytes (Figure 3h). Conversely, in the operated kidney after placebo administration, many red blood cells aggregated in the glomerular capillaries (Figure 3f). Some erythrocyte accumulation was also observed within the lumina of the glomerular capillaries after the administration of albumin and sulodexide (Figure 3, b and d). In addition, some cytoplasmic debris was noted in the urinary space of the renal corpuscles, which was slightly more frequent after sulodexide administration (Figure 3d). However, definitive signs of IRI such as cell necrosis, leukocyte infiltration, or interstitial edema were not observed in our samples.

The lectin histochemical detection was performed at 2 separate observations in 2 days. The MGV of the 4 preparations from the first day varied around the

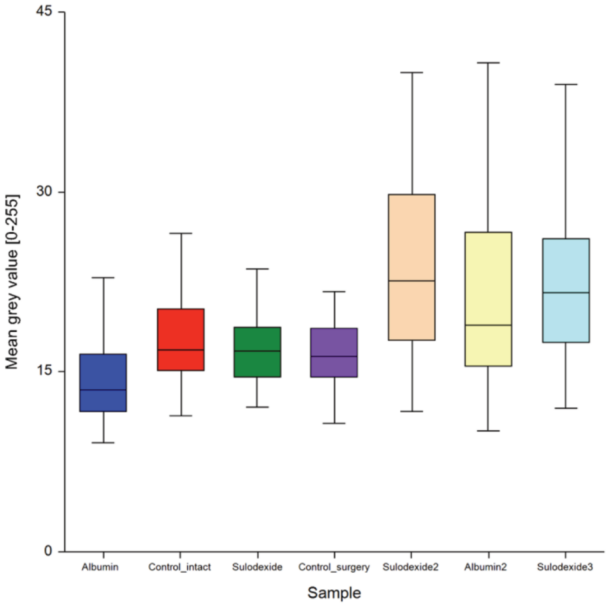
value of 15 (Figure 4). A significant difference was observed between the albumin preparation and the intact control preparation (Z = 4.33). The MGVs of the second and third sulodexide preparations from the second day were significantly different from all preparations from the first day. The statistical differences between the MGV observed on the first and the second day may be caused by certain inconsistencies in the sample preparations affecting the amount of bound lectin or because of coincidence due to the natural variability between different biologic samples. The MGV of the second albumin preparation was significantly different from MGV of the albumin control preparation and the contralateral control preparation from the first observation day. No significant difference in MGV was observed between the protective regimens (albumin vs sulodexide).

Figure 3. Renal Cortex Histology



Images present renal cortex histology (hematoxylin-eosin stain) after the administration of albumin (**a** and **b**), of sulodexide (**c** and **d**), and of placebo (**e, f, g,** and **h**) in the operated kidney (**e** and **f**) and in the contralateral kidney (**g** and **h**). Scale bars are 200 μm (**a, c, e**, and **g**) and 100 μm (**b, d, f**, and **h**).

Figure 4. Lectin Histochemical Detection



Each bar represents the mean gray value (MGV) from lectin fluorescence detection in glomeruli of a single animal. The labels identify individual animals and their assigned group: Albumin and Albumin represent pigs treated with albumin; Sulodexide, Sulodexide2, and Sulodexide3 represent pigs treated with sulodexide; Control_surgery represents pigs from the placebo (control) group that underwent the full surgical protocol; and Control_intact represents pigs from the placebo (control) group with intact contralateral kidney, not subjected to ischemia-reperfusion injury.

Discussion

Interest in EG has recently surged, with recognition of its critical role in vascular health. To our knowledge, this study is the first to explore the potential protective effects of albumin and sulodexide on the EG during organ transplant. It has been established that IRI predominantly targets the vascular endothelium, leading to endothelial dysfunction characterized by cellular swelling, cytoskeletal degradation, and loss of integrity in both the endothelium and the EG.[6] Our findings suggest that sulodexide and albumin may exert a protective effect on the EG during IRI, validating the efficacy of our model.

Of note, the sulodexide group exhibited lower serum syndecan-1 levels shortly after reperfusion versus the placebo group, which aligns with the trend for albumin to reduce urinary glycosaminoglycan levels after reperfusion. These biochemical markers, alon-gside the pathological samples, support the potential of these agents to safeguard the EG. Given these promising indicators, treatment regimens with sulodexide and albumin merit further investigation for the protective capacities in clinical settings.

The therapeutic profile of sulodexide is distinguished by its antithrombotic and profibrinolytic properties, suggesting an active role for modulation of the vascular environment, particularly in the preservation and regeneration of the EG. These pharmacological actions might contribute to the stabilization of the EG, as indicated by the decreased syndecan-1 levels following reperfusion observed in our study. The lower syndecan-1 levels imply that sulodexide could be reinforcing the structural integrity of the EG, potentially curbing the enzymatic degradation and shedding that are hallmarks of IRI. This mechanism may, therefore, be a contributing factor to the protective effects seen with sulodexide in the context of endothelial barrier function and vascular homeostasis.

Albumin has also emerged as a potential protective agent for the EG. Clinical models have demonstrated that albumin, unlike normal saline, is effective in both repairing the EG and reducing plasma syndecan-1 levels. Notably, the effect of albumin to enhance vascular permeability and mitigate leukocyte adhesion is comparable to the effect seen with fresh-frozen plasma, indicating the beneficial role of albumin for vascular integrity.[12] Moreover, recent evaluations in resuscitation fluids emphasize the superior performance of albumin (versus crystalloids and synthetic colloids) to protect and regenerate the EG. These findings position albumin as a potential therapeutic agent for EG preservation, particularly in response to acute vascular injuries and resuscitation scenarios.[13]

We chose to measure serum syndecan-1 and urinary glycosaminoglycan levels as indicators of EG integrity, given that these are components of EG structure. However, these markers are not exclusive to the EG, and the presence of these agents in bodily fluids does not necessarily pinpoint damage to a specific organ. Furthermore, the dynamic nature of the EG, through its continuous cycle of synthesis and degradation, complicates our understanding of its physiology and response to pathological conditions such as IRI. Therefore, the relationship between such injuries during KT and the fluctuations of these EG markers warrants further investigation.

The strengths of our study lie in its targeted approach to a crucial aspect of KT, that is, the preservation of the endothelial function and EG, factors integral to successful posttransplant outcomes. The porcine model closely parallels human physiology, and our study provides insights that hold promise for translation into clinical practice. Here, we have presented a comparative analysis of 2 therapeutic agents, sulodexide and albumin, and evaluated the protective effects of these agents against IRI. The robustness of our study is enhanced by the inclusion of extensive physiological monitoring, alongside detailed biochemical and pathological evaluations of intervention outcomes.

However, our study had some limitations. Because our study was a pilot study, we had a low number of significant results because of the small number of samples available, primarily used to determine the appropriate assessment methodology. However, differences and trends in individual observations, although not significant, confirmed the success and suitability of the chosen detection methods for the assessment of protective substances and indicate possible beneficial effects for preservation of endothelial function during KT. In addition, our focus was on immediate postintervention outcomes; therefore, the longer-term implications and the sustained effects of the protective treatments remain to be determined. Further research, with a larger cohort and extended observation periods, is necessary to build on the initial findings and fully understand the long-term benefits of these therapeutic interventions.

Conclusions

Sulodexide and albumin may confer protective benefits to the EG in the event of IRI during KT. Although these initial findings reveal potential mitigation of endothelial damage in porcine subjects, more expansive additional studies are needed. Future research should aim to substantiate these results, elucidate the underlying mechanisms, and evaluate the clinical applicability of these interventions to enhance outcomes in KT and potentially other contexts where vascular protection is critical.

References

1. Schnuelle P, Lorenz D, Trede M, Van Der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure. *J Am Soc Nephrol*. 1998;9(11):2135-2141. doi:10.1681/ASN.V9112135
2. Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A, Kaplan B. Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. *Am J Transplant*. 2004;4(10):1662-1668. doi:10.1111/j.1600-6143.2004.00573.x
3. Liew H, Roberts MA, McMahon LP. Markers of the endothelial glycocalyx are improved following kidney transplantation. *Kidney Blood Press Res*. 2021;46(5):581-587. doi:10.1159/000517317

4. Nieuwenhuijs-Moeke GJ, Pischke SE, Berger SP, et al. Ischemia and reperfusion injury in kidney transplantation: relevant mechanisms in injury and repair. *J Clin Med*. 2020;9(1). doi:10.3390/jcm9010253
5. Saat TC, van den Akker EK, IJzermans JN, Dor FJ, de Bruin RW. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? *J Transl Med*. 2016;14:20. doi:10.1186/s12967-016-0767-2
6. Duni A, Liakopoulos V, Koutlas V, Pappas C, Mitsis M, Dounousi E. The endothelial glycocalyx as a target of ischemia and reperfusion injury in kidney transplantation: where have we gone so far? *Int J Mol Sci*. 2021;22(4). doi:10.3390/ijms22042157
7. Alphonsus CS, Rodseth RN. The endothelial glycocalyx: a review of the vascular barrier. *Anaesthesia*. 2014;69(7):777-784. doi:10.1111/anae.12661
8. Aldecoa C, Llau J V, Nuvials X, Artigas A. Role of albumin in the preservation of endothelial glycocalyx integrity and the microcirculation: a review. *Ann Intensive Care*. 2020;10(1):85. doi:10.1186/s13613-020-00697-1
9. Coccheri S, Mannello F. Development and use of sulodexide in vascular diseases: implications for treatment. *Drug Des Devel Ther*. 2013;8:49-65. doi:10.2147/DDDT.S6762
10. Bignamini AA, Chebil A, Gambaro G, Matuška J. Sulodexide for diabetic-induced disabilities: a systematic review and meta-analysis. *Adv Ther*. 2021;38(3):1483-1513. doi:10.1007/s12325-021-01620-1
11. Li T, Liu X, Zhao Z, Ni L, Liu C. Sulodexide recovers endothelial function through reconstructing glycocalyx in the balloon-injury rat carotid artery model. *Oncotarget*. 2017;8(53):91350-91361. doi:10.18632/oncotarget.20518
12. Torres LN, Chung KK, Salgado CL, Dubick MA, Torres Filho IP. Low-volume resuscitation with normal saline is associated with microvascular endothelial dysfunction after hemorrhage in rats, compared to colloids and balanced crystalloids. *Crit Care*. 2017;21(1):160. doi:10.1186/s13054-017-1745-7
13. Milford EM, Reade MC. Resuscitation fluid choices to preserve the endothelial glycocalyx. *Crit Care*. 2019;23(1):77. doi:10.1186/s13054-019-2369-x

Late Effect of Early Phase COVID-19 on Outcomes of Kidney Transplant Recipients Who Survived the Acute Infection

Arthur Gus Manfro,[1] Eduarda Tais Schneider,[2] Leonardo Luigi Adams Backes,[2] Gabriel Petrolli,[2] Rodrigo Fontanive Franco,[1] Carla Elizabeth Oliveira Cezar,[1] Andrea Carla Bauer,[1,2] Roberto Ceratti Manfro[1,2]

Abstract

Objectives: **The COVID-19 pandemic has significantly affected global health, particularly among high-risk populations such as kidney transplant recipients, who have exhibited elevated morbidity and mortality rates. Long-term effects of COVID-19 in kidney transplant recipients who survived the infection are unknown. We evaluated the long-term effects of early phase COVID-19 on patient and graft survival, as well as graft function, in kidney transplant recipients who survived the acute phase of the COVID-19 infection.**

Materials and Methods: **We conducted a prospective, single-center cohort study involving kidney transplant recipients who survived COVID-19 from June 2020 to January 2022. Patients were stratified by disease severity and followed for 24 months. Data on renal function (estimated glomerular filtration rate and urinary protein-to-creatinine ratio) were collected at multiple time points. Statistical analyses were based on χ^2 tests, analysis of variance, generalized additive mixed models, and Kaplan-Meier analyses.**

Results: **Among 1477 kidney transplant recipients, 233 (15.8%) contracted COVID-19, with 60 (25.8%) fatalities. Of the 173 survivors, 50 (28.9%) had mild, 102 (59%) moderate, and 21 (12.1%) severe disease. Severe cases showed significant declines in estimated glomerular filtration rate and higher rates of renal replacement therapy and acute rejection versus mild and moderate cases. Mean loss of glomerular filtration rate in 2 years among patients with severe COVID was 9 mL/min/m². Graft and patient survival rates were also worse in moderate and severe COVID groups.**

Conclusions: **Kidney transplant recipients with moderate and severe COVID-19 experienced significant long-term declines in renal function and increased graft loss and mortality. Understanding these effects is critical for optimizing care for this population.**

Key words: *Glomerular filtration rate, Renal transplantation, Severe acute respiratory syndrome coronavirus 2*

Introduction

The COVID-19 pandemic has profoundly affected global health and resulted in unprecedented mortality rates in the modern era.[1] Among high-risk populations, the morbidity and mortality rates associated with the disease have been particularly severe, especially before the widespread availability of vaccines.[2,3] Solid-organ transplant recipients, notably kidney transplant recipients (KTRs), experienced adverse outcomes during the pandemic. Mortality rates in KTR populations ranged from 20% to 30% across various cohorts.[4,5] Compared with the general population, KTRs exhibited higher mortality rates, even when accounting for similar disease severity.[6] Furthermore, among KTRs who survived the infection, ongoing concerns remain regarding long-term outcomes, particularly given that many of these patients experienced acute kidney injury (AKI) and were required to taper or temporarily discontinue their immunosuppression medications.[7,8]

Although the acute effects of COVID-19 on KTRs are well documented, the long-term consequences of the infection in this population

From the [1]Division of Nephrology and Kidney Transplantation, Hospital de Clínicas de Porto Alegre; and the [2]School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Acknowledgements: *RCM received a scholarship from the Brazilian Research Council for conducting this work. The other authors have not received any funding or grants in support of the present research or for the preparation of this work. None of the authors declare having potential conflicts of interest regarding this work.*

Corresponding author: *Roberto Ceratti Manfro, Division of Nephrology and Kidney Transplantation, Hospital de Clínicas de Porto Alegre, Ramiro Barcelos 2350 St, Porto Alegre, Brazil 90410-000*

E-mail: *rmanfro@hcpa.edu.br*

Experimental and Clinical Transplantation (2025) 8: 517-522

DOI: 10.6002/ect.2025.0078

remain underevaluated. Notably, the short-term effect on graft function is significant; reports indicate that 44% of KTRs experienced AKI, 12% required renal replacement therapy, and 8% faced graft loss.[9] However, the literature has yet to comprehensively address late survival and graft outcomes. In this study, we present data on patient and graft survival, as well as the effects on graft function, in a single-center cohort of KTRs who survived the early stages of the COVID-19 pandemic.

Materials and Methods

Study design and population

The present study is a prospective, single-center cohort investigation involving KTRs at the Hospital de Clínicas de Porto Alegre, a large tertiary teaching hospital located in southern Brazil. This research included all KTRs who survived the acute phase of COVID-19 from June 2020 to January 2022. Confirmation of COVID-19 infections was achieved through real-time polymerase chain reaction or specific antigen testing. Patients were followed for a period of 24 months after the COVID-19 diagnosis.

The study received approval from the Institutional Review Board of the Hospital de Clínicas de Porto Alegre and the local ethics committee (CAEE No. 30631820.0.2012.5327). All patients provided consent to participate in the study, and personal information was anonymized. All living donations were performed according to Brazilian legislation and medical ethical standards; all donors were >18 years old, related to the recipient (family up to 4th degree and spouses), and without any financial compensation.

Demographic and clinical information for the patients was obtained from clinical records or directly from patients during follow-up appointments. Baseline measurements of creatinine, estimated glomerular filtration rate (eGFR) (using the CKD-EPI equation),[10] and urinary spot protein-to-creatinine ratio (P/C) were collected 1 month before COVID-19 diagnosis. Follow-up assessments of creatinine, eGFR, and P/C were conducted at 6 months, 12 months, 18 months, and 24 months after diagnosis.

Kidney transplant recipients were stratified according to the severity of COVID-19 as follows. (1) Mild disease patients did not require hospital admission. (2) Moderate disease patients were admitted to a clinical ward for COVID-19 treatment without the need for invasive ventilation. (3) Severe disease patients were admitted to the intensive care unit and required mechanical ventilation support. Survival of acute infection was defined as symptom recovery and release from hospitalization of those admitted to hospital care in the acute infection episode.

Statistical analyses

We used χ^2 tests and analysis of variance to compare demographic characteristics and clinical outcomes among COVID-19-affected KTRs stratified by disease severity, with post hoc analyses performed to adjust for multiple comparisons using Tukey tests. We used generalized additive mixed models to estimate and compare the smoothed trajectories of eGFR and urinary P/C across severity groups over time. This analysis combines the flexibility of the generalized additive models with the ability to consider random effects; by evaluating repeated assessments, the model constructs a locally estimated scatterplot smoothing trajectory curve for each patient and then combines these individual trajectories in a regression model accounting for group differences. We used Kaplan-Meier models to evaluate patient and graft survival. We used R software[11] for all statistical analyses, incorporating the tidygam, ggplot2, and survival packages.[12-14] $P < .05$ was the threshold of statistical significance.

Results

Demographic traits and clinical outcomes

During the study period, a total of 1477 KTRs were followed at our center. Among these, 233 patients (15.8%) developed confirmed COVID-19 infections, and 60 patients (25.8%) died from the disease. Among the 173 COVID-19 patients who survived, 50 (28.9%) were classified as having mild disease, 102 (59%) as having moderate disease, and 21 (12.1%) as having severe disease. All deaths occurred in patients classified in the severe disease group. Most patients were middle-aged, predominantly White race, and had undergone transplant approximately 7 years before their COVID-19 diagnosis. Demographic and clinical characteristics of this cohort are detailed in Table 1.

On average, patients with mild disease exhibited higher eGFRs and lower urinary P/C ratios than

Table 1. Demographic and Clinical Characteristics of the Kidney Transplant Recipient Cohort

Characteristic, mean (SD)	COVID Severity			ANOVA		Mild vs Moderate		Mild vs Severe		Moderate vs Severe	
	Mild	Moderate	Severe	Z	*P*	MD	*P*	MD	*P*	MD	*P*
Age, y	48.4 (12.6)	51.9 (12.0)	53.4 (11.8)	1.8	.168	-3.4	.230	-5.0	.256	-1.5	.855
BMI	27.5 (5.8)	30.0 (5.8)	29.8 (5.7)	1.6	.211	-1.5	.295	-2.3	.282	-0.8	.835
Years after transplant	6.9 (5.7)	7.2 (5.7)	6.9 (7.4)	0.1	.928	-0.3	.937	0.0	.999	0.3	.964
Baseline serum creatinine , mg/dL	1.3 (0.5)	1.7 (0.9)	1.6 (0.8)	4.3	.015	-0.4	.010	-0.3	.407	0.1	.738
Baseline eGFR, mL/min/m^2	65.5 (22.8)	54.2 (24.4)	54.2 (20.0)	4.9	.009	13.2	.007	11.3	.166	-1.9	.941
Baseline P/C, mg/g	0.2 (0.2)	0.5 (0.7)	0.6 (1.2)	3.7	.028	-0.3	.067	-0.4	.052	-0.2	.622
Characteristic, No. (% of total), standardized residual	**Mild (n = 50)**	**COVID Severity Moderate (n = 102)**	**Severe (n = 21)**			**χ2**	**P**				
Male sex	25 (50.0), -0.3	52 (51.0), -0.3	15 (71.4), 1.1			3.21	.201				
White race	43 (86.0), 0.1	87/101 (86.1), 0.1	16 (76.2), -0.4			1.41	.494				
Comorbidities											
Hypertension	43 (86.0), 0.0	87 (85.3), 0.0	18 (85.7), 0.0			0.01	.993				
Diabetes	14 (28.0), -0.8	40 (39.2), 0.8	6 (28.6), -0.5			2.26	.324				
CVD	6 (12.0), -0.8	18 (17.6), 0.2	5 (23.8), 0.8			1.62	.445				
Liver disease	2 (4.0), -0.4	6 (5.9), 0.3	1 (4.8), -0.1			0.25	.882				
Lung disease	2 (4.0), -0.4	7 (6.9), 0.7	0 (0.0), -1.0			1.87	.393				
Autoimmune disease	3 (6.0), 1.0	3 (2.9), -0.3	0 (0.0), -0.9			1.79	.407				
Cancer	2 (4.0), 0.2	4 (3.9), 0.2	0 (0.0), -0.9			0.86	.651				
Etiology of CKD											
Hypertension	3 (6.0), -0.5	7 (6.9), -0.4	4 (19.0), 1.8			3.89	.143				
Diabetes	7 (14.0), -0.5	20 (19.6), 0.7	2 (9.5), -0.8			1.65	.437				
Glomerulopathy	11 (22.0), 0.8	17 (16.7), -0.2	2 (9.5), -0.9			1.68	.431				
Polycystic	4 (8.0), -0.4	12 (11.8), 0.6	1 (4.8), -0.7			1.23	.541				
Urologic	4 (8.0), 0.1	9 (8.8), 0.5	0 (0.0), -1.3			1.97	.373				
Unknown, other	21 (42.0), 0.2	37 (36.3), -0.7	12 (57.1), 1.2			3.22	.200				
Donor (deceased)	40 (80.0), -0.2	83 (81.4), -0.1	20 (95.2), 0.6			2.68	.262				
Immune suppression											
FK + AZA	2 (4.0), -0.2	4 (3.9), -0.3	2 (9.5), 1.0			1.30	.522				
FK + MPA	41 (82.0), 0.1	82 (80.4), 0.0	16 (76.2), -0.2			0.32	.854				
FK + mTOR	0 (0.0), -0.8	2 (2.0), 0.8	0 (0.0), -0.5			1.41	.494				
CsA + MPA	3 (6.0), 0.1	6 (5.9), 0.0	1 (4.8), -0.2			0.05	.977				
MPA + mTOR	1 (2.0), 0.6	1 (1.0), -0.2	0 (0.0), -0.5			0.58	.746				
Other	3 (6.0), -0.3	7 (6.9), 0.0	2 (9.5), 0.5			0.29	.867				
Outcomes											
Hemodialysis	0 (0.0), -2.3	5 (4.9), -1.9	14 (66.7), 7.7			76.63	<.001				
Graft rejection	0/45 (0.0), -1.2	2/99 (2.0), -0.6	3 (14.3), 3.0			10.80	.005				
Immune suppression tapering	18 (36.0), -2.9	83 (81.4), 1.3	21 (100.0), 1.6			43.22	<.001				

Abbreviations: ANOVA, analysis of variance; AZA, azathioprine; BMI, body mass index; CKD, chronic kidney disease; CsA, cyclosporine A; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FK, tacrolimus; MD, mean difference; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin inhibitors; P/C, urinary protein-to-creatine ratio

$P < .05$ is statistically significant.

patients with more severe disease. Clinical outcomes varied significantly among the groups, as anticipated; that is, the requirement for renal replacement therapy was notably higher in the severe disease group (67%) versus moderate (5%) and mild (0%) groups ($\chi^2 = 76.63$, $P < .001$). In addition, the incidence of acute cellular rejection was greater in patients with severe forms of the disease (14%) versus moderate (2%) and mild (0%) cases ($\chi^2 = 10.80$, $P = .005$). All acute cellular rejections were confirmed by kidney biopsies. Furthermore, the frequency of immunosuppression tapering was significantly influenced by disease severity, with rates of 100% for severe, 81% for moderate, and 36% for mild cases ($\chi^2 = 43.22$, $P < .001$). The most common tapering strategy was the discontinuation of the antiproliferative medication (mycophenolic acid). Most patients in the intensive care unit suspended both calcineurin inhibitor and antiproliferative medication. In all oxygen-dependent patients, corticosteroid dosing was increased according to COVID-19 treatment protocols (at least 6 mg oral dexamethasone or equivalent). Two patients in the severe disease group presented biopsy-confirmed recurrence of native kidney diseases; of these, 1 patient was diagnosed with focal and segmental glomerulonephritis and 1 patient with pauci-immune vasculitis.

Trajectories of estimated glomerular filtration rate and urinary protein-to-creatinine ratio

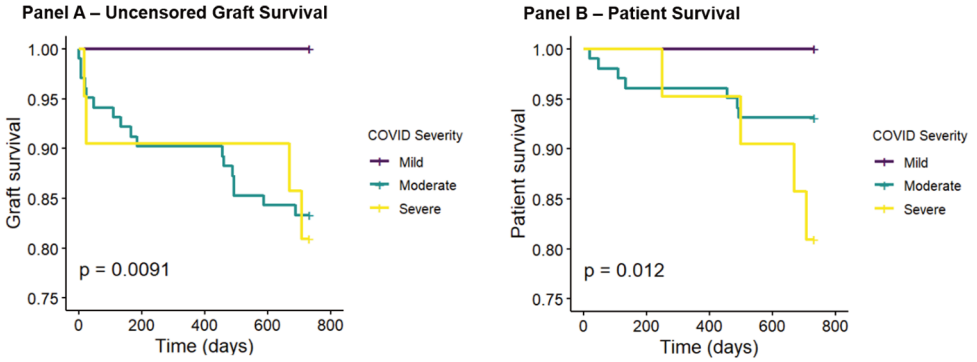
The evaluation of eGFRs revealed that both the moderate and severe groups exhibited lower baseline renal function. Notably, only the severe group demonstrated a marked decline in eGFR during the follow-up period, particularly within the first 6 months after infection. Patients in the severe group experienced a median loss of eGFR of 9 mL/min/1.73 m^2, whereas the mild and moderate groups maintained stable eGFRs. Generalized additive mixed models indicated a significant interaction between eGFR, severity group, and time (F = 1.26, *P* = .01). Conversely, the interaction of urinary P/C ratio, severity group, and time was not statistically significant (F = 0, *P* = .58). The eGFR and proteinuria trajectories are illustrated in Figure 1.

Graft and patient survival

Uncensored graft survival rates over the 24-month follow-up period differed significantly across severity groups. In the mild group, 100% of grafts remained functional, versus 83% in the moderate group and 81% in the severe group (*P* = .009). There were also 11 death-censored graft losses, with 9 because of end-stage allograft kidney disease and 2 from graft rejection. Over the following period, 5 acute cellular rejections were confirmed by biopsy, of which 3 cases were successfully treated with corticosteroid and thymoglobulin pulses and 2 cases led to graft losses. A similar trend was observed for patient survival, with rates of 100% in the mild group, 93% in the moderate group, and 83% in the severe group (*P* = .012). Of the 11 KTRs who died during follow-up, 5 deaths (45%) were due to non-COVID-related sepsis, 1 due to a cardiovascular event, 1 due to malignancy, and 4 due to unknown causes. There was no statistical significance for distinct causes of death among the group comparisons.

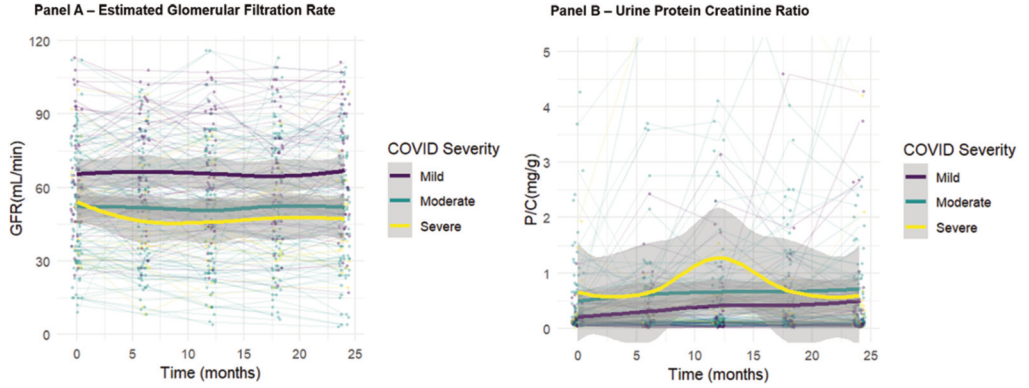
Kaplan-Meier survival statistics are illustrated in Figure 2.

Figure 1. Kaplan-Meier Survival of Kidney Grafts and Kidney Transplant Recipients in Follow-Up



Kaplan-Meier survival statistics for (A) graft and (B) kidney transplant recipients in follow-up, showing higher rates of graft loss and patient mortality in kidney transplant recipients with moderate and severe COVID-19. Graft losses are not censored for death in the present analysis, and Kaplan-Meier graphics are censored in the 75th percentile. *P* values are shown in the bottom left corners.

Figure 2. Distinct Trajectories of Glomerular Filtration Rate and Urine Protein-to-Creatinine Ratio Over Time



Abbreviations: GFR, glomerular filtration rate; P/C, urinary protein-to-creatine ratio

Discussion

Here, we demonstrated that acute early phase COVID-19 infection has distinct late effects on KTR, largely influenced by disease severity. Although mild cases were associated with neither significant acute distress nor long-term complications, severe cases resulted in a temporary increase in proteinuria, long-term deterioration of renal function, and a higher incidence of graft loss and death. Previous studies have already highlighted the severe outcomes of acute COVID-19 infection in KTR, with mortality rates reaching 20% to 30% in several cohorts.[15] Shorter follow-up studies of KTRs with COVID-19 also showed generally good outcomes for survivors with mild to moderate disease and more detrimental recovery for those affected with severe COVID-19.16 To our knowledge, our study is the longest follow-up cohort study of KTRs with COVID-19. Our findings contribute to the body of knowledge, showing that medium-term to long-term consequences of the pandemic persist in KTRs who survive the acute phase of infection.

Our data demonstrated that late clinical outcomes in KTRs who survived the acute COVID-19 infection episode were profoundly influenced by the acute disease severity. Mild cases exhibited stable outcomes, with no episodes of rejection, stable eGFR, and no graft losses or deaths, behaving almost as if unaffected by the disease. Patients with moderate disease severity showed intermediate outcomes, characterized by a low frequency of acute rejection and stable eGFR but with a notable incidence of graft failure and postinfection mortality. As hypothesized, recipients with severe COVID-19 experienced the worst outcomes, including a significantly higher incidence of rejection, graft loss, and mortality, along with substantial eGFR decline over 2 years versus the mild group. These findings may be attributed not only to the direct effects of the disease, such as cytokine storms, immuno-modulation, and the need for advanced life support (eg, mechanical ventilation, vasoactive drugs, hemodialysis), but also to alterations in immunosuppression regimens that were indicated in the acute phase of the disease. In virtually all severe cases, immunosuppression was greatly reduced or even discontinued, which may have potentially triggered alloimmune responses that contributed to subsequent rejection and graft loss.[17,18]

Extensive research has demonstrated significant long-term detrimental effects of COVID-19 on mortality and morbidity within the general population. Survivors of COVID-19, particularly those who experience severe disease that requires hospitalization, face markedly higher risks of adverse outcomes versus uninfected individuals. For instance, a study from 2022 indicated that individuals discharged after hospitalization for COVID-19 had a 2.2-fold increased risk of rehospitalization or death and a 4.8-fold increase in all-cause mortality versus control cases from the general population.[19] Another investigation revealed that nearly 30% of hospitalized patients succumbed within 6 months, with higher mortality rates observed among older patients and those who required mechanical ventilation.[20] Among survivors of acute disease, loss of renal clearance has been observed in general populations infected by COVID-19.[21,22] In addition, the increasingly recognized post-COVID syndrome, also known as long COVID, presents an important concern.[23] This syndrome has also been studied in KTRs, showing significant prevalence and effect in morbidity and labor activities.[24]

Our study produced relevant information on the subject; among the strengths are the prospective evaluation of a cohort of KTRs affected by COVID-19, the multiple measurements of renal function, and the follow-up over an extended period. However, our study had some limitations that included the single-center cohort, which may limit the generalizability of the findings. Nonetheless, the data were collected at an established transplant center that uses updated KTR management practices, suggesting that the results are likely applicable to broader populations. Also, the observations were made before the widespread availability of COVID-19 vaccines and the emergence of less pathogenic variants of the virus, which is substantially different from the present scenario. The reported cases were analyzed during the period from 2020 to 2022, a period when the most prevalent COVID-19 variants were alpha, beta, and gamma. Despite this, a considerable proportion of KTRs worldwide were infected under similar conditions, and many are still under medical care, potentially presenting comparable outcomes.

In conclusion, our cohort study demonstrated that KTRs with moderate and severe COVID-19 infections experienced significant declines in renal function, with higher rates of graft loss and mortality.

Given that many KTRs were infected during the early stages of the pandemic, understanding the long-term effects shown in these patients may be important for informing clinical practice and optimizing patient care.

References

1. COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. *Lancet*. 2022;399(10334): 1513-1536. doi:10.1016/S0140-6736(21)02796-3
2. Banerjee D, Popoola J, Shah S, Ster IC, Quan V, Phanish M. COVID-19 infection in kidney transplant recipients. *Kidney Int*. 2020;97(6):1076-1082. doi:10.1016/j.kint.2020.03.018
3. Russell CD, Lone NI, Baillie JK. Comorbidities, multimorbidity and COVID-19. *Nat Med*. 2023;29(2):334-343. doi:10.1038/s41591-022-02156-9
4. Jager KJ, Kramer A, Chesnaye NC, et al. Results from the ERA-EDTA Registry indicate a high mortality due to COVID-19 in dialysis patients and kidney transplant recipients across *Europe. Kidney Int*. 2020;98(6):1540. doi:10.1016/j.kint.2020.09.006
5. Requião-Moura LR, Sandes-Freitas TV, Viana LA, et al; COVID-19-KT Brazil. High mortality among kidney transplant recipients diagnosed with coronavirus disease 2019: Results from the Brazilian multicenter cohort study. *PLoS One*. 2021;16(7):e0254822. doi:10.1371/journal.pone.0254822
6. Caillard S, Chavarot N, Francois H, et al; French SOT COVID Registry. Is COVID-19 infection more severe in kidney transplant recipients? *Am J Transplant*. 2021;21(3):1295-1303. doi:10.1111/ajt.16424
7. Nahi SL, Shetty AA, Tanna SD, Leventhal JR. Renal allograft function in kidney transplant recipients infected with SARS-CoV 2: an academic single center experience. *PLoS One*. 2021;16(6): e0252979. doi:10.1371/journal.pone.0252979
8. Bajpai D, Deb S, Bose S, et al. Recovery of kidney function after AKI because of COVID-19 in kidney transplant recipients. *Transpl Int*. 2021;34(6):1074-1082. doi:10.1111/tri.13886
9. Chen JJ, Kuo G, Lee TH, et al. Incidence of mortality, acute kidney injury and graft loss in adult kidney transplant recipients with coronavirus disease 2019: Systematic review and meta-analysis. *J Clin Med*. 2021;10(21):5162. doi:10.3390/jcm10215162
10. Levey AS, Stevens LA, Schmid CH, et al; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.7326/0003-4819-150-9-200905050-00006
11. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Published online 2023. https://www.R-project.org/
12. Coretta S. Tidygam: tidy prediction and plotting of generalised additive models. Published online May 9, 2023. Accessed November 3, 2024. https://CRAN.R-project.org/package=tidygam
13. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016. Springer-Verlag. Accessed November 3, 2024. https://ggplot2.tidyverse.org/
14. Therneau TM, Lumley T, Elizabeth A, Cynthia C. survival: Survival Analysis. Published online June 5, 2024. Accessed November 3, 2024. doi:10.32614/CRAN.package.survival (https://CRAN.R-project.org/package=survival)
15. Kremer D, Pieters TT, Verhaar MC, et al. A systematic review and meta-analysis of COVID-19 in kidney transplant recipients: Lessons to be learned. *Am J Transplant*. 2021;21(12):3936-3945. doi:10.1111/ajt.16742
16. Duivenvoorden R, Vart P, Noordzij M, et al. Clinical, functional, and mental health outcomes in kidney transplant recipients 3 months after a diagnosis of COVID-19. *Transplantation*. 2022;106(5):1012-1023. doi:10.1097/TP.0000000000004075
17. Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium. *Am J Transplant*. 2020;20(11):3140-3148. doi:10.1111/ajt.16185
18. Demir E, Ucar ZA, Dheir H, et al. COVID-19 in kidney transplant recipients: a multicenter experience from the first two waves of pandemic. *BMC Nephrol*. 2022;23(1):183. doi:10.1186/s12882-022-02784-w
19. Bhaskaran K, Rentsch CT, Hickman G, et al. Overall and cause-specific hospitalisation and death after COVID-19 hospitalisation in England: a cohort study using linked primary care, secondary care, and death registration data in the OpenSAFELY platform. *PLoS Med*. 2022;19(1):e1003871. doi:10.1371/journal.pmed.1003871
20. Günster C, Busse R, Spoden M, et al. 6-month mortality and readmissions of hospitalized COVID-19 patients: a nationwide cohort study of 8,679 patients in Germany. *PLoS One*. 2021;16(8):e0255427. doi:10.1371/journal.pone.0255427
21. Mahalingasivam V, Faucon AL, Sjölander A, et al. Kidney function decline after COVID-19 infection. *JAMA Netw Open*. 2024;7(12): e2450014. doi:10.1001/jamanetworkopen.2024.50014
22. Andrade JAM, Meinerz G, Palma R, et al. Acute kidney injury in critically ill COVID-19 patients in a tertiary hospital: short and long-term kidney and patient outcomes. *J Bras Nefrol*. 2025;47(1): e20240107. doi:10.1590/2175-8239-JBN-2024-0107en
23. Sk Abd Razak R, Ismail A, Abdul Aziz AF, Suddin LS, Azzeri A, Sha'ari NI. Post-COVID syndrome prevalence: a systematic review and meta-analysis. *BMC Public Health*. 2024;24(1):1785. doi:10.1186/s12889-024-19264-5
24. Amorim CEN, Gomes VLT, Cristelli MP, et al. High Prevalence of long-COVID among kidney transplant recipients: a longitudinal cohort study. *Transplantation*. 2022;106(12):2408-2415. doi:10.1097/TP.0000000000004359

Erector Spinae Plane Block for Postoperative Analgesia in Laparoscopic Living-Donor Nephrectomy

Bora Dinc,[1] Ilker Onguc Aycan,[1] Ali Avanaz,[2] Nihal Kiraz,[3] Abdullah Kisaoglu,[2] Necmiye Hadimioglu,[1] Zeki Ertug,[1] Ismail Demiryilmaz[2]

Abstract

Objectives: **Postoperative donor care is being improved with newly applied methods. Recently, because of its effectiveness, erector spinae plane blocks have been increasing in popularity. However, its use for laparoscopic living-donor nephrectomy is not fully known. We evaluated the effectiveness of erector spinae plane blocks in laparoscopic living-donor nephrectomy on postoperative pain and total analgesia consumption in the first 24 hours postsurgery.**

Materials and Methods: **In this randomized, prospective cohort efficiency study, we included 60 donors scheduled for elective nephrectomy. The control group (n = 30) received analgesic medication according to organ transplant ward protocol for postoperative pain treatment; the erector spinae plane block group (n = 30) underwent an erector spinae plane block application with routine analgesic medication practice. We evaluated the efficacy of postoperative pain treatment and total analgesic consumption at postoperative 1, 6, 12, and 24 hours.**

Results: **Among the 60 donors in the study, there were no differences in the verbal numerical rating scale and the Wong-Baker Faces Pain Rating Scale scores between the groups in the first 24 hours. However, total tramadol consumption in the erector spinae plane block group was less (*P* = .003) than in the control group. Regression analysis confirmed that block application was associated with tramadol consumption (*P* = .001).**

Conclusions: **Although erector spinae plane blocks applied under bilateral ultrasonographic guidance did not have any efficacy for pain relief in the first 24 hours postoperation, a decrease was found in analgesic consumption compared with the nonblock group.**

Key words: *Donor wound healing, Pain control, Postoperative pain*

Introduction

Laparoscopic living-donor nephrectomy (LLDN) is a safe procedure and improves the quality of life among donors. The procedure accelerates wound healing and thus facilitates the earlier discharge of donor candidates. Laparoscopic living-donor nephrectomy also requires fewer analgesics compared with the open method.[1] The physiology of postoperative pain has been attributed to tissue damage as a result surgical trauma, inflammation, residual pneumoperitoneum, related biochemical changes, and diaphragm irritation. Phrenic neuropraxia due to irritation is the most prominent cause of pain and subsequently results in shoulder pain.[2]

Pain control is provided by intravenous (IV) analgesics, which are local anesthetics injected into the incision area, and regional techniques after LLDN operations.[2,3] The development of less invasive methods of transplant surgery has also motivated improvements in anesthesia administration methods. Recently, new regional anesthesia techniques for postoperative pain control have become popular. Many blocks are being applied with ultrasonography to reduce pain severity after laparoscopic and open surgeries.[3,4] In 2016, the erector spinae plane block (ESPB) was first described by Forero and colleagues and was successfully used in thoracic and abdominal surgeries.[5] As previously reported, ESPB has been successful in controlling pain, total opioid use, nausea, vomiting, and pruritus, especially following laparoscopic abdominal surgeries.[3-6]

From the [1]Akdeniz University Medical Faculty, Department of Anesthesiology and Reanimation, Antalya, Turkiye; the [2]Akdeniz University Medical Faculty, Department of General Surgery, Antalya, Turkiye; the [3]Akdeniz University Medical Faculty, Department of Nursing, Organ Transplantation Clinic, Antalya, Turkiye

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: *Ilker Onguc Aycan, Akdeniz University Medical School, Department of Anesthesiology and Reanimation, Antalya, Turkey*

Phone: *+90 242 249 6124* ***E-mail:*** *ilkeraycan@gmail.com*

Experimental and Clinical Transplantation (2025) 8: 523-529

DOI: 10.6002/ect.2022.0401

Currently, focus has been on donor care, and especially practices, to improve postoperative quality of life after transplant operations. In our clinic, patient-controlled analgesia, epidural anesthesia, and, in recent years, ESPB applications have been used for postoperative pain control. However, to our knowledge, no prospective cohort study in the literature has investigated the effects of ESPB applications on postoperative pain control, total analgesic consumption, nausea, vomiting, and pruritus following LLDN operations. The primary aim of our study was to investigate the success and effectiveness of ESPB applications according to the results of the pain assessment scales. The secondary purpose was to evaluate the total amount of analgesics (paracetamol and tramadol) administered as routine and rescue therapy in the first 24 hours after surgery.

Materials and Methods

Volunteer study design

This prospective cohort, randomized, single-center study was conducted in patients scheduled for LLDN in 2021 in the organ transplant department of our hospital following the ethical standards of the Declaration of Helsinki. Ethical approval for the protocol of the presented study was provided by the Institutional Review Board of our hospital in Turkey (no. 70904504/123; date of approval: 10/02/2021), and the study was prospectively registered at ClinicalTrials.gov (NCT04867070).

Written informed consent for participation in the study was obtained from all donors before the LLDN operation. All participants were aged 19 to 75 years and classified as I or II according to the American Society of Anesthesiologists (ASA) physical condition classification. The ESPB was explained to the volunteers, and a block was applied if the patients provided their consent. The donors were randomly assigned to the ESPB group or the control group using computer-generated random numbers placed in separate closed envelopes that the study investigator opened before performing the block in the operation room. All ESPBs were performed by the same anesthesiologist. All data collectors were blinded to randomization. Organ transplant nurses evaluated the donor pain status in the recovery ward and organ transplant ward at 1, 6, 12, and 24 hours postoperatively. Pain assessment results were recorded, which was the primary outcome of the study.

The standard perioperative medical analgesic protocol predetermined by our department was applied to all patients scheduled to undergo LLDN. Accordingly, IV analgesic treatments with tramadol (1 mg/kg) and paracetamol (1 g) were routinely applied at the end of surgery and every 8 hours during the postoperative period. Participants with a pain score >5 were given additional IV paracetamol (1 g, maximum of 4 g daily) or IV tramadol (1 mg/kg), aside from routine analgesic treatments. Total analgesic consumption was recorded, which was the secondary outcome of the study. Side effects such as nausea, vomiting, pruritus, and use of antiemetic drugs were also noted. Demographic and other postoperative data were recorded. Participants with bleeding diathesis, use of anticoagulants, use of corticosteroids, use of antipsychotic drugs, allergy to local anesthesia, and any contraindication to peripheral block were excluded from the study.

Donor anesthesia and erector spinae plane block procedure

All donors were administered IV midazolam (0.05 mg/kg) as an anxiolytic after standard anesthesia monitoring in the preoperative period. For the ESPB group, first, the spinous process of the tenth thoracic vertebra was detected with an 8-MHz frequency linear ultrasonography probe in the prone position. Next, the transverse process of the tenth thoracic vertebra was observed by shifting approximately 3 cm laterally from this point, and the insertion area was anesthetized with a cutaneous-subcutaneous local anesthetic. An echogenic, 22-gauge 80-mm block needle (Stimuplex A, B Braun) was positioned in the craniocaudal direction between the transverse process and the erector spinae muscle using the in-plane technique. Normal saline (3 mL) was injected into this area to check the accuracy of the location, and a hydrodissection image was obtained. After confirmation, 20 mL of 0.25% bupivacaine HCl was injected bilaterally, and its distribution in the cephalocaudal direction between the muscle and transverse processes was observed. For the control group, only a standard postoperative pain management protocol (tramadol and paracetamol) was used.

After patients were in a supine position, IV fentanyl (2 μg/kg), IV pentothal (3-5 mg/kg), and IV

rocuronium bromide (0.4-0.6 mg/kg) were used for induction according to the LLDN operation anesthesia protocol. Anesthesia maintenance was provided by 4% to 6% desflurane (medical air 60% in oxygen), IV remifentanil (0.01-0.05 μg/kg/min), and IV rocuronium bromide (0.1 mg/kg infusion). Artery catheterization was performed as a standard to measure invasive blood pressure. All patients were extubated in the operating room, and their follow-ups and treatments were maintained in the recovery room.

Evaluation of pain

The verbal numerical rating scale (vNRS)[7-9] and the Wong-Baker Faces Pain Rating Scale (WBFS)[10] are routinely used for postoperative pain scoring at our center. Accordingly, the number chosen by the donors from 0 (no pain) to 10 (maximal pain) provided the vNRS score, in which patients are shown emoji analogs of the numbers, provided the WBFS score. Pain status of donors was assessed by experienced organ transplant nurses in the postoperative recovery room at 6, 12, and 24 hours postoperatively. All scoring results and analgesic medications were noted.

Laparoscopic living-donor nephrectomy procedure

The patient was placed in a lateral decubitus position, according to the procurement side of the kidney. We used the Hasson technique for the first trocar insertion. After a pneumoperitoneum with 12 to 15 mm Hg of pressure was ensured, then trocars were inserted with the guidance of the camera.

For left nephrectomy, trocars were inserted as follows: a 10-mm camera port from the left periumbilical region (T10 dermatome), a 5-mm trocar in the left midclavicular line from the subcostal region (T7 dermatome), and a 12-mm trocar in the left midclavicular line from the left lower quadrant (T11 dermatome).

The left colon and sigmoid were reflected medially, and the ureter and gonadal veins were identified. The dissection was performed along through the gonadal vein to the renal vein and continued to the identification of the adrenal vein. The renal vein was dissected from the surrounding tissue. In this step, the renal artery was also identified and dissected from the surrounding tissue. After that, the adrenal vein was ligated and cut. The adrenal gland was separated from the kidney, and the upper pole was dissected from the spleen. The ureter was dissected from the psoas muscle. The kidney, with its Gerota fascia, was dissected from the surrounding perirenal fat. By mobilizing the kidney, the renal artery and vein were dissected completely from the surrounding tissue. We used a vascular stapler (ECHELON FLEX powered vascular stapler; Ethicon) for transection of the renal artery and vein and a Hem-O-Lok clip (Weck Closure Systems) for ligation of the ureter distally. The kidney was extracted through a low transverse Pfannenstiel incision (T12 dermatome). For the right nephrectomy, the technique was similar to that for the left side, in addition to securing the gonadal and adrenal veins; in addition, a 5-mm trocar in the midaxillary line at the umbilical level (T10 dermatome) was inserted for liver retraction.

Statistical analyses

We used the Statistical Package for Social Sciences for Windows version 23.0 (IBM) and Microsoft Office Excel version 16.0 for statistical analyses. Categorical variables were compared using the Pearson chi-square test or the Fisher exact test, and data are presented as numbers and percentages. Normally distributed continuous data are presented as mean and SD, and nonnormal data are presented as median and interquartile range. We used the Kolmogorov-Smirnov or Shapiro-Wilk tests to evaluate the normality of the continuous data. We used the Mann-Whitney-Wilcoxon test to compare nonparametric data between the 2 groups. Variables that were used in the data included block group, age, sex, body mass index (BMI), numeric pain scores (at 1, 6, 12, and 24 h into the postoperative period), WBFPS (at 1, 6, 12, and 24 h into the postoperative period), administered total tramadol and paracetamol dosage, ASA score, vomiting, nausea, pruritus, administered dosage of metoclopramide, and the granisetron requirement. We performed multivariate linear regression analyses and Pearson correlation tests to identify the factors associated with analgesic consumption. Variables used in the multivariate regression analysis included block group, sex, age, BMI, and administered total tramadol and paracetamol dosages.

Results

Study participants

Eight-two consecutive donors who underwent LLDN in 2021 were included in the study. Among

these, 60 donors were investigated after exclusion of those who did not meet the inclusion criteria or who had missing follow-up data. The ESPB group comprised 30 donors, and the control group (treated with only postoperative medical analgesics) comprised 30 donors (Figure 1). No differences were found between age distributions and distributions between men and women, ASA score, BMI, and hospital stay (Table 1). No complications were observed in the intraoperative follow-up of either group. All participants were discharged without any major complications after their routine treatment in the postoperative period.

Pain intensity and characteristics

The same anesthesiologist successfully performed all ESPBs in the ESPB group. No block-related complications, such as bleeding or subcutaneous emphysema, were encountered during the intraoperative or postoperative periods. Resting pain for all participants was questioned during the postoperative period (at 1, 6, 12, and 24 h). Aside from routine practice, additional analgesic treatments were ordered if the pain score was >5. When we compared the ESPB group versus the control group at the specified time intervals, we observed no significant differences in vNRS and WBFS scores (Table 2). Two donors described right shoulder pain in the control group, but this description was not encountered in the ESPB group.

Table 1. Demographic Data of Donors

	ESPB Group	Control Group
Age, year	42.6 ±12.6	42 ±11.9
Male/female donor	10/20	7/23
ASA score I, No. (%)	26 (52)	24 (48)
ASA score II, No. (%)	4 (40)	6 (60)
BMI	27.0 (24.6-29.4)	25.3 (23.8-29.4)
Hospital stay, days	5 (5-6)	5 (5-6)

Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index (in kilograms divided by height in meters squared); ESPB, erector spinae plane block

Parametric data are presented as mean ± SD, and nonparametric data are presented as median (interquartile range).

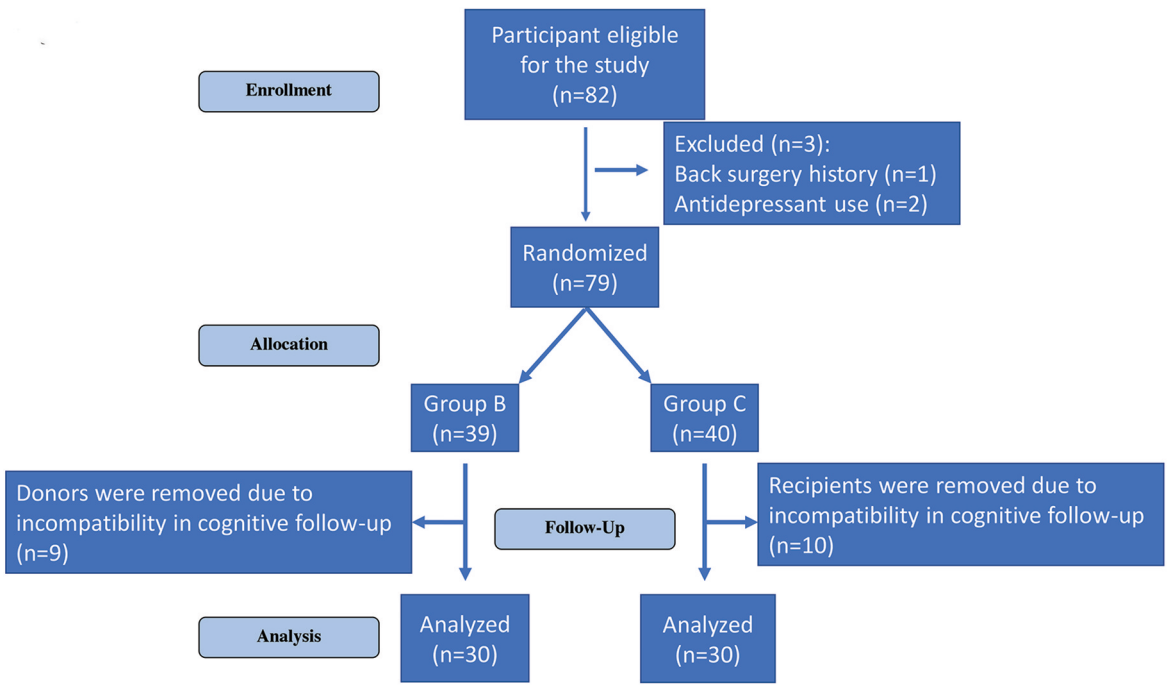
Table 2. Comparison of Pain Assessment Tools According to the Block Application

	ESPB Group	Control Group
vNRS, hour 1	7 (4-8)	6 (5-8)
vNRS, hour 1	5 ± 1.6	4 ± 2.4
vNRS, hour 12	5 ± 2.1	6 ± 2
vNRS, hour 24	3 (2-5)	3 (2-6)
WBFS, hour 1	6 (4-8)	6 (4-8)
WBFS, hour 6	4 ± 1.9	4 ± 2
WBFS, hour 12	5 (3-6)	5 (4-6)
WBFS, hour 24	3 (2-4)	3 (2-5)

Abbreviations: ESPB, erector spinae plane block; vNRS; verbal numerical rating scale, WBFS; Wong-Baker faces pain rating scale

Parametric data are presented as mean ± SD, and nonparametric data are presented as median (interquartile range).

Figure 1. CONSORT Flow Diagram of Study Design



Abbreviations: Group B, erector spinae plane block (ESPB) group; group C, control group

Opioid consumption

Pain management for all donors was done according to the postoperative analgesia protocol of our organ transplant ward. Donors requiring additional analgesia were noted, and, in accordance with level of pain, analgesics were ordered. When we compared analgesic consumption of both groups in the first 24 hours, the mean amount of tramadol used in the control group was higher than in in the ESPB group (P = .003; Table 3).

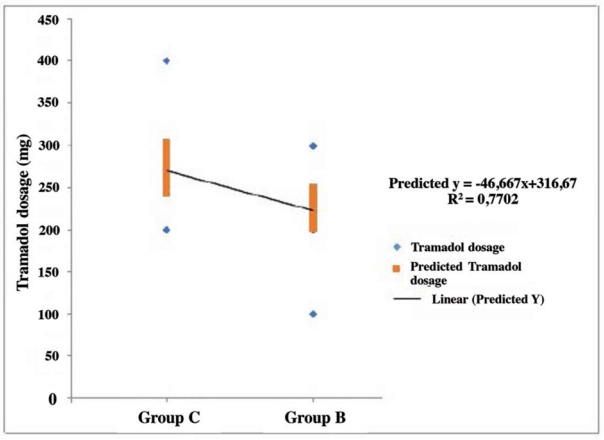
Table 3. Comparison of Analgesic Use and Analgesic-Related Adverse Drug Events

	ESPB Group	Control Group
Tramadol, mg	200 (200-300)*	300 (200-300)
Paracetamol, mg	3000 (2000-3000)	3000 (2750-3000)
Nausea, No. (%)	5 (17)	7 (23)
Vomiting, No. (%)	3 (10)	4 (13.3)
Pruritus, No. (%)	1 (3)	2 (7)

Abbreviations: ESPB, erector spinae plane block
Nonparametric data are presented as median (interquartile range). *P = .003.

We conducted a multiple linear regression to predict tramadol consumption based on age, sex, BMI, block application, and paracetamol consumption. A significant regression equation was found (F[5,54] = 2.74, P = .03), with an R2 of 0.203. The predicted tramadol consumption was calculated as follows: 266.353 – 49.836 (block application) – 0.159 (age) – 0.008 (paracetamol dosage) + 3.754 (sex) + 2.892 (BMI), where sex was coded as 1 = male and 2 = female and block was coded as 1 = not applied and 2 = applied. Only block application was a significant predictor of tramadol consumption. Tramadol consumption had been decreased 49.836 mg with block application (Table 4). Figure 2 shows total and predicted tramadol consumption according to the groups. The regression analysis confirmed that block application was associated with tramadol consumption in the ESPB group (P = .001). We questioned itching, nausea, and vomiting symptoms for all participants and noted antiemetic treatment (metoclopramide, or granisetron) for the first 24 hours (Table 3). We observed no differences between groups in terms of nausea, vomiting, or itching.

Figure 2. Linear Regression Model of Tramadol Consumption



Abbreviations: Group B, erector spinae plane block (ESPB) group; group C, control group

Discussion

The postoperative analgesia regimens used to treat the donors included in our study were standardized for both groups. Bilateral ESPB was applied to the ESPB group before induction of general anesthesia. When we compared pain scores in the postoperative period between groups, no significant differences were shown. We could not find any similar clinical trial investigating the effects of ESPB application in LLDN in the literature. However, in 2 cases, Piliego and colleagues[11] reported that ESPB was advantageous in terms of comfort and the simplicity of application in postoperative pain control following laparoscopic nephrectomy operations. Erector spinae plane block has also been used in renal transplant patients to reduce analgesic consumption.[12,13] As reported, 30 mL of 0.375% of bupivacaine applied unilaterally from the level of the T9 transverse process before the incision resulted in successful perioperative pain control. The studies also reported that vNRS score could decrease to 0 after ESPB was performed with 27 mL of unilaterally applied ropivacaine 0.25% at the T8 to T9 level during the period after renal transplant, with the effect of tramadol continuing for 24 hours.

Epidural anesthesia, which is the gold standard for pain control in abdominal surgeries, is not preferred for living-donor hepatectomies due to

Table 4. Regression Coefficients for Predicting Tramadol Consumption After Laparoscopic Donor Nephrectomy

Variable	B	95% CI	β	t	P
Block application (no = 1, yes = 2)	-49.8	-79.6 to -20.1	-0.42	-3.4	.001

Adjusted R^2 = 0.13 (n = 59, P = .028).

postoperative coagulopathy in living liver donors.[14] Continuous bilateral ESPB at the seventh transverse process level was associated with less analgesic consumption during the postoperative 3 days compared with the control group in right-sided living-donor hepatectomies. However, pain scores were lower than in the control group only at day 2, and postoperative pain scores showed no differences on the day of surgery and at days 1, 3, and 4.15 Similarly, our study did not find a difference in pain scores in the first 24 hours and observed that opioid consumption was lower in the ESPB group.

Erector spinae plane block was applied to 10 patients who underwent a laparoscopic hysterectomy and was found to be a safe procedure.[16] However, similar to our results, in a systemic review investigating the effectiveness of ESPB in reducing postoperative pain after cesarean section, the block showed no significant difference compared with other methods.[17]

Erector spinae plane block is commonly used in laparoscopic cholecystectomy operations in abdominal surgeries[18-20] and has been successful in mitigating postoperative pain and positively affecting total analgesic consumption. In a meta-analysis from Daghmouri and colleagues,6 bilateral ESPB for postoperative pain control in laparoscopic cholecystectomy operations decreased analgesia consumption and was a safe method. However, ESPB has been shown to be effective only for a short time in the postoperative period following percutaneous nephrolithotomy operations, and it was not superior to IV analgesia applications for pain control at 24 hours.[21]

As is well known, ESPB is a paraspinal fascial plane block.[5] The local anesthetic administered between the erector spinae muscle and thoracic transverse processes is spread at 3 to 6 vertebral levels in the craniocaudal direction. This subsequently blocks the spinal nerves in the thoracic and abdominal dorsal and ventral rami and provides somatic-visceral analgesia.[22] According to case reports on laparoscopic nephrectomy and renal transplant, ESPB was effective after being administered to T8 to T9.[11-13] Because our surgical incision level was between T7 and T12 and renal sensation originates from T10 to T11, we performed ESPB at T10.[23] Thus, the multidermatome innervation of the anterior, posterior, and lateral thoracic and abdominal walls at the T7 to T12 levels would have been reduced. According to our hypothesis, inadequate postoperative pain control following LLDN suggests that the application of local anesthetic at the bilateral T10 level may be insufficient for diffusion into the retroperitoneal nerves. Nevertheless, this might be due to the pneumoperitoneum accompanying phrenic neuropraxia. Further studies are needed to support this hypothesis.

The literature indicates that ESPB notably decreases the use of analgesics.[6,17,24] Furthermore, postoperative analgesia strategies aim to provide pain control with the most negligible side effects, especially among donors. In addition to regional techniques, opioid use may lead to adverse effects, such as nausea, vomiting, and itching.[25] In the present study, although there were no significant differences in nausea, vomiting, or antiemetic use between the groups, postoperative 24-hour total tramadol use was lower in the ESPB group versus the control group, which supports the literature. Therefore, ESPB can be considered an option to prevent recurrent opioid use.

Our study had some limitations. Two different pain assessment tools were used in the postoperative period. The aim was to conclude the pain assessment more accurately. However, these tools were used while patients were at rest, and the pain level during movement was not evaluated. We believe that investigating the ESPB's effectiveness during movement will be valuable in terms of ESPB application in future studies on LLDN operations. Furthermore, the total intraoperative amount of remifentanil was not compared between the groups due to adjustments in infusion dosage. Therefore, the effects of ESPB application on pain due to surgery are unknown.

Conclusions

Ultrasonograph-guided bilateral ESPB is a safe and simple method of pain relief that can be used during LLDN surgery. The present study demonstrated that total opioid use over 24 hours decreased in donors who received the block application, thus supporting the literature. However, when we compared pain scores between the ESPB group and control group, there was no significant effect on reducing postoperative pain or increasing donor comfort. Therefore, more studies on the routine use of ESPB in LLDN operations are needed.

References

1. Minnee RC, Idu MM. Laparoscopic donor nephrectomy. *Neth J Med.* 2010;68(5):199-206.
2. Sao CH, Chan-Tiopianco M, Chung KC, et al. Pain after laparoscopic surgery: Focus on shoulder-tip pain after gynecological laparoscopic surgery. *J Chin Med Assoc.* 2019;82(11):819-826. doi:10.1097/JCMA.0000000000000190
3. Kang R, Chin KJ, Kim GS, et al. Bilateral continuous erector spinae plane block using a programmed intermittent bolus regimen versus intrathecal morphine for postoperative analgesia in living donor laparoscopic hepatectomy: a randomized controlled trial. *J Clin Anesth.* 2021;75:110479. doi:10.1016/j.jclinane.2021.110479
4. Rawal N. Current issues in postoperative pain management. *Eur J Anaesthesiol.* 2016;33(3):160-171. doi:10.1097/EJA.0000000000000366
5. Forero M, Adhikary SD, Lopez H, Tsui C, Chin KJ. The erector spinae plane block: a novel analgesic technique in thoracic neuropathic pain. *Reg Anesth Pain Med.* 2016;41(5):621-627. doi:10.1097/AAP.0000000000000451
6. Daghmouri MA, Akremi S, Chaouch MA, et al. Bilateral erector spinae plane block for postoperative analgesia in laparoscopic cholecystectomy: a systematic review and meta-analysis of randomized controlled trials. *Pain Pract*. 2021;21(3):357-365. doi:10.1111/papr.12953
7. Bijur PE, Latimer CT, Gallagher EJ. Validation of a verbally administered numerical rating scale of acute pain for use in the emergency department. *Acad Emerg Med.* 2003;10(4):390-392. doi:10.1111/j.1553-2712.2003.tb01355.x
8. Hjermstad MJ, Fayers PM, Haugen DF, et al. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: a systematic literature review. *J Pain Symptom Manage.* 2011;41(6):1073-1093. doi:10.1016/j.jpainsymman.2010.08.016
9. Bahreini M, Jalili M, Moradi-Lakeh M. A comparison of three self-report pain scales in adults with acute pain. *J Emerg Med.* 2015;48(1):10-18. doi:10.1016/j.jemermed.2014.07.039
10. Stinson JN, Kavanagh T, Yamada J, Gill N, Stevens B. Systematic review of the psychometric properties, interpretability and feasibility of self-report pain intensity measures for use in clinical trials in children and adolescents. *Pain.* 2006;125(1-2):143-157. doi:10.1016/j.pain.2006.05.006
11. Piliego C, Longo F, Agro FE. Erector spinae plane block growing potential: pain management in laparoscopy nephrectomy. *Saudi J Anaesth.* 2020;14(2):275-276. doi:10.4103/sja.SJA_43_20
12. Aytuluk HG, Kara MY. Erector spinae plane block for postoperative analgesia in kidney transplantation: a report of 3 cases. *J Clin Anesth.* 2020;61:109683. doi:10.1016/j.jclinane.2019.109683
13. Temirov T, Ben-David B, Mustafin A, Viderman D. Erector spinae plane block in management of pain after kidney transplantation. *Pain Med.* 2019;20(5):1053-1054. doi:10.1093/pm/pny221
14. Karna ST, Pandey CK, Sharma S, Singh A, Tandon M, Pandey VK. Postoperative coagulopathy after live related donor hepatectomy: incidence, predictors and implications for safety of thoracic epidural catheter. *J Postgrad Med.* 2015;61(3):176-180. doi:10.4103/0022-3859.159419
15. Adelmann D, Khorashadi M, Zhou G, et al. The use of bilateral continuous erector spinae plane blocks for postoperative analgesia after right-sided living donor hepatectomy: a feasibility study. *Clin Transplant.* 2021;35(9):e14413. doi:10.1111/ctr.14413
16. Frassanito L, Zanfini BA, Catarci S, Sonnino C, Giuri PP, Draisci G. Erector spinae plane block for postoperative analgesia after total laparoscopic hysterectomy: case series and review of the literature. *Eur Rev Med Pharmacol Sci.* 2020;24(7):3892-3897. doi:10.26355/eurrev_202004_20855
17. Ribeiro Junior IDV, Carvalho VH, Brito LGO. Erector spinae plane block for analgesia after cesarean delivery: a systematic review with meta-analysis. *Braz J Anesthesiol.* 2022;72(4):506-515. doi:10.1016/j.bjane.2021.09.015
18. Verma R, Srivastava D, Saxena R, et al. Ultrasound-guided bilateral erector spinae plane block for postoperative analgesia in laparoscopic cholecystectomy: a randomized controlled trial. *Anesth Essays Res.* 2020;14(2):226-232. doi:10.4103/aer.AER_41_20
19. Ibrahim M. Erector spinae plane block in laparoscopic cholecystectomy, is there a difference? a randomized controlled trial. *Anesth Essays Res.* 2020;14(1):119-126. doi:10.4103/aer.AER_144_19
20. Kwon HM, Kim DH, Jeong SM, et al. Does erector spinae plane block have a visceral analgesic effect?: a randomized controlled trial. *Sci Rep.* 2020;10(1):8389. doi:10.1038/s41598-020-65172-0
21. Bryniarski P, Bialka S, Kepinski M, Szelka-Urbanczyk A, Paradysz A, Misiolek H. Erector spinae plane block for perioperative analgesia after percutaneous nephrolithotomy. *Int J Environ Res Public Health.* 2021;18(7):3625. doi:10.3390/ijerph18073625
22. Chin KJ, El-Boghdadly K. Mechanisms of action of the erector spinae plane (ESP) block: a narrative review. *Can J Anaesth.* 2021;68(3):387-408. doi:10.1007/s12630-020-01875-2
23. Peate I, Nair M. The kidney: microscopic. In: Peate I, Nair M, ed. *Anatomy and Physiology for Nurses at a Glance.* 1st ed. Wiley-Blackwell; 2015:73-75.
24. Tulgar S, Kapakli MS, Senturk O, Selvi O, Serifsoy TE, Ozer Z. Evaluation of ultrasound-guided erector spinae plane block for postoperative analgesia in laparoscopic cholecystectomy: a prospective, randomized, controlled clinical trial. *J Clin Anesth.* 2018;49:101-106. doi:10.1016/j.jclinane.2018.06.019
25. Shin HJ, Oh AY, Baik JS, Kim JH, Han SH, Hwang JW. Ultrasound-guided oblique subcostal transversus abdominis plane block for analgesia after laparoscopic cholecystectomy: a randomized, controlled, observer-blinded study. *Minerva Anestesiol.* 2014;80(2):185-193.

ARTICLE

Factors Affecting Timeline of Kidney Transplant Evaluation Process in Saudi Arabia

Zainab Habibullah, Muhammed Bukhari

Abstract

Objectives: **Patients who reach the terminal phase of renal disease are candidates for kidney transplant. However, the pretransplant process is substantial and requires time-intensive evaluations. We aimed to investigate the factors that affect the timeline for evaluation of kidney transplants and to identify the challenges and recommendations for improvement of the evaluation process in Saudi Arabia.**

Materials and Methods: **For the first part of the study, we collected retrospective data from all patients who underwent kidney transplant from 2017 to 2022 at King Abdullah Medical City, Makkah, Saudi Arabia. For the second part of the study, we used a cross-sectional design to collect health workers' perceptions of the factors, recommendations, and challenges in the kidney transplant evaluation process at King Abdullah Medical City.**

Results: **The average number of specialty clinic visits was 7.43, and the average number of clinic visits was 21.37. The mean wait time for surgery was 185.5 days (SD, 106.91 days). There was a significant positive correlation between the wait time for surgery and the total number of clinics visited (r = 0.286, *P* = .026).**

Conclusions: **This study provides valuable insights into the factors that may affect kidney transplant evaluation in Saudi Arabia, and our results emphasize the impor-tance of adequate resources and effective teamwork.**

Key words: *End-stage renal disease, Evaluation timeline, Transplantation, Transplant wait time*

From King Abdullah Medical City, Makkah, Kingdom of Saudi Arabia

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: *Zainab Habibullah, King Abdullah medical city, Makkah, KSA*
E-mail: *zainabnsh@yahoo.com*

Experimental and Clinical Transplantation (2025) 8: 530-534

DOI: 10.6002/ect.2025.0121

Introduction

Patients with advanced renal disease are candidates for kidney transplant, which is the treatment of choice. Patients who can locate a living donor have the opportunity to receive a transplant sooner than those awaiting a deceased donor kidney transplant. Furthermore, living donor transplant improves morbidity, cost-effectiveness, and long-term survival rates.

Patients must first undergo a comprehensive and exhaustive kidney transplant evaluation process to be considered a transplant candidate. This process is a multi-step procedure designed to determine whether a person is a suitable candidate for kidney transplant. This process involves several stages, including initial evaluation, medical testing, donor evaluation, and matching.[1,2]

The timeline for the kidney transplant evaluation process can vary depending on several factors, including individual medical history, the availability of donors, and the complexity of the medical tests required. Some patients may be able to complete the process within a few months, whereas other patients may require several years.[3]

In addition to patient factors, hospital factors and health system factors can greatly affect the outcome of the kidney transplant evaluation process, with such factors greatly varying among regions and among hospitals. Patients should work closely with health care teams to understand the hospital factors and system factors that may affect their transplant evaluation process and thereby address potential challenges.[3,4]

No previously published studies have studied the estimated timeline for kidney transplant, the associated challenges, or the opportunities for enhancement of the transplant evaluation process. Therefore, this study aimed to identify factors that may reduce the time required for the transplant evaluation process, to

improve the clinical outcomes of patients who presently receive dialysis in Saudi Arabia.[2]

Materials and Methods

Study design and participants

The first part of our study was the collection of retrospective data for all patients who underwent living related (first-degree relative or spouse) kidney transplant from 2017 to 2022 at King Abdullah Medical City, Makkah, Saudi Arabia. Kidney transplant recipients who had undergone transplant surgery outside our institute were excluded from the study.

The second part of our study was the application of a cross-sectional design to collect health care workers' perceptions of the factors, recommendations, and challenges in the kidney transplant evaluation process at King Abdullah Medical City. This study was approved by the Institutional Review Board of King Abdullah Medical City (approval No. 22-981).

Data collection

Data were collected from medical records, including clinical characteristics (age, sex, ABO blood type, comorbidities, and renal disease), date of start of transplant evaluation process, and date of transplant surgery, in addition to the number and specialty of various clinic visits. All transplants were living related kidney transplants, and all transplants were performed exclusively at our center.

A self-administered questionnaire was used by health care workers to identify challenges and recommendations in the kidney transplant process. The questionnaire included 7 domains with 26 closed-ended questions that required responses on an ordinal scale, as well as 2 open-ended questions that required the respondent to provide their personal view of the challenges and recommendations regarding the transplant evaluation process in Saudi Arabia. The questionnaire was developed from material published in previous studies[5,6] and was electronically distributed to all employees of the King Abdullah Medical City.

Study outcomes

This study investigated factors that affect the timeline for the evaluation of kidney transplants. Moreover, this study identified challenges and recommendations for improvement of the timeline of the transplant evaluation process in Saudi Arabia.

Statistical analyses

We used SPSS software for data analyses, including descriptive and inferential analyses. We used mean values, frequencies, numbers, and standard deviations of the data for descriptive statistics. We measured differences between groups and correlations with inferential statistics.

Results

Patient data collection

Table 1 lists the demographic and clinical characteristics of the patients who underwent kidney transplant. There were more female patients (58.7%) than male patients (41.3%), and most patients (84.75%) were from the city of Makkah.

Table 1. Kidney Transplant Cases

Characteristic	Frequency	Percentage
Sex		
Female	19	41.30%
Male	27	58.70%
City in Saudi Arabia		
Abha	2	4.35%
Aljouf	2	4.35%
Arar	1	2.17%
Jizan	1	2.17%
Makkah	39	84.78%
Taif	1	2.17%
ABO blood type		
A	17	36.96%
AB	4	8.70%
B	5	10.87%
O	20	43.48%
Renal disease		
APCKD	1	2.17%
Congenital	1	2.17%
DM	15	32.61%
Drug induced	2	4.35%
GN	4	8.70%
Hypertension	8	17.39%
Obstruction	1	2.17%
Unknown	14	30.43%

Abbreviations: APCKD, autosomal dominant polycystic kidney disease; DM, diabetes mellitus; GN, glomerulonephritis

Table 2 provides important information on the average characteristics for kidney transplant cases. The results show that the mean age at transplant was 42.48 years, the mean number of specialty clinic visits was 7.43, and the mean number of total clinic visits was 21.37. The mean wait time for surgery was 185.5 days (SD, 106.91 days).

Table 3 shows a positive correlation between wait time to transplant surgery and the total number of clinic visits (r = 0.286, P = .026). This correlation suggests that patients who visited more clinics during their evaluation process tended to experience longer wait times for transplant surgery. However, there was no significant correlation between wait time for surgery and the number of specialty clinics visited (r = 0.213, P = .153), which indicated that the wait time is not affected by the number of different specialty clinics visited.

Table 4 shows the correlation between wait time for transplant surgery and clinical characteristics. The results showed that male sex, Makkah city residency, B blood group type, and autosomal dominant polycystic kidney disease were associated with longer wait times compared with other subgroups. However, these results were not significant.

Health care worker questionnaire

Demographic data of the participants showed 41%, 50%, and 69% of questionnaire respondents were male employees, were members of the nursing staff, and were not from the nephrology department, respectively. Respondents had a mean age and experience of 38.12 years and 5.64 years, respectively (Table 5).

Table 2. Characteristics for Kidney Transplant Cases

Characteristic	Mean Value	SD
Age at transplant, y	42.48	12.31
No. of specialty clinics	7.43	1.78
No. of clinic visits	21.37	10.06
Wait time before surgery, d	185.5	106.91

Table 3. Correlation Between Wait Time for Transplant Surgery and Number of Clinic Visits: Pearson Correlation and *P* Value

Characteristic	Correlation With Wait Time Before Surgeryr	
	r	*P*
Total No. of clinic visits	0.286	.026
Total No. of specialty clinics	0.213	.153

$P < .05$ is significant.

Table 5. Participant Demographics

Characteristic	Value
Sex, No. (%)	
Female	39 (57%)
Male	28 (41%)
Profession, No. (%)	
Nurse	34 (50%)
Physician	33 (49%)
Technician	1 (1%)
Specialty, No. (%)	
Nephrology	20 (29%)
Not nephrology	47 (69%)
Age, mean (SD), y	38.12 (7.85)
Experience, mean (SD), y	5.64 (5.83)

Table 4. Comparison of Wait Times and Clinical Characteristics

Characteristic	Total No. of Clinic Visits		Total No. of Specialty Clinics		Wait Time Before Surgery, d		*P*
	Mean	SD	Mean	SD	Mean	SD	
Sex							.9112
Female	26.05	11.71	156.00	2.04	183.37	94.11	
Male	18.07	7.28	186.00	1.37	187.00	116.81	
City							.0531
Abha	21.00	9.90	18.00	4.24	108.50	13.44	
Aljouf	15.00	8.49	14.00	1.41	80.00	4.24	
Arar	15.00	0.00	7.00	0.00	191.00	0.00	
Jizan	16.00	0.00	5.00	0.00	80.00	0.00	
Makkah	22.18	10.47	291.00	1.71	199.10	109.71	
Taif	15.00	0.00	7.00	0.00	120.00	0.00	
ABO blood type							.506
A	19.53	7.70	121.00	1.50	208.06	138.95	
AB	18.25	7.89	30.00	1.29	140.75	54.54	
B	26.20	6.91	46.00	2.39	217.20	103.27	
O	22.35	12.56	145.00	1.80	167.35	81.29	
Renal disease							.133
APCKD	35.00	0.00	13.00	0.00	290.00	0.00	
Congenital	36.00	0.00	5.00	0.00	228.00	0.00	
DM	23.87	11.33	119.00	1.16	167.40	94.15	
Drug induced	19.00	1.41	16.00	1.41	108.50	47.38	
GN	14.75	9.07	28.00	3.37	160.75	28.79	
Hypertension	16.63	5.24	52.00	1.07	155.63	101.81	
Obstruction	31.00	0.00	10.00	0.00	120.00	0.00	
Unknown	20.93	10.24	99.00	1.33	234.21	134.84	

Abbreviations: APCKD, autosomal dominant polycystic kidney disease; DM, diabetes mellitus; GN, glomerulonephritis

The participants identified heavy workload, poor patient medical condition, and insufficient staff as the highest perceived challenges in the kidney transplant evaluation process in 47%, 46%, and 37% of responses, respectively (Table 6). Moreover, participants identified increases in the amount of equipment and number of beds, improvement of staff education on protocol and guidelines, and an increase in the number of staff as the main recommendations to improve the kidney transplant evaluation process by 51%, 40%, and 37%, respectively (Table 7).

Table 6. Challenges in the Kidney Transplant Evaluation Process

Statement	Frequency	Percentage
Provider workload	32	47%
Patient medical conditions	31	46%
Lack of adequate staff and resources	25	37%
Limited beds	24	35%
Patient incompliance with follow-up appointments	21	31%
Lack of proper knowledge among patients and families	16	24%
Provider incompliance with health care policies and protocols	15	22%
Lack of collaboration by patients' families	13	19%
Lack of collaboration by families	12	18%
Lack of standardized engagement for patients in treatment plans	12	18%
Patients' incompliance with medical instruction	11	16%

Table 7. Recommendations to Improve the Kidney Transplant Evaluation Process

Statement	Frequency	Percentage
Increase the amount of equipment and number beds	35	51%
Educate staff about the protocol and guidelines	27	40%
Increase the number of staff	25	37%
Improve education and engagement of patients and families	21	31%
Timely use of multidisciplinary clinics	19	28%
Audit of protocol compliance	17	25%

Discussion

The results of this study showed that the average wait time for kidney transplant in a Saudi Arabia hospital was 185.5 days (SD, 106.91 days), which is consistent with the results of other studies that have shown a range of 129.4 to 164.7 days.[7,8]

The significant correlation between wait time for surgery and the total number of clinics visited is consistent with several previous studies,[9,10] which may reflect a complex medical history and the need for additional interventions before surgery.

However, our study did not find a significant correlation between the wait time for surgery and the number of specialty clinics visited. These results differ from those of previous studies that reported a correlation between wait times for kidney transplant surgery and the number of visits to specific medical specialties such as cardiology, gastroenterology, and infectious diseases.[11] The lack of this correlation in the present study may be due to differences in the study population or in the evaluation process used to determine the need for specialty clinic visits.

The longer wait times for some subgroups, specifically male patients and patients from the Makkah region, were not statistically significant. However, our study found that patients in blood group B and patients with autosomal dominant polycystic kidney disease had longer wait times, which is consistent with previous research that has identified these factors as potential barriers to timely kidney transplant.[12,13]

Our study sheds light on the challenges faced by Saudi Arabia health care providers during the kidney transplant evaluation process. The participants perceived that workload, medical condition of patients, and lack of sufficient staff were the most substantial challenges. These results are consistent with previous studies that have identified workload and staff issues as substantial barriers to high-quality care during the transplant evaluation process.[14,15]

Our study found that an increase of sufficient materials, a greater number of staff, enhanced unification of protocols and guidelines, and better use of multidisciplinary teams are the main recommendations for improvement of the kidney transplant evaluation process. These findings are consistent with previous research that has emphasized the importance of adequate resources, sufficient staff, and effective teamwork to ensure efficient kidney transplant evaluation and management.[16,17] To overcome the long wait times related to multiple clinic visits, we suggest establishment of 1-day multidisciplinary coordinator clinics, as studied by Formica et al,[18] as well as greater standardization of the protocols and guidelines for evaluation of kidney transplants across Saudi Arabia.[19]

Our study had some limitations. First, this study included a single transplant center in Makkah, Saudi Arabia; therefore, the results may not be generalizable to other populations. Future studies should analyze additional factors that may influence the evaluation process across multiple centers, to compare the evaluation process and wait times for kidney transplant surgery.

Conclusions

This study reviewed the evaluation process for kidney transplants in Saudi Arabia, with an aim to discover factors related to the timeline of the kidney transplant evaluation process, as well as the challenges faced by health care providers. These results informed some recommendations to improve the health care system in Saudi Arabia and other countries, which potentially reduce the wait period and improve the quality of care provided during the kidney transplant evaluation process.

References

1. Massie AB, Leanza J, Fahmy LM, et al. A risk index for living donor kidney transplantation. *Am J Transplant*. 2016;16(7):2077-84. doi: 10.1111/ajt.13709
2. Kasiske BL. OPTN/SRTR 2016 annual data report: deceased organ donation. *Am J Transplant*. 2018;18:434-463. doi:10.1111/ajt.14563.
3. de Jong RW, Stel VS, Heaf JG, Murphy M, Massy ZA, Jager KJ. Non-medical barriers reported by nephrologists when providing renal replacement therapy or comprehensive conservative management to end-stage kidney disease patients: a systematic review. *Nephrol Dial Transplant*. 2021;36(5):848-862. doi:10.1093/ndt/gfz271
4. Sandal S, Charlebois K, Fiore JF, et al. Health professional-identified barriers to living donor kidney transplantation: a qualitative study. *Can J Kidney Health Dis*. 2019;6:2054358119828389. doi:10.1177/2054358119828389
5. Hoffmann SC, Stanley EM, Cox ED, et al. Ethnicity greatly influences cytokine gene polymorphism distribution. *Am J Transplant*. 2002;2(6):560-567. doi:10.1034/j.1600-6143.2002.20611.x
6. Young CJ, Gaston RS. Understanding the influence of ethnicity on renal allograft survival. *Am J Transplant*. 2005;5(11):2603-2604. doi:10.1111/j.1600-6143.2005.01118.x
7. Meier-Kriesche HU, Port FK, Ojo AO, et al. Effect of waiting time on renal transplant outcome. *Kidney Int*. 2000;58(3):1311-1317. doi:10.1046/j.1523-1755.2000.00287.x
8. Bornemann K, Croswell E, Abaye M, et al. Protocol of the KTFT-TALK study to reduce racial disparities in kidney transplant evaluation and living donor kidney transplantation. *Contemp Clin Trials*. 2017;53:52-59. doi:10.1016/j.cct.2016.11.011
9. Husain SA, Brennan C, Michelson A, et al. Patients prioritize waitlist over posttransplant outcomes when evaluating kidney transplant centers. *Am J Transplant*. 2018;18(11):2781-2790. doi:10.1111/ajt.14985
10. Locke JE, Reed RD, Massie A, et al. Obesity increases the risk of end-stage renal disease among living kidney donors. *Kidney Int*. 2017;91(3):699-703. doi:10.1016/j.kint.2016.10.014
11. Kotta PA, Elango M, Papalois V. Preoperative cardiovascular assessment of the renal transplant recipient: a narrative review. *J Clin Med*. 2021;10(11):2525. doi:10.3390/jcm10112525
12. Lim WH, Ooi E, Pilmore HL, et al. Interactions between donor age and 12-month estimated glomerular filtration rate on allograft and patient outcomes after kidney transplantation. *Transpl Int*. 2022;35:10199. doi:10.3389/ti.2022.10199
13. Danovitch GM, Hariharan S, Pirsch JD, et al. Management of the waiting list for cadaveric kidney transplants: report of a survey and recommendations by the Clinical Practice Guidelines Committee of the American Society of Transplantation. *J Am Soc Nephrol*. 2002;13(2):528-535. doi:10.1681/ASN.V132528
14. Concepcion BP, Alasfar S, Levea SL, Singh P, Wiseman A. The transplant nephrology workforce in the united states: current state and future directions. *Adv Chronic Kidney Dis*. 2020;27(4):336-343.e1. doi:10.1053/j.ackd.2020.05.005
15. Israni AK, Zaun D, Rosendale JD, Schaffhausen C, Snyder JJ, Kasiske BL. OPTN/SRTR 2016 Annual Data Report: deceased organ donation. *Am J Transplant*. 2018;18(suppl 1):434-463. doi:10.1111/ajt.14563
16. Fonouni H, Golriz M, Mehrabi A, et al. The role of an interdisciplinary transplant team on living donation kidney transplantation program. *Transplant Proc*. 2010;42(1):137-140. doi:10.1016/j.transproceed.2009.12.038
17. Watford DJ, Cheng XS, Han J, Stedman MR, Chertow GM, Tan JC. Toward telemedicine-compatible physical functioning assessments in kidney transplant candidates. *Clin Transplant*. 2021;35(2):e14173. doi:10.1111/ctr.14173
18. Formica RN Jr, Barrantes F, Asch WS, et al. A one-day centralized work-up for kidney transplant recipient candidates: a quality improvement report. *Am J Kidney Dis*. 2012;60(2):288-294. doi:10.1053/j.ajkd.2012.04.008
19. Pham PT, Pham PA, Pham PC, Parikh S, Danovitch G. Evaluation of adult kidney transplant candidates. *Semin Dial*. 2010;23(6):595-605. doi:10.1111/j.1525-139X.2010.00809.x

Effect of Hepatic Arterial Reconstruction prior to On-Site Normothermic Machine Perfusion in Donation after Circulatory Death Liver Transplant

Jaffe Emily,[1,2] Toshihide Tomosugi,[1] Alban Longchamp,[1] Richard Teo,[1] Shoko Kimura,[1] John Montgomery,[3] Lyle Burdine,[3] James Markmann,[4] Heidi Yeh,[1] Tsukasa Nakamura[1,3]

Abstract

Objectives: **On-site normothermic machine perfusion of the liver may require hepatic arterial reconstruction. The effect of arterial reconstruction on the development of primary ischemic cholangiopathy has not been fully elucidated in liver transplants with organs donated after circulatory death. The aim of this study was to evaluate the effect of normothermic machine perfusion with arterial reconstruction at the onset of ischemic cholangiopathy in liver transplants with organs donated after circulatory death.**

Materials and Methods: **We retrospectively reviewed 93 patients who had received liver transplants donated after circulatory death for the period from 2015 to 2023 at a single institution. The primary endpoint was the onset of primary ischemic cholangiopathy within 1 year after donation after circulatory death liver transplant, excluding secondary ischemic cholangiopathy due to arterial complications.**

Results: **Normothermic machine perfusion was used for 71 cases, whereas standard cold storage was applied for 22 cases. Arterial reconstruction was performed in 14.1% of cases versus 27.3% of cases without normothermic machine perfusion. The cumulative onset of ischemic cholangiopathy was 7.0% versus 27.2% without normothermic machine perfusion (*P* = .013). In the group with normothermic machine perfusion, competing risk analyses demonstrated that the cumulative ischemic cholangiopathy onset rate was significantly higher in the group with arterial reconstruction (30.0%) versus without arterial reconstruction (3.3%) (*P* < .003). Total cold ischemia time and cold ischemia time between liver recovery and normothermic machine perfusion initiation were significantly longer in the group with arterial reconstruction (*P* < .001), without significant differences in arterial flow on normothermic machine perfusion and other relevant factors.**

Conclusions: **In donation after circulatory death liver transplant recipients with normothermic machine perfusion, arterial reconstruction is a risk factor for developing ischemic cholangiopathy, likely mediated by cold ischemia time prolongation.**

Key words: *Artificial perfusion, Cold ischemia time, Extended criteria donor liver, Ischemic cholangiopathy, Liver transplantation*

Introduction

The use of normothermic machine perfusion (NMP) of the liver is rapidly growing in popularity and thereby has enabled the substantial expansion of the donor pool and subsequent number of recipient candidates. Normothermic machine perfusion potentially reduces the risks of critical conditions, including primary nonfunction of the liver, early liver graft dysfunction, and primary ischemic cholangiopathy (IC) in liver transplant (LT).[1,2] However, NMP may still carry unique risks for developing IC, such as increased duration of total cold ischemia time (CIT), increased duration of CIT before initiation of NMP (pre-NMP CIT), multiple rounds of ischemia-reperfusion, or warm ischemia of the liver in NMP conduit malfunction cases.

Hepatic arterial reconstruction (AR) is required in cases for which more than 2 separated arterial conduits exist due to variants of the hepatic artery to avert warm ischemia injury.[3] Two methods are used to prevent warm ischemia injury on NMP: (1) surgical reconstruction with anastomosis, including

From the [1]Division of Transplant Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; the [2]Sackler School of Medicine at Tel Aviv University, Tel Aviv, Israel; the [3]Division of Transplant Surgery, Department of Surgery, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and the [4]Penn Transplant Institute, the University of Pennsylvania, Philadelphia, Pennsylvania, 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: *Tsukasa Nakamura, 4301 W. Markham St. Little Rock, AR, USA*
Phone: *+1 501 686 5164* ***E-mail:*** *tnakamura@uams.edu*

Experimental and Clinical Transplantation (2025) 8: 535-541

DOI: 10.6002/ect.2025.0140

both definitive reconstruction and temporary reconstruction with subsequent definitive reconstruction before implant; or (2) tubing technique to connect the arteries temporarily,[4] in addition to creating multiple arterial outlets in the NMP machine. On-site definitive surgical reconstruction is used at our institution because this procedure can avoid an additional period of CIT. The risks for IC would be higher in cases of on-site NMP with AR, where surgeons and surgical tools are limited and transportation is required. In addition, the presence of variants of the artery and subsequent reconstruction may lead to prolongation of donor hepatectomy time (DHT) and CIT, which has been shown to worsen LT outcomes.[5,6] However, the effect of hepatic AR prior to NMP on the development of IC has not been fully elucidated.

Therefore, we sought to investigate the effects of NMP after AR on the onset of primary IC, excluding secondary IC due to arterial complications, to facilitate safe NMP practices in the field of LT.

Materials and Methods

Study population

We retrospectively enrolled LT recipients who had received donation organs after circulatory death (DCD) during the period from January 2015 to December 2023 at Massachusetts General Hospital. All research was performed in a manner that conformed with the Declaration of Istanbul and the Declaration of Helsinki. The ethics committee of Massachusetts General Hospital approved this study; all enrolled patients provided informed consent at the time of surgery. The DCD LT cases were divided into groups based on the use of NMP (NMP+) versus standard cold storage (NMP-) and LT with AR (AR+) and LT without AR (AR-). The onset of clinically relevant primary IC (non-anastomotic biliary strictures without compromising hepatic arterial flow), diagnosed by magnetic resonance cholangiography and/or endoscopic retrograde cholangiography and/or percutaneous transhepatic cholangiography, was considered the primary endpoint, in the short-term within 1 year after DCD LT, excluding secondary IC due to arterial complications. All patients in this cohort were followed up 1 year after LT. Types of IC were categorized according to a previous study.[7] In our study population there were IC with diffuse necrosis, multifocal progressive IC, and confluence dominant IC. Types of biliary complications (BCs) were defined as anastomotic strictures, excluding IC or bile leak.

Arterial reconstruction

Livers were procured with the entire length of the celiac artery and variant hepatic arteries in a standard fashion. For application of on-site NMP, prior to AR, standard back-table preparation of the liver was performed in all cases. Aberrant arteries were carefully identified and skeletonized. The AR was performed for all replaced hepatic arteries; there was no case where a replaced artery was discarded. As an inflow outlet, the splenic artery stumps or the gastroduodenal artery stumps were used according to the size of aberrant arteries in this study. The AR was performed using 7-0 or 8-0 polypropylene sutures with the aid of surgical loupes on a back table by expert surgeons in microsurgery. Arterial cannulation was performed from the celiac trunk so that a typical aberrant left hepatic artery could be perfused through the left gastric artery without reconstruction. In a case where the left gastric artery arose from the aorta directly, the artery was reconstructed in the same fashion.

Normothermic machine perfusion

A commercially available NMP device (Organ Care System Liver; Transmedics) was used for this study. The NMP was performed according to a previous report,[1] with liver grafts placed on NMP for at least 1.5 hours after resuscitating those temperatures at 34 °C. All transplanted liver grafts showed a downtrend in lactate and production of bile. For NMP liver grafts, pre-NMP CIT was defined as the duration from the recovery of the liver from a donor until NMP initiation. Total CIT was defined as the sum of pre-NMP CIT and the time from pump disconnection to initiation of graft anastomosis in recipients.

Biliary reconstruction

Choledochocholedochostomy was performed in all enrolled cases. In brief, the posterior wall was sutured continuously, and the anterior wall was sutured interruptedly or continuously using 6-0 monofilament sutures without biliary stents.

Statistical analyses

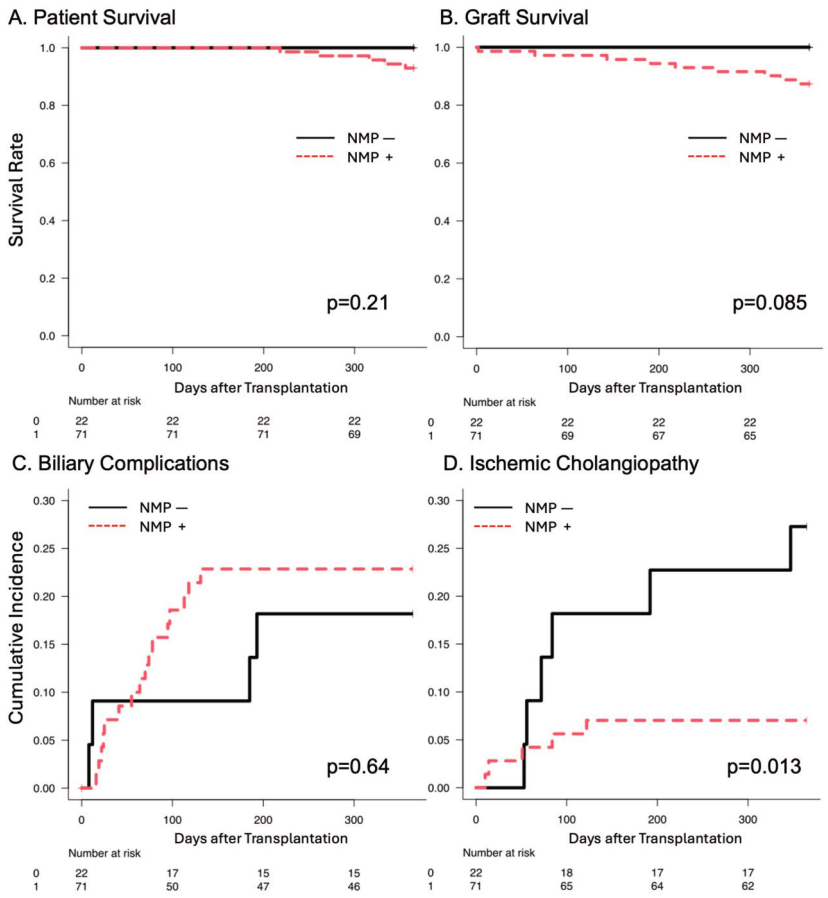
We used the *t* test and Mann-Whitney U test for analyses of mean values (with SD) and median values (with IQR) of continuous variables, respectively. We compared categorical data of each group using the χ^2

test. We calculated cumulative incidence of BC and IC using the Fine-Gray model. In the cumulative incidence calculations, the event was IC onset, and the competing event was death, retransplant without IC, and secondary IC due to arterial issues. $P < .05$ was considered statistically significant. We used R software (version 4.0.4; February 15, 2021) to perform statistical analyses.

Results

For this study, 93 cases, including 22 NMP- cases, were selected. During the follow-up period, no differences were shown in patient survival (Figure 1A) and graft survival (Figure 1B), between the NMP+ and NMP- groups ($P = .21$, $P = .085$, respectively). Importantly, the median donor age in the NMP+ group (38 years; range 26-53 years) was notably higher than that in the NMP- group (29.5 years; range 12-51 years) ($P<.01$) however, no significant differences were shown in donor body mass index (29.0 ± 6.3 vs 28.3 ± 7.1), donor sex (male vs female, 52/19 vs 7/15), the percentage of livers with macrosteatosis greater than or equal to 10% (16.9% vs 4.5%), recipient age (59 years [range, 52-64 y] vs 60 years [range, 45-74 y]), and Model for End-Stage Liver Disease scores (20.3 ± 7.0 vs 17.1 ± 7.1) betweenthe 2 groups. There was no major difference in the cumulative incidence of BC between the NMP+ group at 22.5% (16/71) and the NMP- group at 18.2% (4/22) ($P = .64$) (Figure 1C). Importantly, the cumulative incidence of IC was 7.0% (5/71) for the NMP+ group versus 27.3% (6/22) for the NMP- group ($P<.002$)(Figure 1D)Together, these data suggested that NMP contributed to better outcomes regarding the onset of IC, despite the use of older DCD livers.

Figure 1. Differences in Patient Survival, and Graft Survival, and the Cumulative Incidence of Biliary Complications and Ischemic Cholangiopathy in Patients With or Without Normothermic Machine Perfusion



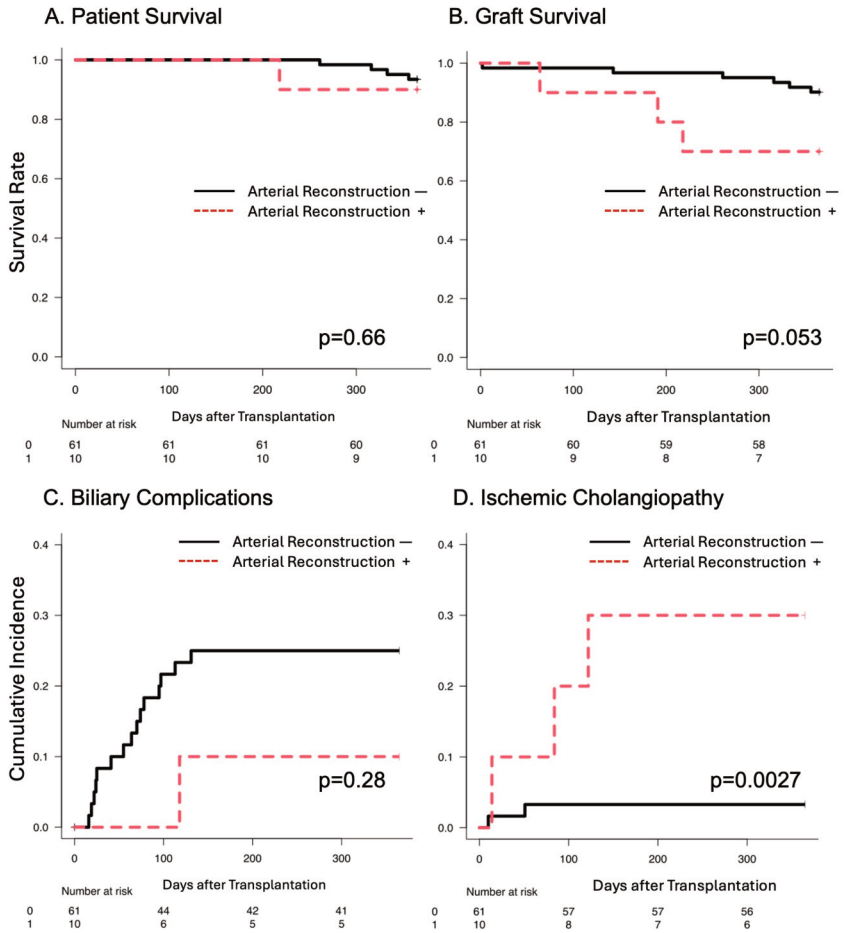
Abbreviations: IC, ischemic cholangiopathy; NMP, normothermic machine perfusion

We analyzed **(A)** patient survival ($P = .21$), **(B)** graft survival ($P = .085$), and **(C)** ischemic-cholangiopathy-free graft survival ($P = .013$) based on the utilization of normothermic machine perfusion. We also analyzed **(D)** biliary complications ($P = .64$) and **(E)** ischemic cholangiopathy ($P < .002$) according to utilization of normothermic machine perfusion.

In total, 17.2% (16/93) of cases required AR: 15 cases had replaced right hepatic artery and 1 case had replaced right hepatic artery and replaced left hepatic artery. We observed that 14.1% of NMP+ cases (10/71) required AR, whereas AR on the back table was performed in 27.3% (6/22) in the NMP- group. The NMP procedure was used in 62.5% (10/16) of AR+ cases. Characteristics of donors and recipients based on AR status (+/-) and IC status (+/-) are shown in Table 1. Both arterial and portal flows, monitored by NMP at 2 hours after initiation of NMP, were comparable between the AR+ and AR- groups (660 ± 140 and 1960 ± 290 mL/min vs 600 ± 100 and 1940 ± 130 mL/min, respectively) (Table 1). In the NMP+ group, no significant differences were shown in patient survival (P = .66) (Figure 2A); however, the overall graft survival of the AR+ group was lower, but not significant, versus the AR- group (P = .053) (Figure 2B).

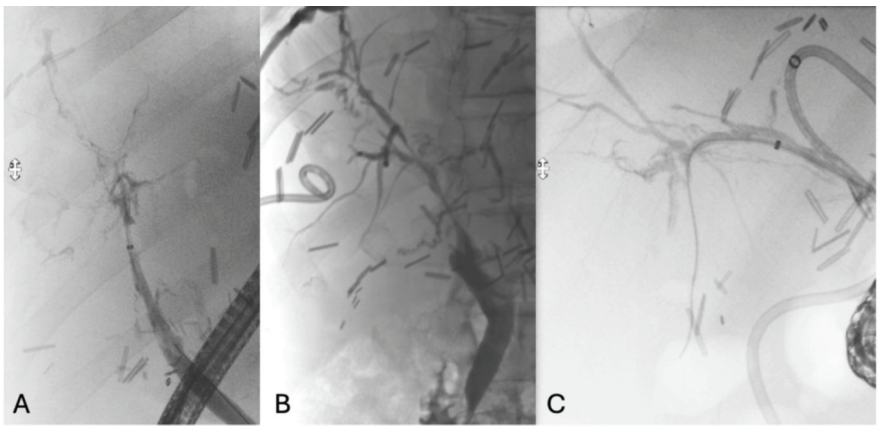
Within the NMP+ group, competing risk analyses demonstrated that AR did not have a significant effect on BC (P = .28) (Figure 2C). However, the cumulative IC onset rate was significantly higher in the AR+ group versus the AR- group (30.0% [3/10] vs 3.3% [2/61], respectively; $P<.003$) (Figure 2D). Regarding the type of IC in the AR+ group, 2 patients were diagnosed with IC with diffuse necrosis (Figure 3, A and B) and 1 with multifocal progressive IC (Figure 3C). These recipients required redo LT due to liver dysfunction. The hepatic arteries of these patients were carefully evaluated by contrast computed tomography and visually observed at the time of redo LT. However, there was no sign that extrahepatic arterial flow was compromised.

Figure 2. Differences in Patient Survival and Graft Survival With or Without Arterial Reconstruction and Competing Risk Analysis of the Effect of Hepatic Arterial Reconstruction in the Group With Normothermic Machine Perfusion



We analyzed **(A)** patient survival (P = .66) and **(B)** graft survival (P = .053) in the normothermic machine perfusion group according to hepatic arterial reconstruction status. The cumulative onset of **(C)** biliary complications (P = .28) and **(D)** ischemic cholangiopathy (P < .003) were analyzed.

Figure 3. Endoscopic Retrograde Cholangiopancreatography Images of Patients Who Developed Different Types of Ischemic Cholangiopathy After Normothermic Machine Perfusion



We observed **(A)** diffuse necrosis (n = 1), **(B)** right-side-dominant diffuse necrosis (n = 1), and **(C)** multifocal progressive ischemic cholangiopathy (n = 1).

Pre-NMP CIT in the AR+ group was significantly longer (167.6 ± 38.6 min) versus the AR- group (112.6 ± 24.6 min) ($P < .001$); however, no significant differences were shown in donor age, donor warm ischemic time, DHT, degree of steatosis, and recipient implant time (Table 1). In the NMP- group, there was no clear difference in CIT between the AR+ (375.2 ± 89.4 min) and AR- groups (329.6 ± 112.7 min) ($P = .39$). On the other hand, both pre-NMP CIT and total CIT were significantly longer in recipients with IC (n = 5), versus in recipients without IC (n = 66) (pre-NMP CIT, 160.6 ± 42.4 vs 117.3 ± 30.4 [P = <.004];

Table 1. Demographics and Clinical Characteristics of Donors and Recipients Based on Hepatic Arterial Reconstruction Status or Ischemic Cholangiopathy Development in the Group With Normothermic Machine Perfusion

	Arterial Reconstruction			Ischemic Cholangiopathy		
	No	Yes	*P*	No	Yes	*P*
Total	61	10		66	5	
Recipient						
Age, y	59 (52-63)	56 (53-64)	.94	59 (30-70)	60 (43-65)	.70
Sex, male/female	43/18	7/3	.99	45/21	5/0	.31
Primary disease, No.			.4			.69
Alcohol	26	4		27	3	
Polycystic liver	1	1		2	0	
MASH	12	3		14	1	
PSC, PBC, or AIH	6	0		7	0	
Hepatitis B or C	8	2		9	1	
Other	8	0		7	0	
MELD 3.0	20.4 ± 7.2	19.4 ± 5.9	.59	20.4 ± 7.0	17.4 ± 5.5	.35
Donor						
Age, y	38 (26-53)	39 (26-50)	.79	37 (14-64)	55 (25-57)	.15
Sex, male/female	44/17	8/2	.72	19/47	0/5	.32
BMI	28.9 ± 6.0	29.6 ± 8.4	.73	29.2 ± 6.2	25.8 ± 7.9	.25
Macrosteatosis ≥10% (%)	14.8 (9/61)	30.0 (3/10)	.36	16.4 (11/67)	25.0 (1/4)	.53
Extubation to death, min	18.8 ± 5.1	20.9 ± 2.8	.22	19.1 ± 5.0	19.4 ± 4.6	.90
Extubation to flush, min	23.0 ± 5.0	24.4 ± 2.9	.4	23.3 ± 4.9	22.2 ± 4.3	.63
Arrest to flush, min	9.6 ± 2.4	8.9 ± 1.9	.4	9.6 ± 2.3	8.2 ± 1.1	.20
DHT, min	39.2 ± 13.9	35.0 ± 12.4	.38	38.0 ± 13.8	46.6 ± 9.3	.18
Pre-NMP CIT, min	112.6 ± 24.6	167.6 ± 38.6	<.001	117.3 ± 30.4	160.6 ± 42.4	<.004
Post-NMP CIT, min	14.0 ± 10.7	29.7 ± 27.2	.01	14.7 ± 11.5	36.4 ± 34.9	<.002
Total CIT, min	126.7 ± 28.8	197.3 ± 39.0	<.001	132.0 ± 35.9	197.0 ± 27.5	<.001
Portal flow at 2 h, mL/min	1960 ± 290	1940 ± 130	.85	1950 ± 280	2030 ± 160	.54
Arterial flow at 2 h, mL/min	660 ± 140	600 ± 100	.24	640 ± 130	710 ± 140	.26
Recipient implant time, min						
HV to PV	52.4 ± 16.9	55.6 ± 15.7	.58	53.5 ± 17.2	55.8 ± 17.8	.89
PV to HA	45.2 ± 16.2	40.3 ± 11.8	.37	46.9 ± 17.0	43.0 ± 12.0	.66

Abbreviations: AIH, autoimmune hepatitis; BMI, body mass index; CIT, cold ischemia time; DHT, donor hepatectomy time; HA, hepatic artery; HV, hepatic vein; MASH, metabolic dysfunction-associated steatohepatitis; MELD, Model for End-Stage Liver Disease; NMP, normothermic machine perfusion; PBC, primary biliary cirrhosis; PSC, primary sclerosis cholangitis; PV, portal vein
Values are presented as frequency (with %), mean values (±SD), or median values (with IQR).

total CIT, 197.0 ± 27.5 vs 132.0 ± 35.9 min [P <.001]). Arterial flow on NMP was also equivalent between the group with IC (640 ± 130 mL/min) versus the group without IC (710 ± 140 mL/min) (P = .26) (Table 1). A receiver operator characteristic curve analysis showed that target pre-NMP CIT at 113 minutes yielded 100% sensitivity and 57.6% specificity with the area under the curve of 0.85 to predict the IC onset. Overall, these results suggested that AR with NMP is a risk factors for the onset of IC, mostly due to the prolongation of CIT.

As a control, within the NMP- group, AR did not contribute to the onset of BC (AR+ group, 20% [2/10] and AR- group, 26.2% [16/61] (P = .72). There was also a higher cumulative IC onset rate in the AR+ group (33.3% [2/6] vs the AR- group 6.3% [1/16]), although it was not significant due to the limited sample size.

Discussion

Several parameters have been recognized as liver viability markers, including lactate, bile pH, and the amount of bile in the context of NMP.[8,9] However, bile duct ischemic insult may not result in disturbance in these parameters immediately, because hepatocytes can still be viable and ischemic damage of the bile duct may not become apparent within few hours.[10] Thus, it is difficult to predict the onset of IC relying on these parameters alone and it is necessary to identify other risk factors for primary IC.

Based on our findings, hepatic AR prior to NMP was a risk factor for developing primary IC in DCD LT. We further demonstrated that the increased risk of primary IC after AR prior to NMP is related to an increase in CIT. In our study, 3 of 5 cases were consistent with prolonged CIT related IC, because the entire liver grafts were affected. One case of IC could have been a result of AR malfunction, since the patient's IC manifested as right lobe dominant diffuse necrosis. Excluding this case based on the assumption of warm ischemic damage while on NMP, our analysis still found the same effect of CIT on IC (not shown). It can be argued, therefore, that the prolongation of CIT is one of the risk factors for IC.

The procedure of AR itself can prolong pre-NMP CIT, and the existence of variant arteries may also increase the DHT, depending on the recovery procedures. Other studies have also suggested that longer CIT is associated with worse graft outcomes without NMP.[6,11] However, the ideal CIT specifically for DCD liver grafts with NMP had not previously been investigated, making our investigation of vital importance. The receiver operating characteristic curve analysis demonstrated that the minimization of pre-NMP CIT is essential because the risk of IC increase when pre-NMP CIT reaches 113 minutes, according to our cohort. In other words, there was no primary IC case with CIT less than 113 minutes.

Several studies have reported that prolonged DHT negatively affects graft and patient survival of DCD LT without NMP.[5,12] Furthermore, DHT is advocated to be less than 22 to 60 minutes.[5,12-14] Although the NMP+ group did not show a significant effect of DHT on IC, the entire cohort demonstrated that recipients with IC had longer DHT versus those without IC. Thus, it is likely that a larger cohort might demonstrate a significant effect of DHT on the development of IC with the utilization of NMP. Regardless of CIT and DHT, warm ischemic injury due to improper reconstruction and positioning that limits arterial flow on NMP can increase the risk of IC, although this was not a focus of our present study.[15]

Several countermeasures could address the potential associated risk factors discussed above. First, it is reasonable to employ 2 recovery surgeons so that a surgeon at the back table can begin the procedure immediately after the liver is recovered, to minimize CIT, while the other surgeon completes the donor surgery. Second, especially for DCD with no preoperative vascular imaging, liver and pancreas (the head of the pancreas) en bloc recovery, including the superior mesenteric artery with an aortic cuff and the hepatogastric ligament, would be beneficial to minimize DHT and the chance of injuries in aberrant arteries. It can be argued that additional dissection on the back table does not cause a significant prolongation in CIT, considering the time frame of DHT versus the duration of the back table. Third, design of reconstruction and precise anastomosis are essential. Finally, positioning on the NMP should be carefully checked, and all problems, such as twists or bleeding, should be addressed in a timely manner. As an alternative, if materials and surgeons to perform a proper reconstruction are limited, then a tubing technique could be an alternative strategy for on-site AR and NMP. Furthermore, it would be also useful to create multiple outlets for hepatic arteries and cannulate the main and aberrant hepatic arteries, respectively. This facilitates the definitive reconstruction after NMP.

Our study had several limitations, including the small study sample. There are other variables that we did not assess due to the small sample size that could affect LT outcomes and IC development. For example, younger donor age has been shown to have a protective effect on LT outcomes with longer CIT.[16] Further analysis with the accumulated data may enable us to use portable NMP selectively. Some other factors affecting the development of BC are not clear, as the rates of BC were similar between the NMP+ and NMP- cohorts.

Of note, the use of NMP in LT has numerous advantages, such as broadening the donor pool not to exclude elderly donors and increasing the utilization rate of other marginal livers.[17,18] However, our study showed that an unintended consequence when using NMP is the increased risk of IC in patients who underwent AR. Safe, quick reconstruction and investigation of the liver grafts on NMP are essential to prevent future IC. To maximize the beneficial effects of NMP, awareness of these consequences and implementation of reasonable countermeasures are crucial.

References

1. Longchamp A, Nakamura T, Uygun K, Markmann JF. Role of machine perfusion in liver transplantation. *Surg Clin North Am*. 2024;104:45-65. doi:10.1016/j.suc.2023.07.001
2. Markmann JF, Abouljoud MS, Ghobrial RM, et al. Impact of portable normothermic blood-based machine perfusion on outcomes of liver transplant: the OCS Liver PROTECT randomized clinical trial. *JAMA Surg*. 2022;157:189-198. doi:10.1001/jamasurg.2021.6781
3. Hiatt JR, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases: *Ann Surg*. 1994;220:50-52. doi:10.1097/00000658-199407000-00008
4. Li SF, Nave JB, Hostetler C, et al. Using IV-tubing "bridge" from splenic artery to superior mesentery artery to create a single arterial cannulation for normothermic machine perfusion when donor liver has replaced right hepatic artery. *Am J Case Rep*. 2023;24:e940437. doi:10.12659/AJCR.940437
5. Bekki Y, Kozato A, Kusakabe J, et al. Impact of the donor hepatectomy time on short−term outcomes in liver transplantation using donation after circulatory death: a review of the US national registry. *Clin Transplant*. 2022;36:e14778. doi:10.1111/ctr.14778
6. Mathur AK, Heimbach J, Steffick DE, Sonnenday CJ, Goodrich NP, Merion RM. Donation after cardiac death liver transplantation: predictors of outcome. *Am J Transplant*. 2010;10:2512-2519. doi:10.1111/j.1600-6143.2010.03293.x
7. Croome KP, Mathur AK, Aqel B, et al. Classification of distinct patterns of ischemic cholangiopathy following DCD liver transplantation: distinct clinical courses and long-term outcomes from a multicenter cohort. *Transplantation*. 2022;106:1206-1214. doi:10.1097/TP.0000000000003928
8. Melandro F, De Carlis R, Torri F, et al. Viability criteria during liver ex-situ normothermic and hypothermic perfusion. *Medicina (Kaunas)*. 2022;58(10):1434-1446. doi:10.3390/medicina58101434
9. Mergental H, Stephenson BTF, Laing RW, et al. Development of clinical criteria for functional assessment to predict primary nonfunction of high-risk livers using normothermic machine perfusion. *Liver Transpl*. 2018;24:1453-1469. doi:10.1002/lt.25291
10. Li J, Lu H, Zhang J, Li Y, Zhao Q. Comprehensive approach to assessment of liver viability during normothermic machine perfusion. *J Clin Transl Hepatol*. 2022;11:466-479. doi:10.14218/JCTH.2022.00130
11. Paterno F, Guarrera JV, Wima K, et al. Clinical implications of donor warm and cold ischemia time in donor after circulatory death liver transplantation. *Liver Transpl*. 2019;25:1342-1352. doi:10.1002/lt.25453
12. Farid SG, Attia MS, Vijayanand D, et al. Impact of donor hepatectomy time during organ procurement in donation after circulatory death liver transplantation: the United Kingdom experience. *Transplantation* 2019;103:e79-e88. doi:10.1097/TP.0000000000002518
13. Hobeika MJ, Glazner R, Foley DP, et al. A step toward standardization: results of two national surveys of best practices in donation after circulatory death liver recovery and recommendations from The American Society of Transplant Surgeons and Association of Organ Procurement Organizations. *Clin Transplant*. 2020;34:e14035. doi:10.1111/ctr.14035
14. Goussous N, Alvarez-Casas J, Dawany N, et al. Ischemic cholangiopathy postdonation after circulatory death liver transplantation: donor hepatectomy time matters. *Transplant Direct*. 2021;8:e1277. doi:0.1097/TXD.0000000000001277
15. Mergental H, Laing RW, Kirkham AJ, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun*. 2020;11:2939. doi:10.1038/s41467-020-16251-3
16. Grąt M, Wronka KM, Patkowski W, et al. Effects of donor age and cold ischemia on liver transplantation outcomes according to the severity of recipient status. *Dig Dis Sci*. 2016;61:626-635. doi:10.1007/s10620-015-3910-7
17. Shubin AD, Feizpour CA, Hwang CS, Hanish SI, Raschzok N, Wang BK. Normothermic machine perfusion for older transplant recipients. *Artif Organs*. 2023;47:1184-1191. doi:10.1111/aor.14519
18. Shen C, Cheng H, Zong T, Zhu H. The role of normothermic machine perfusion (NMP) in the preservation of ex-vivo liver before transplantation: a review. *Front Bioeng Biotechnol*. 2023;11:1072937. doi:10.3389/fbioe.2023.1072937

Recurrence of Hepatocellular Carcinoma After Liver Transplant: A Single-Center Experience

Aydan Farzaliyeva,[1] Mehmet Nezir Ramazanoglu,[1] Adem Safak,[2] Arzu Oguz,[1] Zafer Akcali,[1] Ozden Altundag,[1] Mehmet Haberal[2]

Abstract

Objectives: **Hepatocellular carcinoma is the fourth leading cause of cancer-related mortality worldwide, and almost all patients have simultaneous cirrhosis. For patients with hepatocellular carcinoma concurrent with cirrhosis, the best treatment option is liver transplant. With expansion of transplant criteria and increased use of liver transplant for treatment, median survival and recurrence rates in patients with hepatocellular carcinoma have also increased. Here, we evaluated tumor recurrence characteristics of hepatocellular carcinoma after liver transplant, treatments given, and survival periods.**

Materials and Methods: **We retrospectively analyzed data of 512 patients who underwent transplant from January 2000 to December 2023 at Baskent University (Ankara, Turkey). We evaluated recurrence patterns, time to recurrence, and treatment survival outcomes among patients with or without recurrence.**

Results: **Of 204 adult patients, 63 underwent transplant because of hepatocellular carcinoma. Of the 63 patients, 16 (25%) developed recurrence after liver transplant. Only 1 of the patients who developed recurrence was still alive at the time of this report. Of 16 patients, 50% had local and distant recurrence, 31% had distant metastasis, and 19% developed only local recurrence. Among patients, median overall survival was 65 months. Median survival was significantly lower in the recurrent group than in the nonrecurrent group (33 vs 49 mo; *P* = .001). Median time to recurrence was 11.6 months. Of the 63 patients, 32 patients (50.7%) underwent liver transplant by use of the expanded criteria developed in our center.**

Conclusions: **Hepatocellular carcinoma requires a multidisciplinary approach. Although advances in interventional radiology, surgery, and medical oncologic treatment have prolonged survival in patients with hepatocellular carcinoma, hepatocellular carcinoma recurrence is still associated with poor prognosis. Management of recurrence remains an issue, with not enough data and single guidelines for management of hepatocellular carcinoma recurrence in immunosuppressed transplant recipients.**

Key words: *Immunosuppression, Metastasis, Survival*

Introduction

Hepatocellular carcinoma (HCC) accounts for 80% of primary liver cancers; incidence and mortality of HCC are still increasing globally, making it the fourth leading cause of cancer-related deaths worldwide.[1] At the time of diagnosis, <25% of HCC cases are resectable, often because of underlying liver disease and inadequate hepatic reserve.[2] Liver transplant (LT) is the most effective treatment option for nonmetastatic or unresectable HCC, particularly in the presence of cirrhosis. In the late 1990s, Mazzaferro and colleagues developed the first transplant criteria, which is now known as the Milan criteria. Criteria include patients with cirrhosis having a single tumor of 5 cm or less, those with no more than 3 tumors, each no larger than 3 cm in diameter, and those without extrahepatic manifestations and gross vascular invasion.[3]

As use of LT for HCC treatment has increased, criteria have expanded and indications have been extended through less restrictive requirements for the size and number of tumors. Despite the application of updated criteria, recurrence of HCC has been reported to occur in about 10% to 15% of LT recipients and most commonly in the first 2 years after LT. Recurrence typically is accompanied by significant mortality and limited potential for a

From the [1]Department of Medical Oncology and the [2]Department of General Surgery, Division of Transplantation, Baskent University Faculty of Medicine, Ankara, Türkiye

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: *Aydan Farzaliyeva, Baskent University, Department of Medical Oncology, Yukarı Bahçelievler, Mareşal Fevzi Çakmak Cd. 10. Sk. No:45, 06490 Bahçelievler, Ankara, Türkiye*

E-mail: *aydanflv@gmail.com*

Experimental and Clinical Transplantation (2025) 8: 542-545

DOI: 10.6002/ect.2025.0041

cure.4 The prognosis of patients with recurrent HCC after LT remains poor, and there are several areas of uncertainty in the management of these patients. Improving the understanding of the contributing factors of HCC recurrence after LT remains urgent. In this study, we evaluated tumor recurrence characteristics of HCC patients after LT, the treatments given, and the survival outcomes of this patient group in our center.

Materials and Methods

From January 2003 to December 2023, 512 LTs were performed in our center (Baskent University Ankara Hospital, Ankara, Türkiye). Among patients who underwent LT, 63 received transplants because of HCC. Patient follow-up was done by a multidisciplinary team. We obtained details of patient transplant procedures, demographic characteristics, and laboratory and radiologic reports from hospital records. We retrospectively assessed the patient tumor status at the time of diagnosis, tumor recurrence patterns, time to recurrence, treatment strategies used, and survival outcomes. Posttransplant recurrence was diagnosed by alpha-fetoprotein (AFP) levels and imaging studies with or without histologic confirmation. Overall survival was defined as the time from the date of surgery to death (from any cause) or last follow-up. We used the Kaplan-Meier method to generate survival curves and calculate 3- and 5-year overall survival rates for recurrent and nonrecurrent groups, Survival periods were compared between recurrent and nonrecurrent groups. $P < .05$ was considered statistically significant.

Results

Between 2003 and 2023, 512 patients underwent LT. Among 204 adult patients, 63 underwent LT because of HCC. Median age was 55 years (range, 30-68 y), and 57 patients (90%) were men. In 32 patients (51%), LT was performed in accordance with expanded criteria developed by our center. Of 63 patients, 35 (55%) had living donors and 20 (45%) had deceased donors. The most common etiology was hepatitis B virus (HBV) infection (n = 26); in 23 patients, the etiology for HCC was unknown. Hepatitis C virus (HCV) infection was detected in 5 patients, HBV and hepatitis D virus in 3, HBV and HCV in 2, alcohol-induced cirrhosis in 1, and autoimmune hepatitis in 1 patient.

Of 63 patients, 16 (25%) developed HCC recurrence after LT. Fifteen patients (94%) who developed recurrence were men. Only 1 of the patients who developed recurrence was still alive at the time of this report. Of 16 patients with recurrence, 50% had local and distant recurrence, 31% (n = 5) had distant metastasis, and 19% (n = 3) developed only locoregional recurrence. The most frequent sites of distant metastasis were lungs and bones. Interventional procedures like radiofrequency ablation, transarterial radioembolization, and transcatheter arterial chemoembolization were performed in 8 patients with recurrence. Only 4 patients received systemic treatment. Median overall survival was 65 months for all 63 LT patients (Figure 1). Median time to recurrence was 11.6 months.

Median survival was significantly lower in the recurrent group than in the nonrecurrent group (33 vs 149 mo; $P = .001$) (Figure 2). The 3- and 5-year survival rates for the nonrecurrent group were 74% and 63%, respectively. However, for the recurrent group, the 3- and 5-year survival rates were 43% and

Figure 1. Overall Survival in 63 Patients Who Underwent Liver Transplant Recipients Because of Hepatocellular Carcinoma

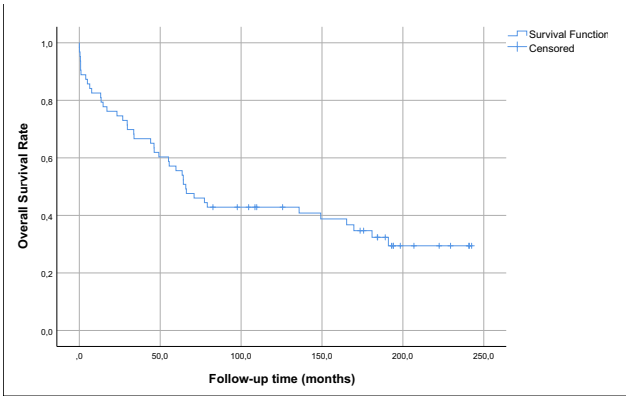
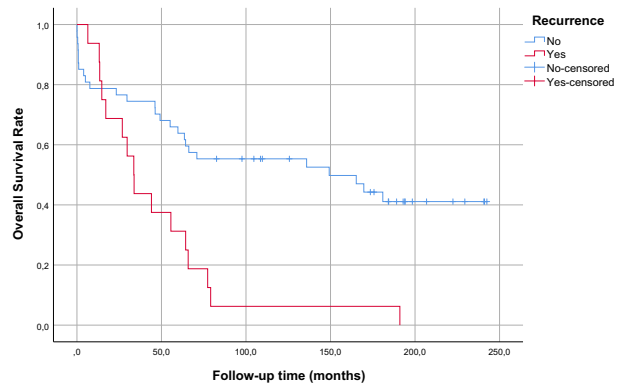


Figure 2. Survival in Patients Who Underwent Liver Transplant Recipients Because of Hepatocellular Carcinoma With and Without Recurrence of Hepatocellular Carcinoma



31%, respectively. During follow-up, 8 patients (12.6%) developed secondary malignancies (skin, lung, and hematological cancers).

Discussion

Liver transplant is the best treatment option for HCC in patients with cirrhosis. The criterium for transplant is still a subject of discussion worldwide. The Milan and other expanded criteria consider morphologic features like tumor number and size but not histopathologic characteristics, such as grade and differentiation of the tumor. Despite adherence to the criteria for LT in HCC patients, up to 20% to 30% of transplanted HCC patients may develop tumor recurrence that negatively affects their survival.[5]

In our center, we use the expanded Baskent University transplant criteria for patients with nonmetastatic HCC. Regardless of tumor number and size, these criteria include patients without major vascular invasion and without distant metastases.

In the previous report from our center of 49 adult patients who had LT, 17 patients (26.5%) had HCC recurrence during follow-up. Median overall survival was 64.3 months for all included patients. Median time to recurrence was 12.6 months. The 3- and 5-year survival rates were 80% and 68% in the nonrecurrent group and 52% and 17% in the recurrent group, respectively.6 In the present study, the recurrence rate of HCC was 25% after LT and the median survival rates were significantly lower for the recurrent patient group compared with the nonrecurrent group. The recurrence rate obtained in our study was consistent with the literature, and this reflects that transplant criteria are mainly based on tumor burden characteristics (size and number) and do not necessarily reflect the biological behavior of HCC.

Several studies have reported and correlated the added value of AFP level as a useful predictor of HCC recurrence. In patients, high AFP levels before LT were found to be associated with worse survival rate after transplant.[7,8]

In our study, the median time to posttransplant recurrence was 11.6 months. This result aligns with other studies in the literature that reported a median time to recurrence ranging from 7 to 36 months. In the report from Fernandez-Sevilla and colleagues of 493 patients transplanted for HCC, 70 patients (14.2%) developed recurrence after a median disease-free interval of 17 months.[9] Among patients with recurrence, 70% of recurrences were diagnosed within the 2 years after LT. As shown in our study, most recurrences were extrahepatic (lung, lymph nodes, and bone); intrahepatic recurrences were observed in only 2 patients (2.8%).[9]

Timing of HCC recurrence is variable; in most cases, timing is 1 to 3 years after LT. Early recurrence, defined as those occurring less than 1 year after LT, is frequently associated with significantly worse prognosis, possibly the result of HCC micrometastasis engraftment.[10] Considered the timing of HCC recurrence reported in the literature, post-LT surveillance should be more intense for the first 3 years after LT. As shown in our study, lung and bones are the most frequent metastatic sites of recurrence.11 This indicates the need for close follow-up of these sites by radiologic imaging techniques, especially during the first 2 to 3 years after LT.

We found that median survival was significantly lower in the recurrent group than in the nonrecurrent group (33 vs 149 mo, respectively; $P = .001$). In other words, survival was almost 5 times better in the nonrecurrent group than in patients who developed recurrence. Recurrence of HCC after LT leads to poor survival outcomes, as already reported, with a median survival of less than 2 years.[12,13] Survival after recurrence observed in our study was one of the longest reported, showing that a multidisciplinary approach, including local therapies, is effective in this group of patients.

In our study, 3- and 5-year survival rates were 43% and 31%, respectively. Kim and colleagues reported 1-year, 3-year, and 5-year survival rates of 65.2%, 34.0%, and 20.5%, respectively, in the 151 patients who had HCC recurrence after LT. In that study, multivariable Cox analysis showed that grafts from living donors, recurrence-free interval of more than 9 months, AFP levels of ≥100 ng/mL at the time of recurrence, recurrence in bone, and everolimus treatment within 3 months after recurrence were related with improved survival after recurrence.[14]

The immune system is the primary defense mechanism against cancer, and the role of immunosuppressive regimens in the risk of HCC recurrence after LT has been previously examined. However, the optimal immunosuppression regimen to reduce the frequency of HCC recurrence and improve survival

has not yet been determined and still represents a matter of debate. Calcineurin inhibitors, such as tacrolimus and cyclosporine, create a permissive environment for the growth of cancer cells because of impairment of the recipient's immune system.[15] Calcineurin inhibitors have been shown to be associated with increased recurrence rates of posttransplant HCC, whereas mammalian target of rapamycin inhibitors have been shown to inhibit HCC growth in vitro and in animal models.[16]

Among our included study patients, 25% received systemic treatment, with the most commonly used agent being sorafenib. Recurrence of HCC can be considered as a systemic disease because of persistence of malignant cells after resection of the affected liver. Therefore, HCC recurrence after LT often requires systemic therapy.

In the past few years, the combination of the anti-programmed cell death ligand 1 (PD-L1) antibody atezolizumab and the anti-vascular endothelial growth factor antibody bevacizumab and the combination of the anti-PD-L1 antibody durvalumab with a single "priming" dose of the anticytotoxic T-lymphocyte-associated protein 4 antibody tremelimumab showed prolonged overall survival compared with sorafenib and became the first-line treatment option in patients with advanced HCC. Despite these promising results with immunotherapy, the use of immune checkpoint inhibitors after transplant may expose liver recipients to the risk of allograft rejection and graft loss.[17] Therefore, tyrosine kinase inhibitors such as sorafenib and lenvatinib remain the optimal choice in systemic first-line therapy.

Conclusions

Advances in interventional radiology, surgery, and medical oncology treatments have led to prolonged survival in patients with HCC. Despite these developments, HCC recurrence is still associated with poor prognosis. Although immune checkpoint inhibitors have become the first-line treatment for advanced HCC worldwide, the use of these agents in the treatment of posttransplant HCC remains controversial. At the same time, the choice of immunosuppressive regimens is still a matter of debate. Management of HCC recurrence remains a big issue, and few data and no single guideline for management of HCC recurrence management in immunosuppressed recipients remain as limitations.

References

1. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol*. 2019;16(10):589-604. doi:10.1038/s41575-019-0186-y
2. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med*. 2011;365(12):1118-1127. doi:10.1056/NEJMra1001683
3. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med*. 1996;334(11):693-699. doi:10.1056/NEJM199603143341104
4. Kim B, Kahn J, Terrault NA. Liver transplantation as therapy for hepatocellular carcinoma. *Liver Int*. 2020;40(suppl 1):116-121. doi:10.1111/liv.14346
5. Badwei N. Challenges related to clinical decision-making in hepatocellular carcinoma recurrence post-liver transplantation: is there a hope? *World J Transplant*. 2024;14(3):96637. doi:10.5500/wjt.v14.i3.96637
6. Rahatli S, Ayvazoglu Soy EH, Oguz A, Altundag O, Moray G, Haberal M. Single-center experience of recurrence patterns and survival analyses of patients with hepatocellular carcinoma and liver transplant. *Exp Clin Transplant*. 2020;18(2):201-205. doi:10.6002/ect.2020.0046
7. Ling Q, Liu J, Zhuo J, et al. Development of models to predict early post-transplant recurrence of hepatocellular carcinoma that also integrate the quality and characteristics of the liver graft: a national registry study in China. *Surgery*. 201827:S0039-S6060(18)30079-5. doi:10.1016/j.surg.2018.01.022
8. Kim SJ, Kim JM. Prediction models of hepatocellular carcinoma recurrence after liver transplantation: a comprehensive review. 2022;28(4):739. *Clin Mol Hepatol*. 2022;28(4):739-753. doi:10.3350/cmh.2022.0060
9. Fernandez−Sevilla E, Allard MA, Selten J, et al. Recurrence of hepatocellular carcinoma after liver transplantation: is there a place for resection? *Liver Transpl*. 2017;23(4):440-447. doi:10.1002/lt.24742
10. Sapisochin G, Goldaracena N, Astete S, et al. Benefit of treating hepatocellular carcinoma recurrence after liver transplantation and analysis of prognostic factors for survival in a large Euro-American series. *Ann Surg Oncol*. 2015;22(7):2286-2294. doi:10.1245/s10434-014-4273-6
11. Escartin A, Sapisochin G, Bilbao I, et al. Recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc*. 2007;39(7):2308-2310. doi:10.1016/j.transproceed.2007.06.042
12. Kornberg A, Küpper B, Tannapfel A, et al. Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. *Eur J Surg Oncol*. 2010;36(3):275-280. doi:10.1016/j.ejso.2009.10.001
13. Valdivieso A, Bustamante J, Gastaca M, et al. Management of hepatocellular carcinoma recurrence after liver transplantation. *Transplant Proc*. 2010;42(2):660-662. doi:10.1016/j.transproceed.2010.02.014
14. Kim M, Rhu J, Choi GS, Kim JM, Joh JW. Risk factors for poor survival after recurrence of hepatocellular carcinoma after liver transplantation. *Ann Surg Treat Res*. 2021;101(1):28-36. doi:10.4174/astr.2021.101.1.28
15. Maluccio M, Sharma V, Lagman M, et al. Tacrolimus enhances transforming growth factor-β1 expression and promotes tumor progression. *Transplantation*. 2003;76(3):597-602. doi:10.1097/01.TP.0000081399.75231.3B
16. Schumacher G, Oidtmann M, Rueggeberg A, et al. Sirolimus inhibits growth of human hepatoma cells alone or combined with tacrolimus, while tacrolimus promotes cell growth. *World J Gastroenterol*. 2005;11(10):1420-1425. doi:10.3748/wjg.v11.i10.1420
17. Kittai AS, Oldham H, Cetnar J, Taylor M. Immune checkpoint inhibitors in organ transplant patients. *J Immunother*. 2017;40(7):277-281. doi:10.1097/CJI.0000000000000180

ARTICLE

Liver Fibrosis and Quality of Life in Liver Transplant Recipients Over 10 Years: A Cross-Sectional Study Using Transient Elastography

Nilay Daniş,[1] Hüseyin Döngelli,[2] Tarkan Ünek,[3] Tufan Egeli,[3] Cihan Ağalar,[3] Mücahit Özbilgin,[3] Mesut Akarsu[1]

Abstract

Objectives: **Liver transplant has significantly improved the survival of patients with end-stage liver disease, yet long-term transplant recipients often face challenges related to graft function and well-being. We aimed to evaluate the clinical role of vibration-controlled transient elastography for assessment of liver fibrosis and steatosis, with a focus on fibrosis and steatosis, in liver transplant recipients who were over 10 years posttransplant. In addition, we aimed to identify factors that influence liver function and quality of life in these patients.**

Materials and Methods: **This prospective, crosssectional study included 105 liver transplant recipients. Vibration-controlled transient elastography measurements (controlled attenuation parameter and liver stiffness measurement) were used to assess liver steatosis and fibrosis, and the Short Form 36 quality of life questionnaire was use to evaluate overall health of patients. Demographic data, medical history, and laboratory results were also collected. Generalized linear models identified significant factors that may affect liver function and quality of life.**

Results: **No significant differences were observed between liver transplant recipients with living donors versus recipients with deceased donors with regard to fibrosis, quality of life, or other factors. The study found that diabetes mellitus (controlled attenuation parameter: *P* = 0.278; 95% CI, 0.193-0.363; *P* < .001) and history of biopsy-proven rejection (liver stiffness measurement: β = 0.814; 95% CI, 0.653-0.975; *P* < .001) were key factors associated with greater severity of liver steatosis and fibrosis. Significant fibrosis was associated with lower physical function scores (β = -0.207; *P* = .040).**

Conclusions: **Vibration-controlled transient elastography is a valuable tool for assessment of liver fibrosis and steatosis in long-term liver transplant recipients and thereby facilitates optimization of posttransplant care and improved outcomes.**

Key words: *Elasticity imaging techniques, Fatty liver, Liver transplantation, Severity of illness index*

Introduction

Liver transplant (LT) is the only curative treatment for end-stage liver disease and markedly improves both survival and quality of life.[1] Liver transplant is indicated for various conditions, including cirrhosis due to hepatitis B virus (HBV) or hepatitis C virus (HCV), alcoholic liver disease, and metabolic dysfunction-associated steatohepatitis, as well as acute liver failure, hepatocellular carcinoma within transplant criteria, and certain metabolic liver diseases.[2] Recently, indications for LT have expanded to include select cases of unresectable colorectal liver metastases, intrahepatic cholangiocarcinoma, and neuroendocrine liver metastases.[2]

Despite advances in surgical techniques and immunosuppression regimens, long-term graft survival remains a clinical challenge due to complications such as chronic rejection, metabolic syndrome, and progressive graft fibrosis.[3-6] Acute rejection, which typically occurs within the first year after transplant, is generally well controlled with immunosup-pression therapy.[3,4] In contrast, chronic rejection, albeit less common, can lead to irreversible bile duct injury and graft failure.[5,6] Therefore, long-term posttransplant care must focus on immunosuppression optimization, management of cardiovascular and metabolic risks, and monitoring for graft fibrosis and rejection.[7,8]

From the [1]Department of Gastroenterology, the [2]Department of Internal Medicine, and the [3]Department of General Surgery, Dokuz Eylul University Hospital, Izmir, Türkiye

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: *Hüseyin Döngelli, Dokuz Eylul Universitesi Hastanesi, Department of Internal Medicine, Mithatpasa St. No:56 Balcova/Izmir, 35000 Türkiye*
Phone: *+90 538 335 23 93* ***E-mail:*** *drhuseyindongelli@gmail.com*

Experimental and Clinical Transplantation (2025) 8: 546-554

DOI: 10.6002/ect.2025.0114

Graft fibrosis and hepatic steatosis are major determinants of long-term liver function in LT recipients.[9,10] Fibrosis results from chronic injury and excessive extracellular matrix deposition, whereas steatosis reflects fat accumulation in hepatocytes and is often associated with metabolic risk factors such as obesity and type 2 diabetes mellitus (T2DM).[9] The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) is high among patients with T2DM, and posttransplant metabolic syndrome may accelerate both steatosis and fibrosis in the graft.[8,9]

Vibration-controlled transient elastography (VCTE) is a noninvasive imaging modality that quantifies liver stiffness and hepatic fat content.[11] It provides 2 parameters: liver stiffness measurement (LSM, in kPa) for fibrosis assessment and the controlled attenuation parameter (CAP, in dB/m) for steatosis quantification.[11-13] The VCTE modality has demonstrated good diagnostic performance for evaluation of graft fibrosis and steatosis in LT recipients and is a practical alternative to liver biopsy.[12,13]

Several studies have explored the use of VCTE in posttransplant settings, including the assessment of HCV-related fibrosis, acute and chronic rejection, metabolic liver disease, and cardiovascular outcomes.[14-19] In addition, fibrosis scoring systems such as fibrosis 4 (FIB4), aspartate aminotransferase-to-platelet ratio index (APRI), and nonalcoholic fatty liver disease fibrosis score (NFS) have been evaluated for the utility of these scoring systems versus VCTE in this patient population.[16,20]

Our present study evaluated the clinical utility of VCTE for assessment of graft fibrosis and steatosis in LT recipients more than 10 years after transplant. The >10-year cutoff was chosen so that we could focus on long-term survivors, a growing yet understudied cohort, for whom chronic complications become increasingly relevant. We also investigated factors that influence liver graft function and quality of life, as reported by patients, using validated instruments.

Materials and Methods

Patients and data collection

This study was conducted among patients regularly followed at the Liver Transplantation Clinic of Dokuz Eylul University who met specific eligibility criteria. A total of 145 patients met the inclusion criteria. During their routine follow-up visits, patients underwent both the Short Form 36 (SF-36) quality of life questionnaire and VCTE measurements.

Eleven patients were excluded because of lack of follow-up data during the study period (from June 2024 to January 2025). Twenty-two patients declined to complete either the SF-36 or VCTE, and 7 were excluded based on other predefined exclusion criteria. These criteria were chosen because of the potential influence on both VCTE measurements and laboratory results used to calculate fibrosis scores. The final analysis included 105 patients.

Patients who were more than 10 years after LT were selected, and this criterion facilitated our focus on long-term graft health and patient-reported outcomes, which minimized the confounding effects of early posttransplant complications or acute changes in liver function.

Inclusion and exclusion criteria

Inclusion criteria included the following: liver transplant recipients who received grafts from either living or deceased donors; for recipients of living donor transplants, donors were either biologically related up to the fourth degree or legal spouses and were at least 18 years old at the time of donation. Other inclusion criteria included more than 10 years after LT, no missing clinical or laboratory data, and follow-up and treatment performed at our LT clinic.

Exclusion criteria included the following: age <18 years at the time of the study, presence of malignant or infiltrative diseases involving the liver, morbid obesity (defined as body mass index [BMI] ≥40 kg/m^2), and incomplete VCTE and SF-36 questionnaire.

We collected demographic data such as age, sex, and BMI, as well as LT-related information including donor type, transplant age, biopsy-proven rejection history, and the primary disease causing liver failure prior to transplant. Comorbid conditions, such as hypertension and DM, were also recorded. Laboratory data included creatinine, international normalized ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and bilirubin, all of which were routinely obtained during follow-up.

The obtained laboratory and clinical data were used to calculate fibrosis indexes, including FIB4 (FIB4 = [Age × AST]/[Platelets × √ALT]), APRI (APRI = [AST/40]/Platelets), and NFS (NFS = -1.675 + 0.037 × Age + 0.094 × BMI + 1.13 × DM + 0.99 × AST/ALT ratio - 0.013 × platelets).

Ethics and informed consent

The study was approved by the Non-Interventional Research Ethics Committee of Dokuz Eylul University on May 8, 2024 (Decision No. 2024/16-21). All procedures adhered to the ethical principles outlined in the 2013 Declaration of Helsinki and the 2018 Declaration of Istanbul. Written informed consent was obtained from all participants to ensure confidentiality and voluntary participation.

This study included both recipients with living donors and recipients with deceased donors; all living donors were biologically related up to the fourth degree or were spouses and were ≥18 years old at the time of donation.

Vibration-controlled transient elastography measurement

Patients fasted for at least 3 hours before VCTE. Measurements were performed by H. Döngelli and N. Daniş using a standardized protocol. At least 10 valid measurements were obtained per patient.

Liver fibrosis was assessed from median values of LSM (in kPa). Only results with a median value <20% were included. Significant fibrosis was defined as LSM >7.0 kPa. Liver steatosis was assessed from the CAP (in dB/m), and results were averaged.

Short Form 36 quality of life questionnaire

Health-related quality of life was evaluated using the SF-36, a validated questionnaire covering 8 domains: physical function, role limitations due to physical health, bodily pain, general health perceptions, vitality, social function, role limitations due to emotional problems, and mental health. Higher scores represent better perceived health. Patients completed the SF-36 during follow-up visits, and assistance was provided if needed.

Statistical analyses

We used SPSS software (version 25.0) for statistical analyses. Descriptive statistics were used to summarize demographic, clinical, and laboratory data. Continuous variables were expressed as means (with SD) or medians (with IQR) depending on the distribution. Categorical variables were presented as frequencies and percentages. We compared continuous variables with independent t tests or Mann-Whitney U tests for nonnormally distributed data and analyzed categorical variables with χ^2 tests.

Univariate generalized linear models (GLMs) were used to examine associations between independent variables (eg, age, sex, BMI, comorbidities) and outcomes (VCTE parameters and SF-36 scores). Multivariate GLMs were then conducted to identify independent predictors of liver fibrosis and steatosis. Clinical factors associated with quality of life were evaluated using only univariate GLMs, as our aim was to explore potential associations without overfitting the model given the sample size limitations. The results of the univariate and multivariate GLM analyses were reported as regression coefficients (β) and *P* values to quantify the strength and significance of associations between independent variables and outcomes.

Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic accuracy of VCTE parameters (CAP and LSM) and fibrosis indexes (FIB4, APRI, NFS). The ROC analyses were presented using area under the curve (AUC) values with corresponding 95% CI and *P* values. $P < .05$ was considered statistically significant.

Results

Baseline clinical characteristics and comparison between deceased donor and living donor liver transplant recipients

A total of 105 LT recipients were included, with a mean age of 59.3 ± 13.3 years; 63.8% were male. The median time since transplant was 15 years (IQR, 5 years). The most frequent primary etiologies for transplant were coinfection with HBV and hepatitis D virus (24.8%), HBV alone (22.9%), and other causes (16.2%) (Table 1).

For deceased donor recipients (n = 58) versus living donor recipients (n = 47), there were no significant differences in age, sex, BMI, or time since transplant. The prevalence of comorbidities such as hypertension (40.0%) and DM (30.5%) was similar across both groups. Laboratory parameters, including platelet count, AST, ALT, bilirubin, creatinine, albumin, and international normalized ratio, also showed no statistically significant differences.

Health-related quality of life scores, as assessed by the SF-36, were comparable across all domains between deceased donor and living donor recipients (Table 1).

Fibrosis and steatosis assessments using noninvasive methods also did not differ significantly between groups. Specifically, FIB4, APRI, and VCTE para-meters, including CAP and LSM, showed no

significant variation. The prevalence of significant fibrosis (defined as LSM >7.0 kPa) was 24.1% in the deceased donor group versus 27.7% in the living donor group (P = .681).

Table 1. Baseline Clinical Characteristics and Comparison of Patients With Deceased Versus Living Donor Transplants

Characteristic	Deceased Donor (n = 58)	Living Donor (n = 47)	Total (n = 105)	*P*
Age, mean ± SD, y	59.8 ± 12.4	58.7 ± 14.4	59.3 ± 13.3	.675
Male sex, No. (%)	34 (58.6)	33 (70.2)	67 (63.8)	.219
BMI, mean ± SD	25.9 ± 4.5	26.9 ± 4.1	26.4 ± 4.3	.298
Posttransplant period, median (IQR), y	16 (5)	14 (6)	15 (5)	.071
Primary disease, No. (%)				NA
Alcohol	4 (6.9)	4 (8.5)	8 (7.6)	
HBV alone	10 (17.2)	14 (29.8)	24 (22.9)	
HBV+HDV coinfection	15 (25.9)	11 (29.8)	26 (24.8)	
HCV	7 (12.1)	5 (10.6)	12 (11.4)	
Fulminant hepatitis/drug	2 (3.4)	1 (2.1)	3 (2.9)	
Autoimmune hepatitis	0 (0)	3 (6.4)	3 (2.9)	
PSC	6 (10.3)	0 (0)	6 (5.7)	
PBC	0 (0)	1 (2.1)	1 (1.0)	
Wilson disease	1 (1.7)	2 (4.3)	3 (2.9)	
Biliary atresia	1 (1.7)	0 (0)	1 (1.0)	
Hemochromatosis	1 (1.7)	0 (0)	1 (1.0)	
Other causes	11 (19.0)	6 (12.8)	17 (16.2)	
HCC prior to transplant, No. (%)	10 (17.2)	12 (25.5)	22 (21.0)	.299
Hypertension, No. (%)	25 (43.1)	17 (36.3)	42 (40.0)	.471
DM, No. (%)	19 (32.8)	13 (27.7)	32 (30.5)	.572
Laboratory findings, median (IQR)				
Platelets, $\times 10^3$ cells/μL	216 (110)	226 (102)	219 (106)	.885
AST, IU/L	22 (14)	22 (11)	22 (12)	.946
ALT, IU/L	19 (12)	22 (12)	20 (12)	.190
Total bilirubin, mg/dL	0.7 (0.4)	0.8 (0.5)	0.8 (0.4)	.224
Direct bilirubin, mg/dL	0.15 (0.10)	0.15 (0.15)	0.15 (0.14)	.300
Creatinine, mg/dL	1.0 (0.4)	1.0 (0.5)	1.0 (0.4)	.757
Albumin, g/dL	4.3 (0.4)	4.3 (0.5)	4.3 (0.4)	.715
INR	1.0 (0.1)	1 (0.1)	1.0 (0.1)	.192
SF-36 results, median (IQR)				
Physical function	80 (42)	80 (30)	80 (33)	.969
Physical role limitations	100 (50)	100 (50)	100 (50)	.645
Bodily pain	78 (43)	90 (52)	78 (43)	.947
General health	55 (25)	50 (20)	55 (25)	.346
Vitality	60 (15)	65 (15)	60 (15)	.969
Social function	75 (25)	75 (25)	75 (25)	.846
Emotional role limitations	67 (67)	100 (33)	100 (33)	.552
Mental health	56 (14)	56 (16)	56 (14)	.823
History of biopsy-proven rejection, No. (%)				.738
Yes	12 (20.7)	11 (23.4)	23 (21.9)	
No	46 (79.3)	36 (76.6)	82 (78.1)	
Fibrosis index, median (IQR)				
FIB4	1.35 (1.10)	1.27 (1.72)	1.29 (0.92)	.495
APRI	0.27 (0.21)	0.26 (0.25)	0.26 (0.23)	.923
FAST				
Agile 4				
VCTE findings, median (IQR)				
CAP, dB/m	216 (78)	226 (70)	222 (75)	.979
LSM, kPa	5.4 (3.1)	5.1 (4.3)	5.3 (3.3)	.762
Significant fibrosis, No. (%)	14 (24.1)	13 (27.7)	27 (25.7)	.681

Abbreviations: ALT, alanine transferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate transferase; BMI, body mass index (measured as kg/m^2); CAP, controlled attenuation parameter; DM, diabetes mellitus; FAST, FibroScan (Echosens) AST score; FIB4, fibrosis 4 score; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; INR, international normalized ratio; LSM, liver stiffness measurement; NA, not applicable; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SF-36, Short Form 36 quality of life questionnaire; VCTE, vibration-controlled transient elastography
$P < .05$ was considered statistically significant.

A history of biopsy-proven rejection was similarly distributed (20.7% for deceased donor group vs 23.4% for living donor group; P = .738). Of the 23 patients with rejection history, 19 had experienced rejection within the first year, and 1 patient had experienced late rejection (year 11) due to patient nonadherence to immunosuppression regimen.

Factors associated with controlled attenuation parameter and liver stiffness measurement in generalized linear models

In multivariate analysis, higher BMI (β = 0.018; 95% CI, 0.009-0.027; P < .001) and presence of DM (β = 0.197; 95% CI, 0.110-0.283; P < .001) were independently associated with higher CAP values, indicating that metabolic factors are significantly linked with hepatic steatosis (Table 2).

A borderline association between biopsy-proven rejection and CAP was observed in univariate analysis (P = .047), but this was not significant in the multivariate model (P = .052).

Regarding LSM (in kPa), a history of rejection was the strongest independent predictor (β = 0.793; 95% CI, 0.632-0.954; P < .001), suggesting that prior rejection episodes contribute to graft fibrosis. Although transplant age showed an association with increased stiffness in univariate analysis (P = .041), this did not persist in multivariate analysis (P = .241). Neither BMI nor DM were independently associated with LSM (Table 2).

Factors associated with the Short Form 36 quality of life scores

Multivariate analysis revealed that male sex was significantly associated with higher SF-36 scores in the domains of general health (β = 0.149, P = .016), social function (β = 0.197, P = .006), and role limitations due to emotional problems (β = 0.199, P = .010), suggesting possible sex-related differences in perceived quality of life (Table 3).

Importantly, the presence of significant fibrosis (LSM >7.0 kPa) was associated with lower physical function scores (β = -0.207, P = .040). We also noted a nonsignificant trend toward lower scores for role limitations due to physical problems (β = -0.143, P = .092). No independent associations were found between SF-36 scores and BMI, CAP, LSM, or donor type (Table 3).

Table 2. Generalized Linear Regression Analysis of Related Parameters With Controlled Attenuation Parameter and Liver Stiffness Measurement

Variable	Univariate		*P*	Multivariate		*P*
	β	95% CI		β	95% CI	
CAP						
Age	0.003	0.001 to 0.007	.064	0.001	-0.002 to 0.004	.653
Male sex	-0.068	-0.163 to 0.027	.162	-0.055	-0.132 to 0.022	.164
BMI	0.028	0.018 to 0.037	<.001	0.018	0.009 to 0.027	<.001
Preoperative MELD score	0.002	-0.006 to 0.010	.625			
ABO incompatibility	0.045	-0.091 to 0.180	.520			
History of rejection	0.111	0.002 to 0.221	.047	0.087	-0.001 to 0.174	.052
Living donor	-0.004	-0.097 to 0.089	.938			
Posttransplant period	0.007	-0.005 to 0.019	.273			
Hypertension	0.107	0.015 to 0.199	.023	0.006	-0.077 to 0.088	.890
DM	0.278	0.193 to 0.363	<.001	0.197	0.110 to 0.283	<.001
LSM						
Age	-0.001	-0.008 to 0.007	.860	-0.004	-0.010 to 0.001	.128
Male sex	-0.049	-0.245 to 0.147	.622	-0.013	-0.163 to 0.137	.864
BMI	0.002	-0.021 to 0.024	.888	-0.003	-0.021 to 0.015	.735
Preoperative MELD score	0.005	-0.012 to 0.022	.552	-0.003	-0.015 to 0.009	.640
ABO incompatibility	0.353	0.084 to 0.622	.010	0.115	-0.091 to 0.321	.274
History of rejection	0.814	0.653 to 0.975	<.001	0.782	0.620 to 0.944	<.001
Living donor	-0.008	-0.197 to 0.182	.936			
Posttransplant period	0.024	0.001 to 0.047	.041	0.010	-0.008 to 0.028	.263
Hypertension	0.113	-0.078 to 0.304	.247			
DM	0.201	0.001 to 0.402	.051	0.083	-0.079 to 0.245	.315
CAP	0.001	<0.001 to 0.003	.077			

Abbreviations: BMI, body mass index; β, beta coefficient; CAP, controlled attenuation parameter; DM, diabetes mellitus; LSM, liver stiffness measurement; MELD, Model for End-Stage Liver Disease
P < .05 was considered statistically significant.

Table 3. Generalized Linear Regression Analysis of Related Parameters With Results From the Short Form 36 Quality of Life Questionnaire

Variable	PhF	Physical Role	Bodily Pain	General Health	Vitality	SoF	Emotional Role	Mental Health
Age								
β	-0.008	-0.004	-0.003	-0.001	0.003	-0.002	-0.001	-0.001
P	.016	.178	.272	.789	.075	.388	.643	.975
Male sex								
β	0.031	0.118	0.108	0.149	0.043	0.197	0.199	0.062
P	.743	.146	.112	.016	.283	.006	.010	.161
BMI								
β	-0.014	-0.005	-0.010	-0.006	-0.004	-0.009	0.003	-0.002
P	.205	.610	.194	.435	.430	.288	.759	.760
Living donor								
β	0.144	0.088	-0.009	-0.059	-0.007	0.027	0.091	0.008
P	.105	.242	.890	.334	.851	.709	.234	.857
CAP								
β	<0.001	<0.001	-0.001	<0.001	<0.001	-0.001	-0.001	<0.001
P	.679	.547	.125	.582	.380	.079	.073	.472
LSM								
β	-0.009	-0.008	-0.006	-0.009	-0.004	-0.008	-0.008	-0.004
P	.441	.387	.480	.278	.482	.388	.425	.539
Significant fibrosis								
β	-0.207	-0.143	-0.046	-0.098	0.014	-0.076	-0.066	0.003
P	.040	.092	.535	.156	.754	.351	.445	.958

Abbreviations: BMI, body mass index; β, beta coefficient; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; PhF, physical function; SoF, social function
P < .05 was considered statistically significant.

Receiver operating characteristic analysis of fibrosis scores and controlled attenuation parameter for prediction of significant fibrosis

Among the clinical fibrosis scores, APRI showed the highest predictive ability with an AUC of 0.763 (95% CI, 0.657-0.869; *P* < .001), followed by FIB4 with an AUC of 0.732 (95% CI, 0.624-0.840; *P* < .001). In contrast, CAP had a more modest predictive power for fibrosis, with an AUC of 0.643 (95% CI, 0.519-0.767; *P* = .027) (Table 4).

Table 4. Receiver Operating Characteristic Analysis of Fibrosis Scores and Controlled Attenuation Parameter in Predicting Significant Fibrosis

Variable	Significant Fibrosis		*P*
	AUC	95% CI	
CAP	0.643	0.519-0.767	.027
FIB4	0.732	0.624-0.840	<.001
APRI	0.763	0.657-0.869	<.001
NFS	0.692	0.578-0.806	.003

Abbreviations: APRI, aspartate aminotransferase versus platelet ratio index; AUC, area under curve; CAP, controlled attenuation parameter; FIB4, fibrosis 4 score; NFS, nonalcoholic fatty liver disease fibrosis score
$P < .05$ was considered statistically significant.

Discussion

This study provides important insights into the metabolic and immunological factors that may affect liver graft health and patient-reported outcomes in long-term LT recipients. Our findings emphasize that DM and higher BMI are independent determinants of hepatic steatosis, and a history of biopsy-proven acute rejection is the most robust predictor of increased liver stiffness, indicating progressive graft fibrosis. Furthermore, significant fibrosis, as assessed by VCTE, was associated with diminished physical function, whereas male sex was linked to better quality of life in multiple domains, including general health, social function, and emotional well-being.

Acute rejection remains a clinically significant complication following LT, with reported incidence ranging from 14.8% to 34.9%; acute rejection predominantly occurs within the first year posttransplant.[3,4,21,22] Several factors have been identified as risk factors for the development of acute rejection, including ABO incompatibility, advanced recipient age, deceased liver donors, lack of aspirin use, primary biliary cirrhosis, primary sclerosing cholangitis, HCV infection, and female sex.[3,4,21,22] Furthermore, long-term outcomes in patients who experience acute rejection have been associated with reduced graft survival and patient survival.[4,21] Although our study was not designed to directly assess acute rejection episodes, we found a 21.9% prevalence of biopsy-confirmed rejection, which aligned with prior reports. Notably, no difference in rejection rates was observed between living donor recipients and deceased donor recipients, and no effect of donor type on fibrosis, steatosis, or quality of life was identified.

Previously published reports have established that MASLD is increasingly prevalent in LT recipients, with posttransplant hepatic steatosis rates reported between 12% and 88%.[9,23-28] Several risk factors for posttransplant hepatic steatosis have been identified, including DM, higher BMI, preoperative alcoholic cirrhosis, preexisting donor graft steatosis, HCV infection, sirolimus use, and female sex.[23-26] Although some previously published studies have reported that posttransplant hepatic steatosis increases the risk of de novo cirrhosis or hepatic fibrosis, others studies have found no significant effect on overall survival or fibrosis progression.[23,28] The long-term effects of posttransplant hepatic steatosis on hepatic fibrosis and mortality remain a subject of debate, although the prevailing view suggests that posttransplant hepatic steatosis contributes to fibrosis progression.

In our study, we used VCTE (CAP) to assess hepatic steatosis, and DM and increased BMI were found to be independently associated with hepatic steatosis. Unlike the results from previous studies, our findings did not identify a significant association between hepatic steatosis and factors such as sex, pretransplant HCV infection, or alcoholic cirrhosis.[27,28] These discrepancies likely reflect differences in cohort composition, as we exclusively included recipients with more than 10 years after transplant. Our findings underscore the central role of metabolic factors, particularly T2DM and obesity, to promote hepatic steatosis in the long-term. Although our analysis was cross-sectional, a trend between CAP (steatosis) and LSM (fibrosis) was observed in univariate models, which supports our hypothesis that steatosis may contribute to fibrosis progression in the long-term.

In a study that investigated the prevalence of hepatic steatosis and hepatic fibrosis in the posttransplant period, the prevalence of significant fibrosis was reported to be 33%, with pretransplant HCV-related cirrhosis identified as a risk factor for significant hepatic fibrosis.[10] Another study evaluated the diagnostic accuracy of VCTE for detection of posttransplant hepatic fibrosis and found that, based on histopathology assessment via liver biopsy, the prevalence of significant fibrosis was 22%, and VCTE demonstrated a sensitivity of 90% for detection of significant fibrosis.[13] Additional risk factors for posttransplant hepatic fibrosis reported in the literature include DM, increased BMI, male sex, low immunosuppression levels, deceased donor transplant, and older donor age.[29-31] In our study, however, biopsy-proven acute rejection history was the only independent factor associated with post-transplant hepatic fibrosis. Previous studies have

demonstrated that acute rejection is linked to long-term graft failure.[4,21] Although most of the patients had unremarkable liver function test results, our study confirmed that fibrosis can progress subclinically, reinforcing the value of noninvasive imaging in long-term monitoring. We also evaluated the performance of noninvasive fibrosis indexes for detection of significant fibrosis (as defined by VCTE). Both FIB4 and APRI demonstrated good diagnostic accuracy, consistent with recent meta-analyses that have validated these tools in posttransplant populations.[20]

With regard to quality of life, our findings partially aligned with prior research. A recent biopsy-based study has reported that hepatic steatosis was associated with impaired quality of life and that fibrosis was associated with poorer general health in posttransplant patients.[32] Similarly, a study in patients with T2DM has identified hepatic fibrosis, as evaluated with VCTE, and obesity as key factors that erode quality of life.[33] To our knowledge, our present study is the first study to assess the relationship between quality of life and hepatic steatosis/fibrosis as evaluated with VCTE in LT recipients.

We observed that significant fibrosis may adversely affect the physical role domain of the SF-36 quality of life survey, whereas male sex appeared to be associated with better scores in general health, emotional role limitations, and social function domains. This negative association between fibrosis and physical role limitations aligns with findings in MASLD, where higher degrees of fibrosis have been linked to increased fatigue, thereby impairing physical function.[34,35] It is plausible that similar mechanisms were present in our cohort. Furthermore, although histopathology was not performed, we hypothesize that a subset of patients within the significant fibrosis group may have progressed to early-stage liver cirrhosis, which could further contribute to the observed decline in quality of life.[36] This underscores the potential of graft fibrosis to impair daily activities and physical well-being in long-term LT recipients. As fibrosis advances, fatigue, decreased exercise tolerance, and physical limitations may arise, all of which diminish quality of life.

Limitations

This study had several limitations that should be acknowledged. First, the cross-sectional design limited the ability to evaluate longitudinal changes in liver fibrosis, steatosis, and quality of life in the long-term and as such prevents conclusions about disease progression or response to interventions. A prospective follow-up study could provide more robust insights. Second, VCTE, despite its noninvasive nature, is subject to variability due to operator dependency, probe selection, and patient-related factors such as obesity and hepatic congestion, all of which potentially degrade measurement accuracy.

In addition, although fibrosis indexes such as FIB4, APRI, and NFS were included for comparison, these scores were originally developed for nontransplant populations and may not be fully reliable in LT recipients, necessitating further validation by histopathology. The absence of liver biopsy data in this study prevented direct histological correlation, which is essential to distinguish fibrosis from other graft-related complications such as rejection or recurrent disease. Furthermore, potential confounding factors, including immunosuppression regimens, patient adherence to medication regimens, and unmeasured metabolic variables, may have influenced both liver function and quality of life outcomes.

The fact that VCTE measurements were performed by only 2 operators may have influenced the results. Restriction of the study population to LT recipients more than 10 years posttransplant may have reduced the applicability of the results to patients in earlier stages of follow-up, as graft function, complication rates, and quality of life parameters can vary substantially over time.

Finally, as a single-center study conducted at a tertiary referral hospital, the results may not be fully representative of LT recipients seen in other types of health care settings. Future multicenter, longitudinal studies that include histopathology validation and a broader patient population are needed to refine the clinical utility of VCTE in posttransplant assessment.

Conclusions

Vibration-controlled transient elastography is a valuable tool for assessment of liver fibrosis and steatosis in long-term LT recipients and provides critical insights beyond standard liver function tests. This study highlights the observation that hepatic steatosis and fibrosis can progress even when conventional biomarkers remain within normal ranges. In addition, DM emerged as a key factor associated with liver graft function, emphasizing

the need for metabolic monitoring in posttransplant care. Quality of life was influenced by both fibrosis and demographic factors, underscoring the importance of a holistic approach to patient management. Given its ability to detect subclinical graft changes, VCTE should be integrated into routine posttransplant monitoring to optimize long-term outcomes.

References

1. Ivanics T, Abreu P, De Martin E, Sapisochin G. Changing trends in liver transplantation: challenges and solutions. *Transplantation*. 2021;105(4):743-756. doi:10.1097/TP.0000000000003454
2. Zanetto A, Shalaby S, Gambato M, et al. New indications for liver transplantation. *J Clin Med*. 2021;10(17):3867. doi:10.3390/jcm10173867
3. Wei Q, Wang K, He Z, Ke Q, Xu X, Zheng S. Acute liver allograft rejection after living donor liver transplantation: risk factors and patient survival. *Am J Med Sci*. 2018;356(1):23-29. doi:10.1016/j.amjms.2018.03.018
4. Levitsky J, Goldberg D, Smith AR, et al. Acute rejection increases risk of graft failure and death in recent liver transplant recipients. *Clin Gastroenterol Hepatol*. 2017;15(4):584-593.e2. doi:10.1016/j.cgh.2016.07.035
5. Choudhary NS, Saraf N, Saigal S, et al. Revisiting chronic rejection following living donor liver transplantation in the tacrolimus era: a single center experience. *Clin Transplant*. 2018;32(2):10.1111/ctr.13161. doi:10.1111/ctr.13161
6. Baumann AK, Schlue J, Noyan F, et al. Preferential accumulation of T helper cells but not cytotoxic T cells characterizes benign subclinical rejection of human liver allografts. *Liver Transpl*. 2016;22(7):943-955. doi:10.1002/lt.24427
7. Dunn MA, Rogal SS, Duarte-Rojo A, Lai JC. Physical function, physical activity, and quality of life after liver transplantation. *Liver Transpl*. 2020;26(5):702-708. doi:10.1002/lt.25742
8. Cheung A, Levitsky J. Follow-up of the post-liver transplantation patient: a primer for the practicing gastroenterologist. *Clin Liver Dis*. 2017;21(4):793-813. doi:10.1016/j.cld.2017.06.006
9. Silva AC, Nogueira P, Machado MV. Hepatic steatosis after liver transplantation: a systematic review and meta-analysis. *Liver Transpl*. 2023;29(4):431-448. doi:10.1097/LVT.0000000000000060
10. Shaked O, Demetris J, Levitsky J, et al. Impact of donor and recipient clinical characteristics and hepatic histology on steatosis/fibrosis following liver transplantation. *Transplantation*. 2022;106(1):106-116. doi:10.1097/TP.0000000000003681
11. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol*. 2003;29(12):1705-1713. doi:10.1016/j.ultrasmedbio.2003.07.001
12. Winters AC, Mittal R, Schiano TD. A review of the use of transient elastography in the assessment of fibrosis and steatosis in the post-liver transplant patient. *Clin Transplant*. 2019;33(10):e13700. doi:10.1111/ctr.13700
13. Siddiqui MS, Idowu MO, Stromberg K, et al. Diagnostic performance of vibration-controlled transient elastography in liver transplant recipients. *Clin Gastroenterol Hepatol*. 2021;19(2):367-374. doi:10.1016/j.cgh.2020.03.067
14. Barrault C, Roudot-Thoraval F, Tran Van Nhieu J, et al. Non-invasive assessment of liver graft fibrosis by transient elastography after liver transplantation. *Clin Res Hepatol Gastroenterol*. 2013;37(4):347-352. doi:10.1016/j.clinre.2012.11.003
15. Rigamonti C, Fraquelli M, Bastiampillai AJ, et al. Transient elastography identifies liver recipients with nonviral graft disease after transplantation: a guide for liver biopsy. *Liver Transpl*. 2012;18(5):566-576. doi:10.1002/lt.23391
16. Beckebaum S, Iacob S, Klein CG, et al. Assessment of allograft fibrosis by transient elastography and noninvasive biomarker scoring systems in liver transplant patients. *Transplantation*. 2010;89(8):983-993. doi:10.1097/TP.0b013e3181cc66ca
17. Crespo G, Castro-Narro G, García-Juárez I, et al. Usefulness of liver stiffness measurement during acute cellular rejection in liver transplantation. *Liver Transpl*. 2016;22(3):298-304. doi:10.1002/lt.24376
18. Lee CK, Nastasio S, Mitchell PD, et al. Transient elastography assessment of liver allograft fibrosis in pediatric transplant recipients. *Pediatr Transplant*. 2020;24(6):e13736. doi:10.1111/petr.13736
19. Baral A, Garg S, Nguyen M, et al. Vibration-controlled transient elastography-based parameters predict clinical outcomes in liver transplant recipients. *Clin Gastroenterol Hepatol*. 2024;22(12):2424-2431.e4. doi:10.1016/j.cgh.2024.06.020
20. Bhat M, Tazari M, Sebastiani G. Performance of transient elastography and serum fibrosis biomarkers for non-invasive evaluation of recurrent fibrosis after liver transplantation: a meta-analysis. *PLoS One*. 2017;12(9):e0185192. doi:10.1371/journal.pone.0185192
21. Tang LCY, Chetwood JD, Lai MSM, et al. Incidence, epidemiology, and outcomes of acute allograft rejection following liver transplantation in Australia. *Liver Transpl*. 2024;30(10):1039-1049. doi:10.1097/LVT.0000000000000375
22. Oberkofler CE, Raptis DA, Müller PC, et al. Low-dose aspirin confers protection against acute cellular allograft rejection after primary liver transplantation. *Liver Transpl*. 2022;28(12):1888-1898. doi:10.1002/lt.26534
23. Adali G, Bilgic NM, Kalaman AE, Ozturk O, Ozdil K. Prevalence and predictors of metabolic-associated fatty liver disease in liver transplant recipients: a cross-sectional prospective study. *Hepatol Forum*. 2023;4(3):129-134. doi:10.14744/hf.2023.2023.0032
24. Galvin Z, Rajakumar R, Chen E, et al. Predictors of de novo nonalcoholic fatty liver disease after liver transplantation and associated fibrosis. *Liver Transpl*. 2019;25(1):56-67. doi:10.1002/lt.25338
25. Satapathy SK, Tran QT, Kovalic AJ, et al. Clinical and genetic risk factors of recurrent nonalcoholic fatty liver disease after liver transplantation. *Clin Transl Gastroenterol*. 2021;12(2):e00302. doi:10.14309/ctg.0000000000000302
26. Kim H, Lee K, Lee KW, et al. Histologically proven non-alcoholic fatty liver disease and clinically related factors in recipients after liver transplantation. *Clin Transplant*. 2014;28(5):521-529. doi:10.1111/ctr.12343
27. Narayanan P, Mara K, Izzy M, et al. Recurrent or de novo allograft steatosis and long-term outcomes after liver transplantation. *Transplantation*. 2019;103(1):e14-e21. doi:10.1097/TP.0000000000002317
28. Vallin M, Guillaud O, Boillot O, Hervieu V, Scoazec JY, Dumortier J. Recurrent or de novo nonalcoholic fatty liver disease after liver transplantation: natural history based on liver biopsy analysis. *Liver Transpl*. 2014;20(9):1064-1071. doi:10.1002/lt.23936
29. Grancini V, Cogliati I, Alicandro G, et al. Assessment of hepatic fibrosis with non-invasive indices in subjects with diabetes before and after liver transplantation. *Front Endocrinol (Lausanne)*. 2024;15:1359960. doi:10.3389/fendo.2024.1359960
30. Angelico R, Spada M, Liccardo D, et al. Allograft fibrosis after pediatric liver transplantation: incidence, risk factors, and evolution. *Liver Transpl*. 2022;28(2):280-293. doi:10.1002/lt.26218
31. Mak LY, Fung J, Lo G, et al. Impact of liver graft steatosis on long-term post-transplant hepatic steatosis and fibrosis via magnetic resonance quantification. *Front Med (Lausanne)*. 2025;11:1502055. doi:10.3389/fmed.2024.1502055
32. Karam V, Sebagh M, Rifai K, et al. Quality of life 10 years after liver transplantation: the impact of graft histology. *World J Transplant*. 2016;6(4):703-711. doi:10.5500/wjt.v6.i4.703
33. Michel M, Funuyet-Salas J, Doll M, et al. Impairment of health-related quality of life among people with type 2 diabetes and advanced liver fibrosis. *Sci Rep*. 2024;14(1):21650. doi:10.1038/s41598-024-72105-8

34. Younossi ZM, Stepanova M, Anstee QM, et al. Reduced patient-reported outcome scores associate with level of fibrosis in patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol.* 2019;17(12):2552-2560.e10. doi:10.1016/j.cgh.2019.02.024
35. Yilmaz Y, Toraman AE, Alp C, et al. Impairment of patient-reported outcomes among patients with non-alcoholic fatty liver disease: a registry-based study. *Aliment Pharmacol Ther.* 2023;57(2):215-223. doi:10.1111/apt.17301
36. Polis S, Fernandez R. Impact of physical and psychological factors on health-related quality of life in adult patients with liver cirrhosis: a systematic review protocol. *JBI Database System Rev Implement Rep.* 2015;13(1):39-51. doi:10.11124/jbisrir-2015-1987

A Rare Case of Plasmablastic Myeloma After Renal Transplant

Sabir Sulejman,[1] Ardian Memeti,[1] Vlora Sadiku,[1] Galina Severova,[1] Gazmend Amzai,[2] Nikola Gjorgjievski,[1] Gordana Petrushevska,[3] Goce Spasovski[1]

Abstract

Posttransplant lymphoproliferative disorders are a serious complication after solid-organ transplant, with a reported incidence from 2% to 20%. Plasma cell neoplasms in solid-organ transplants represent a rare but increasingly serious complication after solid-organ transplant. We report a case of plasmablastic myeloma, a very rare variant of multiple myeloma with aggressive course and poor prognosis. Few such cases have been reported in the literature in patients after solid-organ transplant. A 41-year-old male patient received a renal transplant from a living unrelated donor (wife) in 2019. After transplant, he was given triple immunosuppression therapy (mycophenolic acid, tacrolimus, prednisolone). In September 2024, 56 months after transplant, the patient presented to our nephrology department due to confusion, weight loss, loss of appetite, and gastric discomfort. Laboratory results showed hypercalcemia, elevated serum creatinine, and thrombocytopenia with anemia, without apparent blood loss. As a result of persistent hypercalcemia and elevated serum creatinine levels, treatment with hemodialysis was initiated. Polymerase chain reaction results were negative for Epstein-Barr virus. After preliminary preparation, bone marrow biopsy was performed, which revealed infiltration by cells with blastic morphology. Immunohistochemical analysis confirmed the finding with more than 80% of cells positive for CD138; the remaining marker tests were mostly negative. The day after the bone marrow biopsy, the patient had progressive deterioration in his health, with severe malaise and disorientation; he developed acute heart failure and pulmonary edema. An urgent hemodialysis was initiated, but it was unsuccessful. The patient died on day 10 of hospitalization. Posttransplant lymphoproliferative disorders and other malignant neoplasms pose a serious posttransplant complication in patients with challenging diagnoses due to overlapping features with other posttransplant complications; such patients most often experience a rapid and atypical course, due to high doses of immunosuppressants.

Key words: *Plasma cell neoplasms, Posttransplant complications, Posttransplant lymphoproliferative disorders, Solid-organ transplantation*

Introduction

Posttransplant lymphoproliferative disorders (PTLD) are a heterogenous group of neoplasms, with an incidence from 2% to 20% with 90% being mostly B-cell neoplasms. Plasma cell neoplasm (PCN) is a rare but increasingly serious complication following solid-organ transplant (SOT); PCNs constitute a distinct and less common entity among PTLDs. The clinical presentation of PCNs may be quite atypical, and accurate diagnosis is a challenge because of overlapping features with other posttransplant complications. According to some studies, the incidence of multiple myeloma is approximately 3 cases per 100 recipients of SOTs. Plasmablastic myeloma (PBM) is a rare subtype of multiple myeloma comprising plasmablasts, which are immature plasma cells that exhibit clear round nuclei with small amounts of cytoplasm. We report a case of PBM, which is a very rare variant of multiple myeloma with aggressive course and poor prognosis.

Case Report

A 41-year-old male patient, with kidney disease of unknown etiology, received a renal transplant from a living unrelated donor (wife) in 2019. One year prior to transplant, he had been on hemodialysis. He received a quadruple immunosuppression protocol,

From the [1]University Clinic for Nephrology and the [2]University Clinic for Hematology, and the [3]Institute of Pathology, Faculty of Medicine, Saints Cyril and Methodius University in Skopje, Skopje, North Macedonia

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: *Sabir Sulejman, Faculty of Medicine, Saints Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia*
Phone: *+389 71652167* ***E-mail:*** *suleymans@live.com*

Experimental and Clinical Transplantation (2025) 8: 555-559

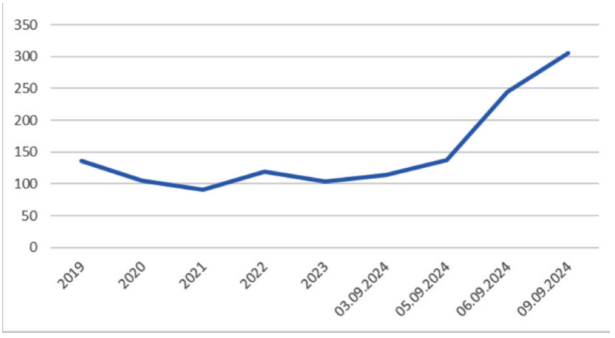
DOI: 10.6002/ect.2025.0148

including induction therapy with anti-thymocyte globulin and triple maintenance immunosuppression therapy (mycophenolic acid, tacrolimus, and prednisolone). In the first 6 months after transplant, a mild elevation in serum creatinine levels was observed (Figure 1), from 150 to 200 μmol/L. Afterward, serum creatinine levels normalized. Two years later, the patient developed mild proteinuria (1.4 g/diuresis), mild anemia and thrombocytopenia, worsening of graft function with serum creatinine from 320 to 175 μmol, calcium 3 mmol/L, and high lactate dehydrogenase and ferritin levels, without deviations in total serum proteins, albumin, and globulin fraction. He also experienced persistent gastric discomfort for the previous 3 months (*Helicobacter pylori* test was negative) as well as confusion. He was referred to a hematologist; however, after the first visit, the patient did not attend further examinations.

In September 2024, 56 months after transplant, the patient presented to our nephrology department due to aggravation of the condition, including confusion, weight loss, loss of appetite, and gastric discomfort. The initial laboratory results showed bicytopenia, impaired graft function with serum creatinine of 247 μmol/L (reference range, 45-109 μmol/L), proteinuria, and hypercalcemia (3.1 mmol/L) (Table 1). Levels of tumor markers carcinoembryonic antigen, CA19-9, and CA72-4 were within references ranges. The only elevated tumor marker was neuron-specific enolase (49.4 ng/mL; elevated from 16.3 ng/mL); we observed that tests for squamous cell carcinoma, carcinoembryonic antigen, α-fetoprotein, β-human chorionic gonadotropin, and prostate-specific antigen were all within the reference ranges.

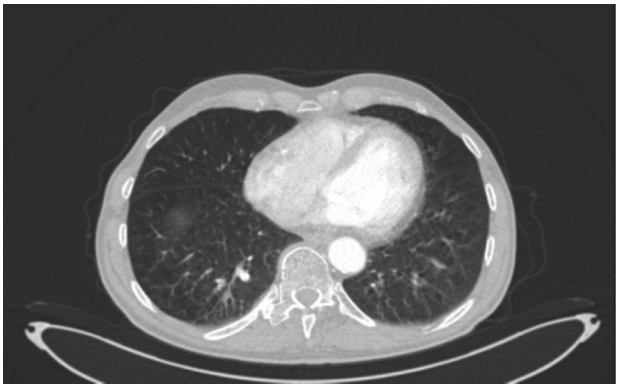
Additional diagnostic procedures were performed. Gastroscopy revealed mild gastritis. Computed tomography scan of the abdomen (Figure 2) did not show any tumor masses in the abdominal cavity but revealed dilated left cardiac cavities, suspected subendocardial infarction of the posterolateral wall of the left ventricle, and pronounced eccentric calcifications along the left anterior descending and circumflex coronary arteries. Because of the patient's agitation and severe general condition, no other radiological examinations were performed to find lytic lesions. An echocardiogram showed elements of pressure overload with left ventricular hypertrophy and/or diastolic dysfunction. Despite treatment with dexamethasone and hydration, hypercalcemia and oligoanuria persisted, and treatment with hemodialysis was initiated. High blood glucose levels were recorded, and insulin therapy was started.

Figure 1. Serum Creatinine Trend After Transplant



Dates are shown on x-axis; creatinine levels (in μmol/L) are shown on y-axis.

Figure 2. Computed Tomography of the Chest



Dilated left cardiac cavities are shown, with suspected subendocardial infarction of the posterolateral wall of the left ventricle.

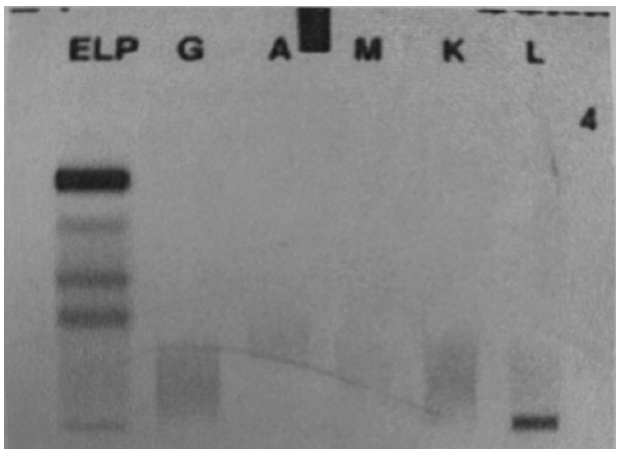
Table 1. Laboratory Results During Hospitalization

Variable	Date						Reference
	Sept. 5	Sept. 7	Sept. 8	Sept. 10	Sept. 11	Sept. 12	Range
RBC, $\times 10^{12}$/L	2.77	2.45	2.60	2.47	2.72	2.79	4.20-5.50
Hemoglobin, g/L	87	78	81	79	86	88	120-180
WBC, $\times 10^{9}$/L	8.1	3.9	6.4	5.3	6.0	6.47	4.00-9.00
Platelets, $\times 10^{9}$/L	47	30	36	27	27	33	150-450
Urea, mmol/L	14.3				18.6	18.2	2.7-7.8
Creatinine, μmol/L	247	190		448	526	517	45-109
Uric acid, μmol/L	736			516			150-450
Total protein, g/L	59					57	63-83
Albumin, g/L	41				35	37	35-50
Sodium, mmol/L	134				129	133	137-145
Potassium, mmol/L	3.66				3.10	3.80	3.8-5.5
Calcium, mmol/L	3.10	2.86	3.06	2.91	3.10	2.98	2.1-2.6
CRP, mg/L	29.5				26.5	29.8	≤6

Abbreviations: CRP, C-reactive protein; RBC, red blood cells; WBC, white blood cells

After the observed bicytopenia and consultation with a hematologist, a peripheral blood smear was performed. Serum protein electrophoresis (Figure 3) was positive. Thrombocytopenia and anemic syndrome were managed by substitution with platelet concentrates and erythrocyte mass.

Figure 3. Electrophoresis of γ-Globulin Fraction



Results show an oligoclonal profile in the γ-globulin fraction. The presence of lambda light chains was detected.

On day 8 of hospitalization, after preliminary preparation, a bone marrow biopsy was performed (Figure 4), which revealed infiltration by cells with blastic morphology. The samples were stained with hematoxylin and eosin stain and Giemsa stain. We also performed immunochemical tests for CD138, CD34, CD117, CD20, and CD3. Immunohistochemical analysis confirmed the finding with more than 80% of cells positive for CD138; the test results for the remaining markers were mostly negative. Tests were not available for MUM1, CD38, and CD56. Also, the tests for Ki-67 proliferative index and Epstein-Barr virus (EBV)-encoded RNA in situ hybridization were not available for evaluation in our institutions.

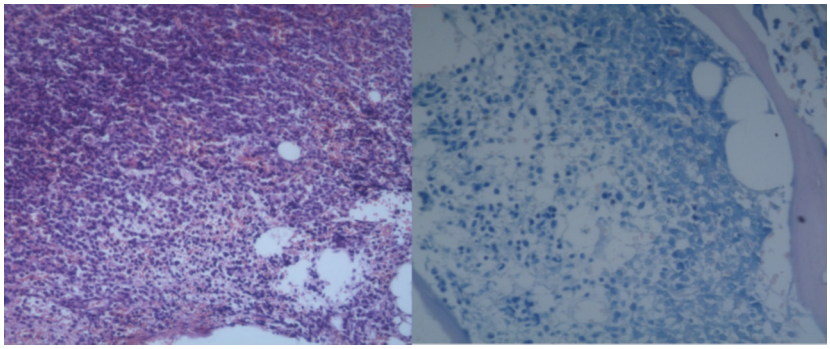
The patient was disoriented and confused throughout the entire hospitalization. A neurological assessment was also performed, which confirmed an acute psycho-organic syndrome. From the beginning of hospitalization, immunosuppression therapy was modified, with discontinuation of mycophenolic acid, reduction of tacrolimus dose to 3 mg daily, and prednisolone at a full dose of 20 mg daily. In the last few days of hospitalization, parenteral dexamethasone 4 mg was also given. As a result of histopathology findings obtained 2 days after the patient's death, antimyeloma therapy was not initiated. The patient had been also treated with antibiotics, intravenous fluids, and diuretics. Urine culture and polymerase chain reaction results were negative for EBV, with <293 copies (cutoff <293 copies). One day later, progressive deterioration in his health occurred, with severe malaise and disorientation, acute heart failure, and pulmonary edema. An urgent hemodialysis was initiated, but it was unsuccessful. The patient died on day 10 of hospitalization.

Discussion

According to some transplant database surveys, PCNs account for 0.2% to 4% of all PTLDs.[1] Compared with the general population, SOT recipients have a 3-fold increased risk of developing PCNs. The pathogenesis of the uncommon types of PCN is still under debate. There is a strong association with EBV infection and an increased risk for PCN in recipients who are EBV seronegative at the time of transplant.

The rare and aggressive variant of multiple myeloma known as PBM is characterized by a predominance of plasmablasts, with a very high mitotic activity and a poor response to conventional therapies. It has an extremely rare occurrence after SOT, with only a few cases described in the literature, and sometimes with early onset after transplant. In the study by Ofori and colleagues, the median time from transplant to diagnosis of PCM was 9.7 years.[1] Sharma and colleagues reported a case of multiple

Figure 4. Bone Marrow Biopsy



Bone marrow biopsy shows infiltration by cells with blastic morphology, with >80% of cells positive for CD138 (left shows hematoxylin and eosin stain, ×200 magnification; right shows Giemsa stain, ×200 magnification).

myeloma that developed early, 15 months after a renal transplant, presenting with back pain radiating in the lower limb.[2] Engels and colleagues reported a median onset of PCN of 3.8 years, with 28 cases (of 140) diagnosed in the first year after transplant.[3]

In SOT recipients, the emergence of PBM could be influenced by chronic immunosuppression, which impairs the immune system and potentially promotes clonal plasma cell expansion. In this population, PTLDs are a common complication, whereas the emergence of PCNs, including PBM, is far less common. Immunosuppressive agents such as calcineurin inhibitors and antimetabolites impair T-cell surveillance, which may cause unchecked proliferation of B cells or plasma cells.

Although EBV is frequently implicated in PTLDs, its role in PBM remains less clearly defined. Only some cases of PBM have shown EBV positivity, suggesting that EBV could play a strong role in the oncogenic process. In terms of clinical presentation, it can present with a wide range of symptoms,[4,5] most often atypical for the underlying disease due to immunosuppression therapy. In general, it poses a diagnostic challenge and in rare cases it can have overlapping features with plasmablastic lymphoma.[6] Both entities demonstrate plasmacytic differentiation and immunophenotypically express plasma cell markers such as CD38, CD138, and MUM1. However, unlike plasmablastic lymphoma, PBM arises in the bone marrow and is often associated with paraproteinemia, hypercalcemia, anemia, renal impairment, and bone lesions.

Regarding the clinical presentation from the available literature, most patients with PCN have presented with malaise, night sweats, abdominal pain, abdominal nodular tumors,[2,5] pathological fracture, vague bone pain, and vertebra compression fracture.[5,7] Another study described sudden onset of memory disorders and speech abnormalities. In some cases of PCN, lytic lesions were also found on radiodiagnostic examinations. In our case, the cardinal symptoms were confusion with disorientation, gastric discomfort, and hypercalcemia with anemia.

Treatment of PBM in SOT recipients poses challenges and is particularly complex, with a need to balance antimyeloma therapy with the risk of graft rejection. Also, reduction of immunosuppression, a mainstay in treatment of PTLDs, may not be effective in cases of PBM; because of its aggressive nature, PBM often requires high-dose chemotherapy and autologous stem cell transplant. These options may be contraindicated due to comorbidities or organ dysfunction. In most cases, switching to mammalian target of rapamycin inhibitors (sirolimus) is recommended.

Several chemotherapeutic options are available for PCN. Bortezomib-based regimens are first-line treatments for most cases of PCN (eg, thalidomide, bortezomib, dexamethasone. In the case described by Sharma and colleagues,[2] the patient was initially treated with the triple protocol of vincristine, adriamycin, and dexamethasone, and a moderate effect was shown after 6 cycles; this treatment was supplemented with a second line of therapy, ie, thalidomide. For aggressive cases like PBM, few therapeutic approaches are available). In CD38-positive cases, daratumumab can be added to the therapy.[7] The triple regimen of cyclophosphamide, bortezomib, and dexamethasone is used most often in extramedullary involvement, and the triple regimen of lenalidomide, cyclophosphamide, and dexamethasone is mostly used in relapsed patients.[6]

Ultimately, the prognosis for this rare disease remains poor, especially in immunocompromised individuals. The rapid and aggressive course of PBM, along with diagnostic delays and limited therapeutic options, contribute to a high mortality rate. In the study by Ofori and colleagues, 5 of the 17 patients died early during the study period.

Conclusions

Posttransplant lymphoproliferative disorders and other malignant neoplasms pose serious posttransplant complications in patients with challenging diagnoses due to overlapping features with other posttransplant complications. The rarity of PCN in SOT recipients has hindered further study. Our case adds to the limited body of literature regarding diagnostic dilemmas, unusual presentation of PBM, and the need for individualized treatment of SOT recipients. Special emphasis should be placed on increasing awareness, vigilant monitoring, and early detection strategies in this group of patients.

References

1. Ofori K, Soderquist CR, Murty VV, et al. The clinical and pathological features of plasma cell myeloma post solid organ transplantation. *Am J Hematol.* 2020;95(12):1531-1541. doi:10.1002/ajh.25988

2. Sharma S, Rana C, Vinod PB, Gupta A. Plasma cell myeloma in a renal transplant recipient: a case report and review of literature. *Indian J Nephrol.* 2011;21(4):270-272. doi:10.4103/0971-4065.78074
3. Engels EA, Clarke CA, Pfeiffer RM, et al. Plasma cell neoplasms in US solid organ transplant recipients. *Am J Transplant*. 2013;13(6):1523-1532. doi:10.1111/ajt.12234
4. Sun X, Peterson LC, Gong Y, Traynor AE, Nelson BP. Post-transplant plasma cell myeloma and polymorphic lymphoproliferative disorder with monoclonal serum protein occurring in solid organ transplant recipients. *Mod Pathol.* 2004;17(4):389-394. doi:10.1038/modpathol.3800080
5. Legeard I, Chevrollier M, Bader G. Diagnosis of a plasmoblastic lymphoma of the mandible after renal transplantation: a case report. *J Oral Med Oral Surg.* 2021;27:46. doi:10.1051/mbcb/2021036
6. Xie X, Wu W, Zhu Y, et al. Successful treatment with lenalidomide of secondary multiple myeloma with extramedullary liver plasmacytoma in a renal transplant recipient: a case report and review of the literature. *Oncol Lett.* 2015;10(5):2931-2936. doi:10.3892/ol.2015.3729
7. Ramadas P, Williams M, Duggan DB. Plasmablastic lymphoma or plasmablastic myeloma: a case of post-transplant lymphoproliferative disorder. *Case Rep Hematol.* 2021;2021:4354941. doi:10.1155/2021/4354941

Case Report

Unexpected Diagnosis of Non-Hodgkin Lymphoma After Liver Transplant: A Case Report of Suspected Neuroendocrine Tumor Recurrence Managed with Robotic Distal Pancreatectomy and Splenectomy

Urjita Das,[1] Srinivasan Muthukrishnan,[2] Miranda James,[3] Seung Tae Lee,[4] Raphael Pascal Henri Meier,[2] Chandra Shekhar Bhati[2]

Abstract

The development of non-Hodgkin lymphoma following liver transplant is rare. We present an unusual case of a 40-year-old female patient with morbid obesity who had undergone a deceased donor liver transplant for an unresectable neuroendocrine tumor of the liver 12 years ago. She presented with a lesion in the tail of pancreas that was suggestive of a recurrent neuroendocrine tumor. She underwent robotic-assisted distal pancreatectomy and splenectomy, which demonstrated a final diagnosis of splenic marginal zone lymphoma. Although there is a high likelihood of recurrence of neuroendocrine tumor after transplant, non-Hodgkin lymphoma and posttransplant lymphoproliferative disorder must be considered in the background of transplant and solid-organ malignancy.

Key words: *Hepatic transplantation, Lymphoproliferative disorders, Neuroendocrine tumor, Splenic marginal zone lymphoma*

Introduction

Posttransplant malignancies are a common long-term complication after liver transplant, and this risk is heightened in the setting of continuous immunosuppression. This is often a major cause of morbidity and mortality in liver transplant recipients. The incidences and outcomes of all cancer types that can occur in liver transplant recipients have not been fully determined. Therefore, greater efforts are needed to capture posttransplant cancer risk patterns so that more optimal surveillance and monitoring of immunosuppression can occur. Moreover, greater awareness is needed for rarer cancer types such as non-Hodgkin lymphoma and posttransplant lymphoproliferative disorder (PTLD). The incidence of PTLD is estimated at 1% to 2% in liver transplant recipients, with non-Hodgkin lymphoma comprising a significant subset of cases. These lymphomas vary in histology and are often driven by Epstein-Barr virus (EBV) infection, although EBV-negative cases are increasingly recognized.

Case Report

A 40-year-old female patient, who received a deceased donor liver transplant 12 years prior for an unresectable well-differentiated neuroendocrine tumor (NET, grade 2) of the liver and was on postoperative immunosuppression therapy (tacrolimus 4 mg twice daily), presented with abdominal pain, 20-lb weight loss, fever, chills, and submandibular and periauricular lymphadenopathy. She was closely followed with hepatology to detect disease recurrence using chromogranin A level as a marker, the results of which were negative to date. Her comorbid conditions were morbid obesity (body mass index 62.8), hepatic steatosis, diabetes, and hypertension. She had a history of cytomegalovirus viremia, which occurred 5 months after transplant, and rejection (Banff score 7), which occurred 14 months after transplant. Computed tomography (CT) scan of the abdomen

From the [1]University of Maryland School of Medicine, Baltimore; the [2]Division of Transplant Surgery and the [3]Department of Pathology, University of Maryland Medical Center, Baltimore; and the [4]Department of Hematology/Oncology, University of Maryland Greenbaum Cancer Center, Baltimore, Maryland, USA

Acknowledgements: *We extend our gratitude to the patient who provided support toward our research efforts and provided her signed and documented consent. 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: *Chandra Shekhar Bhati, University of Maryland Medical Center, 16 South Eutaw Street Suite 100, Baltimore, MD 21201, USA*
Phone: *+1 804 502 9984* ***E-mail:*** *cbhati@som.umaryland.edu*

Experimental and Clinical Transplantation (2025) 8: 560-564

DOI: 10.6002/ect.2025.0117

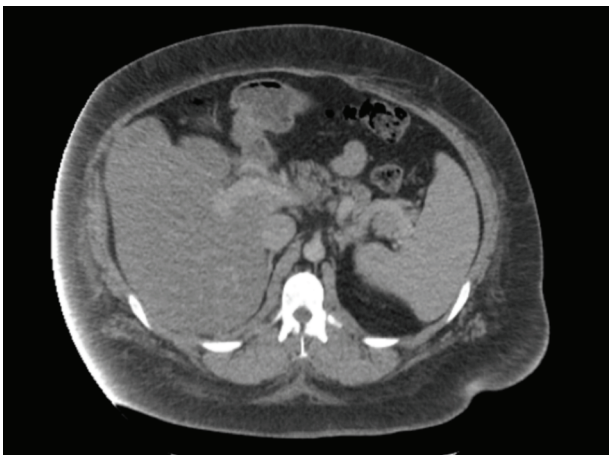
and pelvis showed a 3.2-cm rounded soft tissue 2 density at the splenic hilum distant to the splenic artery and vein, and a 2.1 × 3.1-cm lesion on the tail 4 of the pancreas (Figure 1). Gallium dotatate positron emission tomography (PET) revealed increased metabolic activity of the spleen and pancreas tail lesion suspicious of recurrent or metastatic NET. The spleen was enlarged and measured 18.9 cm. The maximum standardized uptake value was 15.3 for the liver, 15.2 for the pancreatic tail, and 34.8 for the spleen.

Chromogranin A was 39 ng/mL, and alkaline phosphatase was elevated at 152 U/L. Lipase, amylase, total bilirubin, and liver enzymes were within reference ranges. Test results were negative for EBV DNA polymerase chain reaction (PCR), EBV DNA log10 PCR, hepatitis B virus antibody and surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antigen and antibody.

Upper endoscopic ultrasonography revealed 2 hypoechoic, well-defined lesions in the pancreatic tail and splenic hilum, which were further characterized by magnetic resonance imaging. A 2.1 × 3.1-cm hypoenhancing pancreatic tail lesion and an 18.4-cm splenomegaly were observed on magnetic resonance imaging. No intrahepatic biliary ductal dilation or pancreatic ductal dilation was shown, but there was diffuse hepatic steatosis without focal lesions. Fine needle aspiration using a transgastric approach of the celiac node and pancreatic node was performed, and the test results were negative for malignant cells. This case was discussed by the multidisciplinary tumor board and given the high clinical suspicion of recurrent NET. She was scheduled for robotic-assisted distal pancreatectomy and splenectomy.

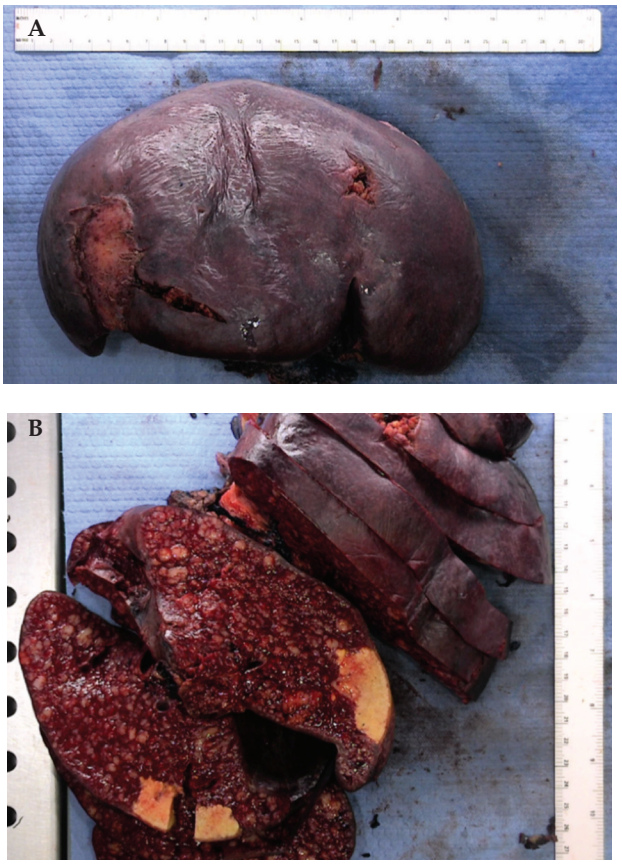
The patient was positioned in a supine, semi-right lateral orientation, with the left arm abducted at a 90-degree angle and the right arm resting alongside the body. We used a robotic surgical system with 4 arms (da Vinci Xi, Intuitive). After a quick diagnostic laparoscopy of the abdominal cavity, 4 robotic trocars were placed. Numerous omental adhesions to the abdominal wall and transplant liver were present, which we released. The gastrocolic omentum was incised, and the lesser sac was entered. The spleen was noted to be enlarged (Figure 2, A and B). A pancreatic tail lesion was noted to be present over the splenic vein. We carefully dissected the mass and excised a biopsy that measured 0.9 × 0.8 × 0.4 cm from the vein and artery, which was sent for frozen section analysis.

Figure 1. Computed Tomography Scan of Splenic and Pancreatic Masses, 4 Months Before Surgery



Computed tomography scan (4 months before surgery) of splenic and pancreatic masses shows homogenous hypoechoic well-defined lesions of the pancreatic tail and splenic hilum.

Figure 2. Spleen



A, Gross spleen intact. **B**, Gross spleen in sections.

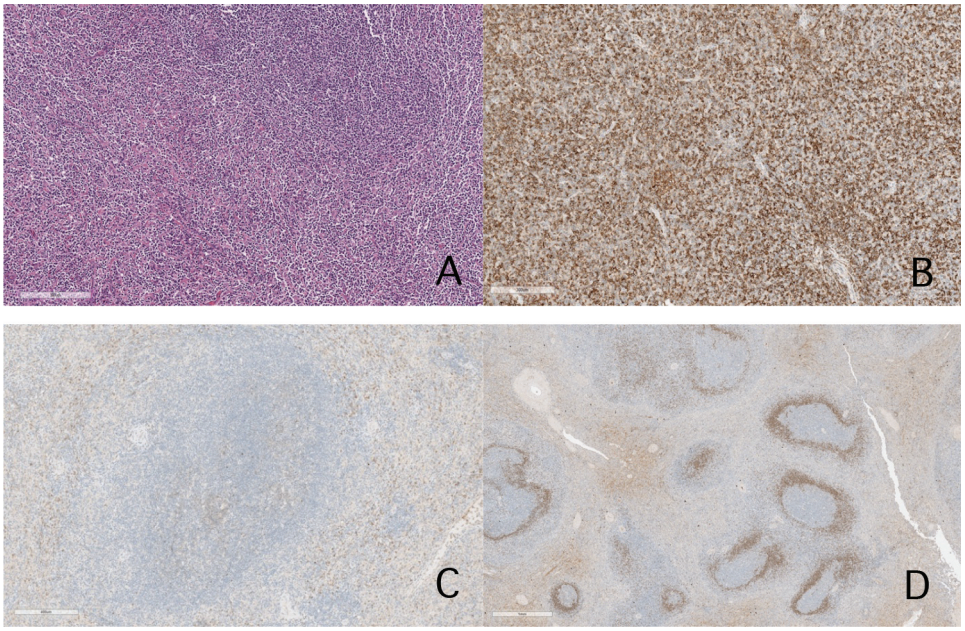
The frozen biopsy result favored neuroendocrine origin, although it was a difficult distinction given history of prior NET, so we proceeded with a distal pancreatectomy and splenectomy. Careful dissection was performed toward the 3 × 2-cm lesion, which

was in close proximity to the splenic artery in the region of the pancreatic tail and appeared white-tan in color and hemorrhagic. The splenic artery was dissected, and a robotic stapler was fired across the splenic artery. The rest of the pancreas with the splenic vein was then divided with the stapler. The spleen was mobilized from its attachments, and the specimen was removed using a left subcostal incision. The spleen measured 28.5 × 18.5 × 11.5 cm, and the distal pancreas measured 11.5 × 9.0 × 5.0 cm. After hemostasis was achieved, a drain was placed, and all ports were closed.

The final pathology report surprisingly confirmed the lesion to be a B-cell lymphoma, most consistent with splenic marginal zone lymphoma (SMZL) with direct extension to the pancreas tail. Hematoxylin and eosin staining showed diffuse infiltration of small lymphoid cells involving the splenic white pulp, red pulp sinuses, and cords, and the pancreatic parenchyma (Figure 3A). Immunohistology staining was positive for CD19 (Figure 3B), CD45, and CD25 (dim), and CD20 kappa and lambda light chain stains showed polytypic plasma cells. The Ki67 proliferation index appeared to increase around 30% to 40%; however, this could have been because of proliferation among the numerous interspersed T cells. Immunostaining on block B12 showed reactive follicles with germinal centers that were positive for CD10 (Figure 3C) and negative for BCL-2. The splenic mass also demonstrated positivity for immunoglobin M and immunoglobin D (Figure 3D) in mantle zones with extrafollicular B-cell staining. Results from Epstein-Barr encoded RNA in situ hybridization, EBV PCR, EBV DNA log10 PCR, and EBV DNA Quant Source were negative for the biopsy. Results from the flow cytometry of the pancreatic mass showed positivity for CD10 lambda restricted B cells on a background of CD20, CD19, and light chain, which confirmed the diagnosis of SMZL.

Postoperatively, the patient was noted to have leukocytosis and thrombocytosis. Her hospital course was unremarkable except for hyperglycemia, and she was discharged on postoperative day 4. Subsequent PET-CT demonstrated generalized lymphadenopathy above and below the diaphragm with mild to moderate intensity of the maximum standardized uptake value. The peripheral blood flow cytometry results were negative for monoclonal B cells. She was started on rituximab (weekly dose of 375 mg/m^2) for 8 cycles and continued on tacrolimus (4 mg twice daily) with a goal of 4 to 6 ng/mL. Her following PET-CT 10 months after the initial PET-CT later showed no metabolic evidence of malignancy or recurrent lymphoma (Table 1).

Figure 3. Pathology Images of Splenic Biopsy



A, Section of spleen shows replacement and infiltration of red and white pulp with small size to medium size lymphoid cells (hematoxylin and eosin stain, ×100). **B,** Splenic lesion shows immunohistochemical staining positive for CD19 (CD19 stain, ×100). **C**, Section of spleen with CD10 highlights cells in a germinal center of a reactive follicle (CD10 stain, ×60). **D**, Spleen with immunoglobin D staining mantle zone cells of the reactive follicles (immunoglobin D stain, ×25).

Table 1. Key Events From Transplant to Diagnosis, Surgical Intervention, and Treatment Follow-Up.

Time Posttransplant	Event
0	Liver transplant for NET.
0-12 y	Surveillance (chromogranin A, imaging).
12 y	Symptom onset of abdominal pain, 20-lb weight loss, fever, lymphadenopathy, chills. CT abdomen and pelvis and MRI abdomen demonstrate splenic and pancreatic lesions, which correlate with PET hypermetabolic activity. Test for EBV was seronegative. FNA sample was negative for malignant cells.
12 y, 2 mo	Underwent robotic-assisted distal pancreatectomy and splenectomy. Pathology diagnosed SMZL. PET-CT demonstrated generalized lymphadenopathy. Rituximab therapy with continuation of tacrolimus.
13 y	At the 10-mo follow-up, PET-CT demonstrated no evidence of disease.

Abbreviations: CT, computed tomography; EBV, Epstein-Barr virus; FNA, fine needle aspiration; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; PET, positron emission tomography; SMZL, splenic marginal zone lymphoma

Discussion

There is an established increased risk of non-Hodgkin lymphoma following solid-organ transplant. However, a low-grade lymphoma is typically not categorized as a PTLD unless the PCR results are positive for EBV DNA. Although SMZL is not traditionally categorized under PTLD in the absence of EBV positivity, it is important to recognize that late onset, EBV-negative indolent PTLD is a well-described entity. The differentiation between de novo SMZL and indolent PTLD in the posttransplant population remains complex and may overlap clinically and histologically. The intraoperative frozen section material of the pancreatic tail lesion was highly challenging to interpret given the history of a NET. However, NET was a favored diagnosis at the time of frozen section analysis because NET has a high rate of post-transplant recurrence.

The liver is the most involved organ affected by NET.[1] Clinical-pathological staging of NET has provided indications for liver transplant to prolong survival and provide curative intent in patients with unresectable tumor bulk, expected survival ≥70%, and a 5-year recurrence-free survival rate greater than 50%.[1] Our patient's younger age (≤45 years old) made her a candidate for liver transplant due to her likelihood of improved survival.[2] Nevertheless, NET relapse in cases of liver metastases has high recurrence rate, ranging from 31.3% to 56.8% at 5 years after liver transplant.[3,4] Patients with unilobar or single metastatic spread without evidence of extrahepatic disease exhibit a 5-year tumor recurrence rate of 80%.[1] Aside from the increase likelihood of disease recurrence, little is known about posttransplant prognostic courses due to the absence of solid data.

The incidence of SMZL following liver transplant is unknown. Diffuse large B-cell lymphoma is the most common non-Hodgkin lymphoma subtype among transplant recipients and individuals infected with HIV.5 A US registry of 175732 solid-organ recipients between 1987 and 2008 demonstrated a bimodal onset of non-Hodgkin lymphoma and PTLD following solid-organ transplant involving patients aged 0 to 34 years or ≥50 years at transplant.[5] The same registry revealed non-Hodgkin lymphoma had the greatest risk among lung recipients, and secondarily kidney and liver recipients.[5] In another population-based study of 540 Finnish liver transplant recipients, 8 cases (1.4%) developed non-Hodgkin lymphoma, of which 4 cases (0.7%) were PTLD.[6] Elevated non-Hodgkin lymphoma risk was associated with male sex, young age, and the immediate posttransplant period in this study.[6] In another population-based study of 2005 adult liver transplant patients, 23 patients (1.1%) were identified with PTLD and 5 patients (0.2%) were identified with B-cell non-Hodgkin lymphoma.[7] Reports of non-Hodgkin lymphoma following liver transplant for NET, in which the likelihood of NET recurrence may appear greater, are insufficient.

Given the potential role of chronic calcineurin inhibitor exposure in lymphoma pathogenesis, tacrolimus had been continued for 12 years after the liver transplant at a reduced dose with a target trough level of 4 to 6 ng/mL; this regimen was monitored closely in consultation with transplant hepatology to balance the risk of rejection and lymphoma progression. Chronic immunosuppression with tacrolimus at reduced doses could have led to suppression of T-cell activation and impaired immune surveillance against oncogenic viruses such as EBV or malignant lymphoid clones, which could have inevitably increased the patient's risk of lymphoma. In a large multicenter, case control study of 2495 living donor transplants, total cumulative exposure, and not just the daily dose, of tacrolimus was an independent risk factor for the development of non-Hodgkin lymphoma following living donor

transplant.[8] Thus, tacrolimus at a dose of 4 mg twice daily for 12 years after liver transplant would result in a higher cumulative exposure that could place a patient at risk for non-Hodgkin lymphoma.

After the patient's splenectomy and pancreatectomy, tacrolimus was restarted at the same dose with addition of rituximab monotherapy. Bone marrow biopsy was not performed because of absence of cytopenias and lack of systemic symptoms. Based on the indolent histology and limited stage of disease, rituximab monotherapy was selected in alignment with National Comprehensive Cancer Network guidelines for SMZL. We used PET-CT imaging for assessment of treatment response and observed complete metabolic remission at 10 months.

The group of PTLDs encompasses a spectrum of lymphoid proliferations, and, although most cases are EBV-positive and polyclonal, late onset monomorphic PTLD may be negative for EBV and histologically indistinguishable from de novo B-cell lymphomas. Splenic marginal zone lymphoma remains a distinct entity characterized by splenic involvement and indolent behavior. In this case, despite the patient's transplant history, the lack of EBV association and histopathological features favored SMZL, although the possibility of EBV-negative PTLD remains part of the differential diagnosis. Thus, it is important for the transplant team, oncologist, and radiologist to be alert to the possibility of non-Hodgkin lymphoma in addition to PTLD for several years after transplant.

References

1. Mazzaferro V, Pulvirenti A, Coppa J. Neuroendocrine tumors metastatic to the liver: how to select patients for liver transplantation? *J Hepatol*. 2007;47(4):460-466. doi:10.1016/j.jhep.2007.07.004
2. Valvi D, Mei X, Gupta M, et al. Younger age is associated with improved survival in patients undergoing liver transplantation alone for metastatic neuroendocrine tumors. *J Gastrointest Surg*. 2021;25(6):1487-1493. doi:10.1007/s11605-020-04708-1
3. Moris D, Tsilimigras DI, Ntanasis-Stathopoulos I, et al. Liver transplantation in patients with liver metastases from neuroendocrine tumors: a systematic review. *Surgery*. 2017;162(3):525-536. doi:10.1016/j.surg.2017.05.006
4. Sposito C, Droz dit Busset M, Citterio D, Bongini M, Mazzaferro V. The place of liver transplantation in the treatment of hepatic metastases from neuroendocrine tumors: pros and cons. *Rev Endocr Metab Disord*. 2017;18(4):473-483. doi:10.1007/s11154-017-9439-7
5. Engels EA, Pfeiffer RM, Fraumeni JF, et al. Spectrum of cancer risk among US solid organ transplant recipients. *JAMA*. 2011;306(17):1891-1901. doi:10.1001/jama.2011.1592
6. Åberg F, Pukkala E, Höckerstedt K, Sankila R, Isoniemi H. Risk of malignant neoplasms after liver transplantation: a population-based study. *Liver Transpl*. 2008;14(10):1428-1436. doi:10.1002/lt.21475
7. Dhillon MS, Rai JK, Gunson BK, Olliff S, Olliff J. Post-transplant lymphoproliferative disease in liver transplantation. *Br J Radiol*. 2007;80(953):337-346. doi:10.1259/bjr/63272556
8. Rodríguez-Perálvarez M, Colmenero J, González A, et al. Cumulative exposure to tacrolimus and incidence of cancer after liver transplantation. *Am J Transplant*. 2022;22(6):1671-1682. doi:10.1111/ajt.17021

Rare Presentation of Posttransplant Lymphoproliferative Disease in a Pediatric Liver Transplant Recipient: Plasmablastic Lymphoma Complicated With Hemophagocytic Lymphohistiocytosis

Burcu Belen Apak,[1] Pamir Işık,[1] Figen Özçay,[2] Oya Balcı Sezer,[2] Pelin Bayık,[3] Lale Olcay,[1] Mehmet Coşkun,[4] Emre Karakaya,[5] Mehmet Haberal[5]

Abstract

A 3-year-old female patient, who had received a liver transplant from her father 1 year previously to treat biliary atresia, was admitted with fever and pancytopenia. History showed Epstein-Barr virus polymerase chain reaction positivity detected in the patient 3 months earlier; the patient received reduced immunosuppression in doses of tacrolimus, and valganciclovir was administered. Physical examination showed lymphadenopathies at the cervical, axillary and inguinal regions with 2 × 2 cm at diameter, splenomegaly of 5 cm, and fever of 39 °C. Considering the clinical setting of immune suppression, the patient was diagnosed with monomorphic posttransplant lymphoproliferative disorder in the form of plasmablastic lymphoma. The pancytopenia of the patient could not be explained with plasmablastic lymphoma; therefore, a concomitant hemophagocytic lymphohistiocytosis was suspected. The patients was changed from tacrolimus to sirolimus followed by 6 days of induction therapy and received a combination regimen of cyclophosphamide, doxorubicin, prednisolone, and vincristine every 21 days for 4 to 6 cycles plus rituximab 375 mg/m²/wk for 4 weeks. For hemophagocytic lymphohistiocytosis, the patient received intravenous immunoglobulin 1 g/kg every week for 4 weeks, followed by monthly therapy during chemotherapy cycles. After 2 courses of chemotherapy and 4 doses of rituximab, lymph nodes and splenomegaly disappeared, blood tests returned to standard levels, and Epstein-Barr virus polymerase chain reaction results were negative. Positron emission tomography computed tomography after 2 cycles showed complete remission of disease. After 5 cycles of chemotherapy, the patient remained well without any complications.

Key words: *Hemophagocytosis, Lymphoma, Posttransplant lymphoproliferative disease*

Case Report

A 3-year-old female patient, who had received a liver transplant from her father 1 year previously to treat biliary atresia, was admitted with fever and pancytopenia. History showed Epstein-Barr virus (EBV) polymerase chain reaction (PCR) positivity detected in the patient 3 months earlier; immunosuppression was reduced in doses of tacrolimus, and valganciclovir was administered. Physical examination showed lymphadenopathies at the cervical, axillary, and inguinal regions with diameter 2 × 2 cm, splenomegaly of 5 cm, and fever of 39 °C.

Laboratory tests revealed hemoglobin 6.8 g/dL, white blood cells 3.24 × 10^3 cells/μL, platelets 68 × 10^3 cells/μL, and absolute neutrophil count 0.9 × 10^3 cells/μL. The biochemical parameters were within reference ranges except for lactate dehydrogenase (1623 U/L) and uric acid (8.5 mg/dL). Epstein-Barr virus PCR revealed 5 × 10^4 copies/mL. Tacrolimus trough level was 13.7 μg/L at the time of admission.

Radiology imaging showed multiple lymphadenopathies in bilateral parailiac, mesenteric,

From the [1]Department of Pediatric Hematology and Oncology, the [2]Department of Pediatric Gastroenterology, the [3]Department of Pathology, the [4]Department of Radiology, and the [5]Department of General Surgery, Organ Transplantation Center, Baskent University Medical Faculty, Ankara, 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: *F. Burcu Belen Apak, Baskent University Medical Faculty Department of Pediatric Hematology and Oncology, Pediatric Hematopoietic Stem Cell Transplantation Unit, Sehit Temel Kuguluoglu Street No 24 Bahcelievler, Ankara, Turkey*
Phone: *+90 532 581 45 51* ***E-mail:*** *draidabb@gmail.com*

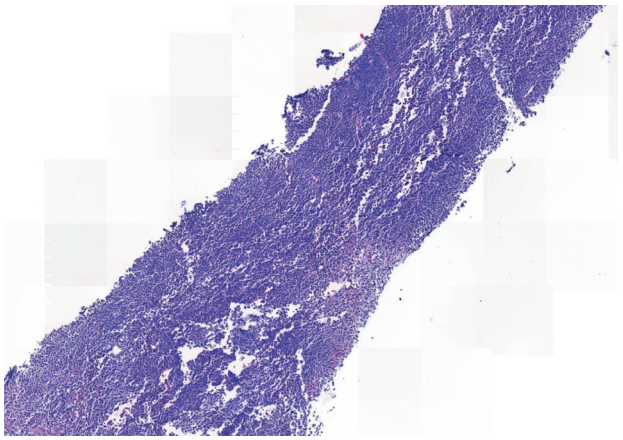
Experimental and Clinical Transplantation (2025) 8: 565-568

DOI: 10.6002/ect.2024.0326

cervical, axillary, paratracheal, pretracheal, subcarinal, and hilar regions with a maximum diameter of 3 × 3 cm. Positron emission tomography of multiple lymph nodes showed increased fluorodeoxyglucose uptake in the bilateral cervical chain, left supraclavicular region, which was more pronounced on the left side, bilateral axillae, left retropectoral area, bilateral perimammary region, mediastinum, bilateral hilar regions, left anterior peridiaphragmatic region, and abdominopelvic region.

Core biopsy from the enlarged lymph node revealed loss of normal lymph node architecture consisting of monotonous cells with intermingling areas of necrosis (Figure 1). On closer inspection, the cells had medium-sized, hyperchromatic nuclei with eccentric and basophilic cytoplasm and showed numerous areas of mitosis (Figure 2). Immunohistochemical studies displayed strong and diffuse CD138 positivity (Figure 3) with a Ki-67 proliferation index reaching 90% (Figure 4). The neoplastic cells showed positive results for the lambda light chain protein, whereas kappa positivity was observed only in a few cells. The results of EBV RNA in situ hybridization for integrated EBV genome were negative.

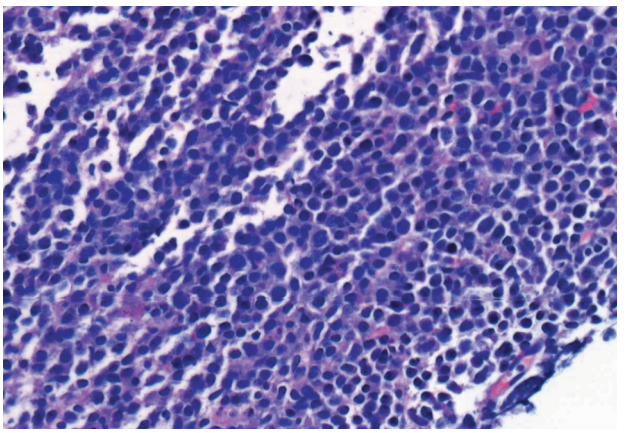
Figure 1. Lymph Node Core Needle Biopsy Shows Loss of Normal Lymph Node Architecture With a Monotonous Infiltration



Hematoxylin and eosin stain; ×40 original magnification.

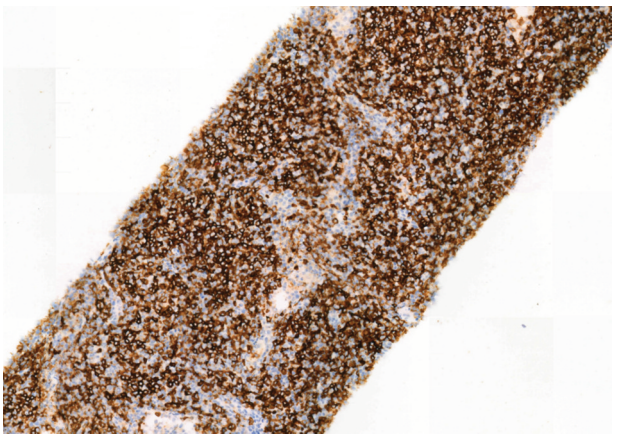
Considering the clinical setting of immune suppression, the diagnosis was monomorphic post-transplant lymphoproliferative disorder (PTLD) in the form of plasmablastic lymphoma (PBL). The bone marrow aspiration and biopsy results were negative for lymphoma infiltration; however, increased levels of histiocytes and hemophagocytosis were detected. The pancytopenia of the patient could not be explained with PBL; therefore, a concomitant hemophagocytic lymphohistiocytosis (HLH) was suspected. With the laboratory tests (pancytopenia; increased ferritin, 5291 μg/L; increased triglyceride, 240 mg/dL) and the prolonged fever, splenomegaly, and hemophagocytosis in bone marrow aspiration, the patient fulfilled the HLH-2009 diagnostic criteria and was diagnosed as PBL-type monomorphic PTLD leading to secondary HLH.

Figure 2. Neoplastic Cells With Medium-Sized, Hyperchromatic Nuclei and Scanty, Eccentric, Basophilic Cytoplasm



Hematoxylin and eosin stain; ×400 original magnification.

Figure 3. Diffuse CD138 Positivity

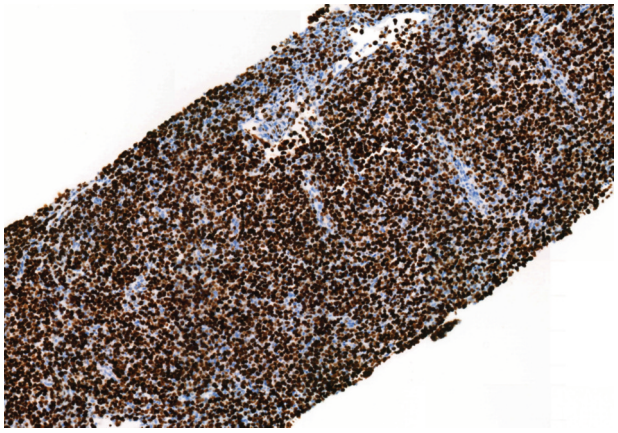


Original magnification, ×100.

The patient was administered reduced immunosuppression with a change from tacrolimus to sirolimus followed by 6 days of induction therapy, as well as a planned combination regimen of cyclophosphamide, doxorubicin, prednisolone, and vincristine (CHOP chemotherapy) every 21 days for 4 to 6 cycles plus rituximab 375 mg/m^2/wk for 4 weeks. For HLH therapy, the patient was given

intravenous immunoglobulin 1 g/kg once a week for 4 weeks, followed by monthly therapy during chemotherapy cycles. After 2 courses of chemotherapy and 4 doses of rituximab, the lymph nodes and splenomegaly disappeared, blood tests returned to standard levels, and EBV PCR showed negative results. The positron emission tomography computed tomography control after 2 cycles revealed complete remission of disease. After 5 cycles of chemotherapy the patient remained well without any complications. A final cycle of chemotherapy was planned (total of 6 cycles), with follow-up after treatment cessation.

Figure 4. Very High Ki-67 Proliferation Index



Original magnification, ×200.

Discussion

Plasmablastic lymphoma is a rare cause of PTLD and was first described as an EBV-associated B-cell neoplasm in patients with HIV.[1] Plasmablastic lymphoma was also reported as a rare cause of PTLD.[2,3] Plasmablastic lymphoma has a strong association with EBV infection, and EBV positivity is found in 80% of patients with PBL. Plasmablastic lymphoma is recognized as a CD20-negative aggressive non-Hodgkin lymphoma by the World Health Organization Classification of Lymphomas.[4] In addition to patients with HIV, PBL can be seen rarely in other immunocompromised patient groups, such as recipients after solid-organ transplant and patients with autoimmune diseases.

Clinically, 53% of patients with PBL present with extranodal involvement; however, our patient was admitted with both nodal and extranodal involvement. In the pathogenesis of PBL, chronic EBV infection (which was also the case in our patient) and acquisition of MYC gene rearrangements seem to play a role.[5] We could not show MYC gene rearrangement in the pathology specimens of our patient. The immunophenotype of the lymph node biopsy of our patient was consistent with typical PBL, with CD79a, CD38, and CD138 positivity, as well as EBV RNA positivity showing EBV association.

In a 2012 study of 195 adult patients reported by the German PTLD Registry, 8 patients (4%) were diagnosed as PBL-PTLD; all of these patients were adult patients with a median age of 47 years (range, 30-67 years) at diagnosis. Although there are rare reports in the literature of cases associated with HIV, we could only find a single case of PBL in an infant with combined living donor small bowel and liver transplant.[6] This was a 14-month-old female baby who had received a transplant to treat ultra-short bowel syndrome and total parenteral nutrition-induced cholestatic liver disease. Five months after transplant, the patient developed multifocal cutaneous and systemic EBV-associated PBL-PTLD and died due to multiorgan failure. Given the rarity of the disease, most data are from patients with HIV-positive PBL.

Literature reviews have indicated poor survival rates in patients with PBL. A study of 112 patients with HIV-positive PBL showed a median overall survival (OS) of 15 months, and another study reported a shorter median OS in 76 patients with HIV-negative PBL.[7,8] The Lymphoma Study Association has reported a large retrospective cohort of 135 patients diagnosed with PBL for the period 2000 to 2015 (56 were positive for HIV, 17 were post-transplant cases, and 62 were immunocompetent patients) with a median OS of 32 months.[9] A recent multicenter study of 80 patients with stage I or II PBL from 13 academic centers in the United States showed a 3-year progression-free survival of 72% (95% CI, 62-83) and OS of 79% (95% CI, 70-89).[10]

Despite these data for HIV-positive PBL and PBL-PTLD in adults, there is no previously published report showing prognosis among pediatric patients. However, our patient demonstrated a complete response to chemotherapy after 2 cycles of rituximab-CHOP, and she was in remission after 5 cycles; yet it remains difficult to foresee the prognosis of our patient. Furthermore, our patient had been admitted with secondary HLH, which further complicated the primary PTLD. However, she responded well to

intravenous immunoglobin therapy and steroids as a part of her PBL chemotherapy, and this response led us to believe that her HLH was associated with her PTLD-lymphoma.

Conclusions

Plasmablastic lymphoma is a rare and aggressive type of non-Hodgkin lymphoma typically seen in the setting of an immunocompromised state, classically associated with HIV infection. Very rarely, PBL may be seen as a type of PTLD. There is only a single reported case of PBL-PTLD following small bowel and liver transplant in an infant. Furthermore, our patient had been admitted with secondary HLH, which further complicated the primary PTLD. Despite the aggressiveness of PBL-PTLD in adults, there is no report showing prognosis of pediatric patients. The rapid response to therapy in our patient was encouraging, but it remains difficult to establish a prognosis in pediatric patients. More studies on PBL-PTLD in pediatric patients are warranted. The initial pancytopenia that cannot be explained by lymphoma bone marrow involvement led to our conclusion that HLH can complicate PTLD cases, and clinicians should be aware of this condition.

References

1. Delecluse HJ, Anagnostopoulos I, Dallenbach F, et al. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood*. 1997;89(4):1413-1420.
2. Borenstein J, Pezzella F, Gatter KC. Plasmablastic lymphomas may occur as post-transplant lymphoproliferative disorders. *Histopathology*. 2007;51(6):774-777. doi:10.1111/j.1365-2559.2007.02870.x
3. Trappe R, Zimmermann H, Fink S, et al. Plasmacytoma-like post-transplant lymphoproliferative disorder, a rare subtype of monomorphic B-cell post-transplant lymphoproliferation, is associated with a favorable outcome in localized as well as in advanced disease: a prospective analysis of 8 cases. *Haematologica*. 2011;96(7):1067-1071. doi:10.3324/haematol.2010.039214
4. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia*. 2022;36(7):1720-1748. doi:10.1038/s41375-022-01620-2. Erratum in: *Leukemia*. 2023;37(9):1944-1951. doi:10.1038/s41375-023-01962-5
5. Ramirez-Gamero A, Martínez-Cordero H, Beltrán BE, Florindez J, Malpica L, Castillo JJ. Plasmablastic lymphoma: 2024 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2024;99(8):1586-1594. doi:10.1002/ajh.27376
6. Apichai S, Rogalska A, Tzvetanov I, Asma Z, Benedetti E, Gaitonde S. Multifocal cutaneous and systemic plasmablastic lymphoma in an infant with combined living donor small bowel and liver transplant. *Pediatr Transplant*. 2009;13(5):628-631. doi:10.1111/j.1399-3046.2008.01026.x
7. Castillo J, Pantanowitz L, Dezube BJ. HIV-associated plasmablastic lymphoma: lessons learned from 112 published cases. *Am J Hematol*. 2008;83(10):804-809. doi:10.1002/ajh.21250
8. Castillo JJ, Winer ES, Stachurski D, et al. HIV-negative plasmablastic lymphoma: not in the mouth. *Clin Lymphoma Myeloma Leuk*. 2011;11(2):185-189. doi:10.1016/j.clml.2011.03.008
9. Tchernonog E, Faurie P, Coppo P, et al. Clinical characteristics and prognostic factors of plasmablastic lymphoma patients: analysis of 135 patients from the LYSA group. *Ann Oncol*. 2017;28(4):843-848. doi:10.1093/annonc/mdw684
10. Hess BT, Giri A, Park Y, et al. Outcomes of patients with limited-stage plasmablastic lymphoma: a multi-institutional retrospective study. *Am J Hematol*. 2023;98(2):300-308. doi:10.1002/ajh.26784

Carriers of m.3243A>G Who Receive Transplant Must be Closely Monitored for Possible Mitochondrial Toxicity of Required Immunosuppressants

Josef Finsterer

Dear Editor:

We were delighted to read the article by Vicinius de Souza and colleagues about a 31-year-old woman with end-stage renal failure due to focal segmental glomerulosclerosis attributable to the m.3243A>G variant in MT-TL1.[1] In addition to nephropathy, the patient phenotypically exhibited hearing impairment.[1] She was initially dependent on hemodialysis for 27 months, after which she received a kidney transplant.[1] The 5-year posttransplant outcome was satisfactory; however, shortly after transplant, she developed diabetes.[1] The study is noteworthy, but several points need to be discussed.

The first point is that the heteroplasmy rate was not reported.[1] Knowledge of the heteroplasmy rate is not only important for the assessment of disease prognosis but also for genetic counseling and family planning. As the patient underwent a kidney biopsy and explant of the diseased kidneys, determination of the heteroplasmy rates, not only in the blood lymphocytes but also in the explanted kidney, would have been useful. Knowledge of the mtDNA copy number and the haplotype, 2 other parameters that greatly determine the phenotype, would have also been interesting. In addition, biochemical studies of the kidney tissue would be useful for analyses of the function of the respiratory chain complexes.

The second point is that the index patient was not prospectively examined for multisystem diseases. This examination should have also been done during the 5-year follow-up period. Mitochondrial diseases (MIDs), including MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), are progressive multisystemic diseases that either occur at the onset of the disease or become multisystemic as the disease progresses. m.3243A>G can manifest not only with miscarriages, diabetes, hearing impairment, or renal insufficiency but also with short stature, epilepsy, stroke-like episode, myopathy, neuropathy, cognitive impairment, cardiomyopathy, endocrine disturbances, and lactic acidosis. Of particular interest is cerebral, endocrine, and cardiac involvement because such involvement strongly influences outcomes of patients with MID. Because multisystem involvement can be subclinical, a prospective search is important.

The third issue is that the type of "stroke" experienced by the index patient's sister was not stated.[1] We should know whether the sister had an ischemic stroke, an intracerebral hemorrhage, a subarachnoid hemorrhage, a venous sinus thrombosis, or a stroke-like episode, the pathognomonic feature of MELAS.[2] Knowing the subtype of stroke is important because the consequences differ considerably between subtypes and because the preventive measures for the different types of stroke are also different.

The fourth point is that it is not understandable why steroids were continued despite the development of diabetes. Although diabetes is a common phenotypic feature of carriers of m.3243A>G,[3] the time course suggests that the onset of steroid treatment also triggered diabetes. Were HbA1c levels ever measured before the kidney transplant? What were the current HbA1c levels, and did the patient develop diabetic nephropathy in the implanted kidney?

From the Neurology Department, Neurology and Neurophysiology Center, Vienna, Austria
Acknowledgements: *The author has not received any funding or grants in support of the presented research or for the preparation of this work and has no declarations of potential conflicts of interest.*
Corresponding author: *Josef Finsterer, Neurology Department, Neurology and Neurophysiology Center, Postfach 20, 1180 Vienna, Austria*
Phone: *+43 1 5861075* ***E-mail:*** *fifigs1@yahoo.de*

Experimental and Clinical Transplantation (2025) 8: 569-570

DOI: 10.6002/ect.2025.0009

In summary, the heteroplasmy rate in clinically affected tissues should be determined in carriers of m.3243A>G to assess prognosis and allow genetic counseling of other family members. Carriers of m.3243A>G who receive transplants should be carefully monitored for disease progression, multisystem involvement, and mitochondrial toxicity of immunosuppressants to prevent early host-transplant reactions.

References

1. Vinicius de Sousa M, Mariani G, Ribeiro Alves MA, Mazzali M. Segmental and focal glomerulosclerosis secondary to MELAS syndrome and long-term outcomes after kidney transplant: case report and literature review. *Exp Clin Transplant*. 2025;23(2):146-150. doi:10.6002/ect.2025.0006
2. Finsterer J. Characteristics of stroke-like lesions on cerebral imaging. *Ideggyogy Sz*. 2023;76(1-2):5-10. doi:10.18071/isz.76.0005
3. El-Hattab AW, Emrick LT, Hsu JW, et al. Glucose metabolism derangements in adults with the MELAS m.3243A>G mutation. *Mitochondrion*. 2014;18:63-69. doi:10.1016/j.mito.2014.07.008

Index to Volume 23 Issue 8

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

Urology Journal

Official Journal of
the Urology and Nephrology Research Center
& the Iranian Urological Association

ISSN 1735-1308
eISSN 1735-546X

Inedexing/Abstracts:
MEDLINE, CABI, DOAJ
WHO-IMEMR, CSA
Google Scholar

Publisher:
Urology and Nephrology
Research Center (UNRC)

Chairman:
Nasser Simforoosh, MD

Editor-in-Chief:
Abbas Basiri, MD

Editorial Office:
No 44, 9th Boustan
Pasdaran, Tehran
1666668111
Tel: +98 21 2259 4204
Fax: +98 21 2259 4204
E-mail: urol_j@unrc.ir
Web site: www.uj.unrc.ir

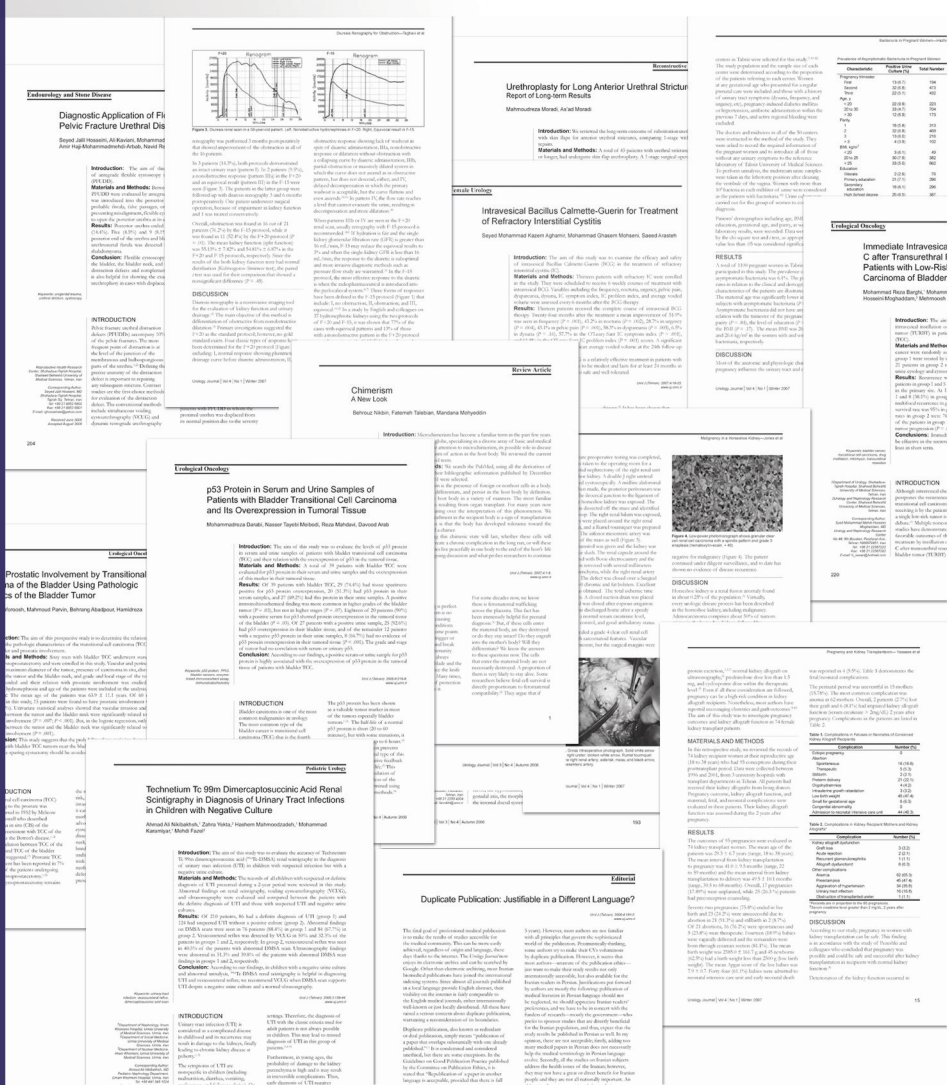
Online Submission:
www.manuscriptonline.com/uj

Visit the online version of
Urology Journal

www.uj.unrc.ir

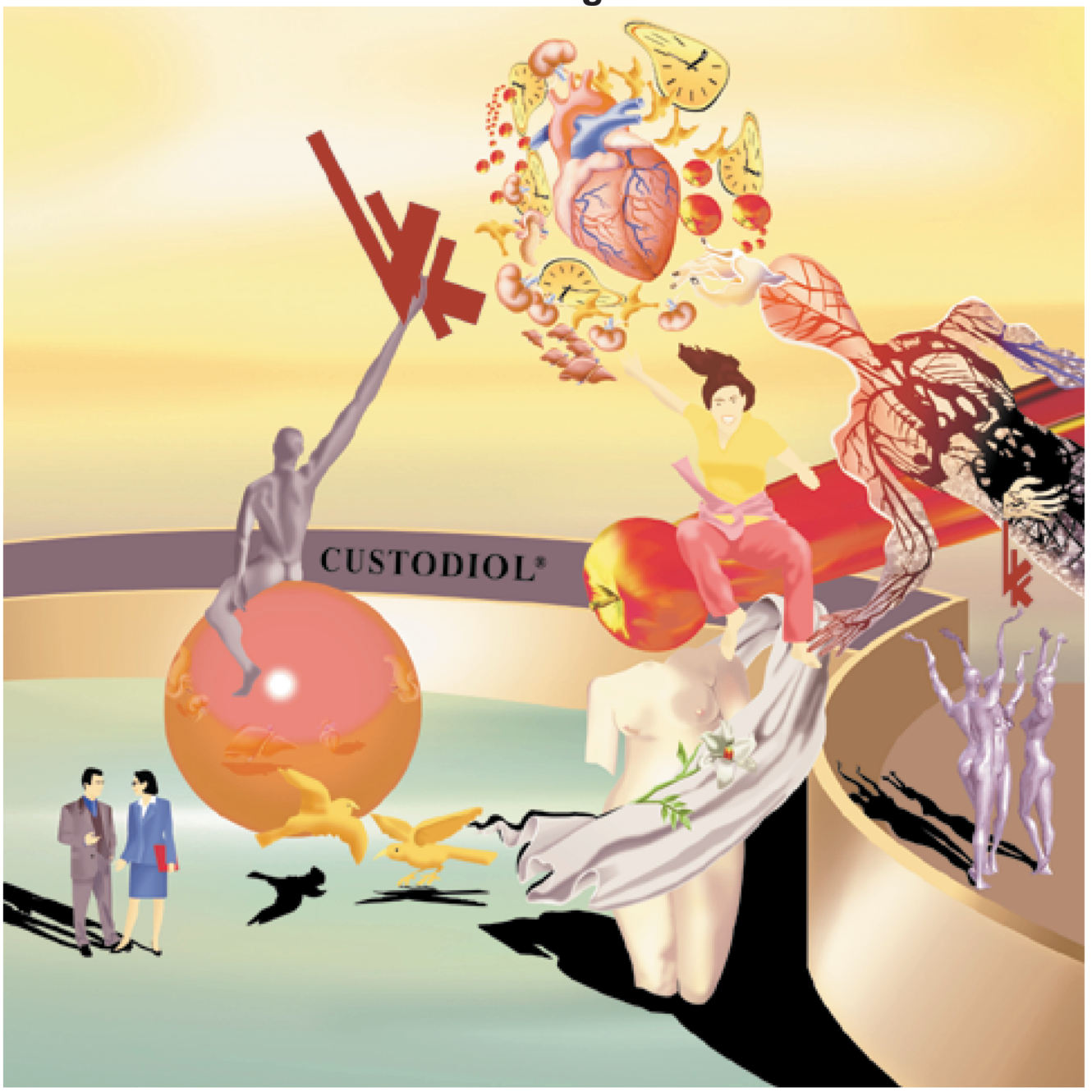
Features:

Open Access
Online Submission
&
Indexed in MEDLINE



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 blood-less 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

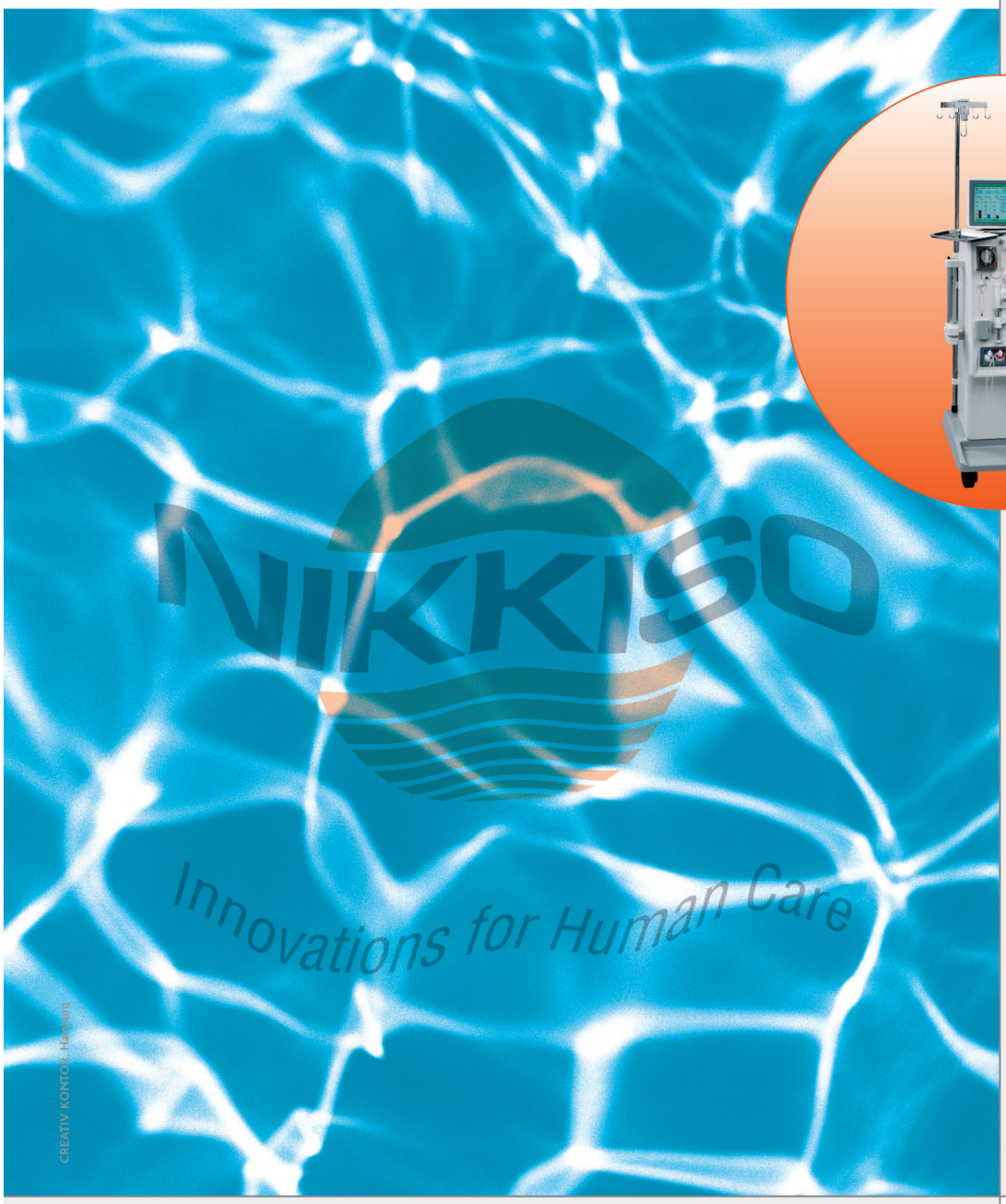
Local Distributor in Turkey: PAG LTD.
11. Sokak No: 40, 06490 Bahçelievler Ankara
Tel: +90 312 - 221 23 26 Fax: +90 312 - 212 49 67
E-mail: baskent@ada.net.tr internet: www.baskentholding.com.tr

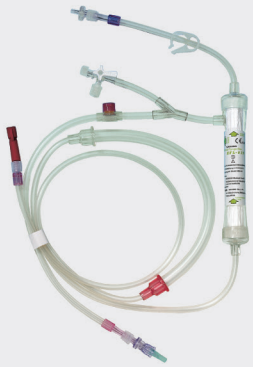
DR. F. KÖHLER CHEMIE

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

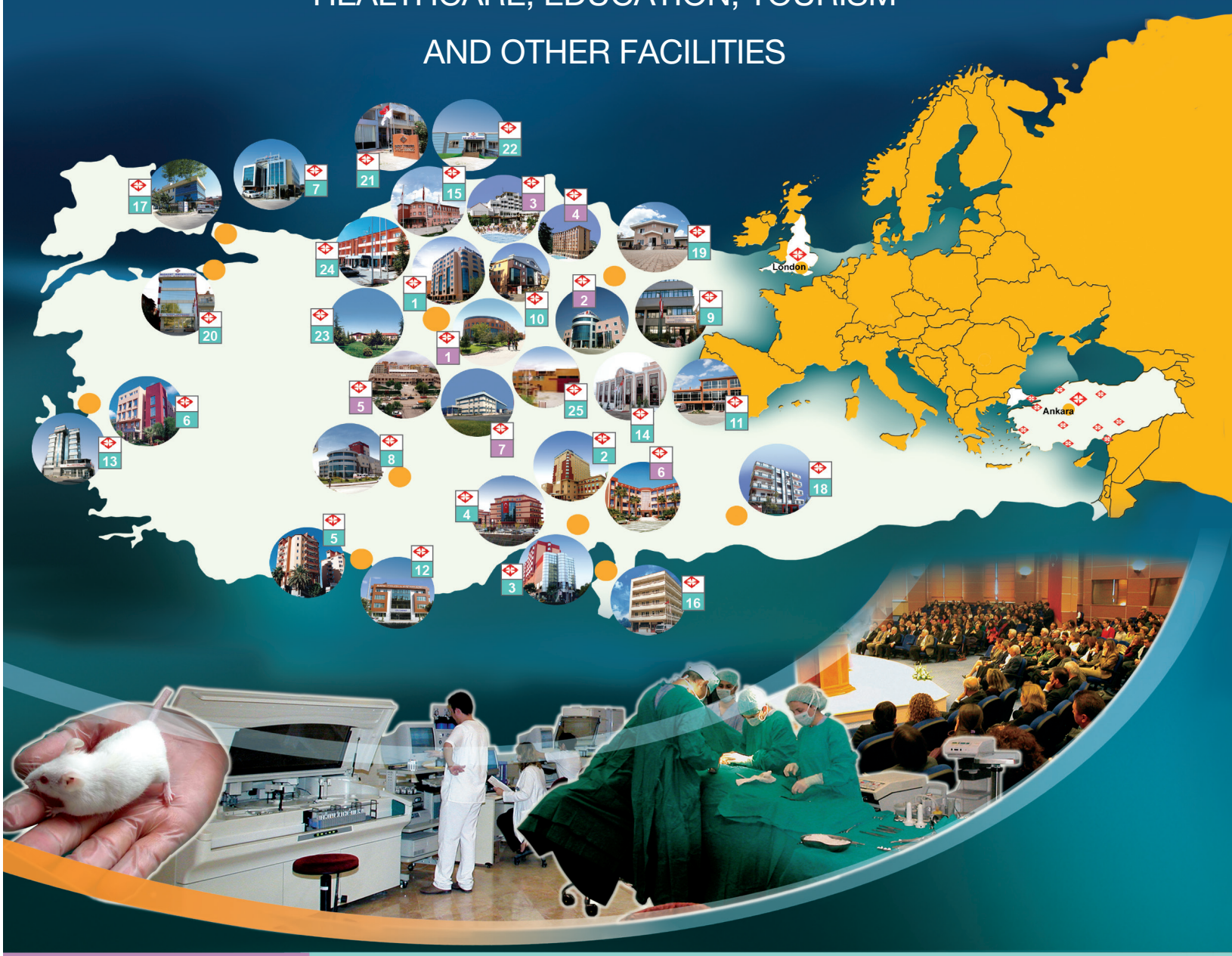
Master Program of Healthcare Management Without Thesis

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