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Experimental and Clinical Transplantation

OFFICIAL JOURNAL OF THE MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION

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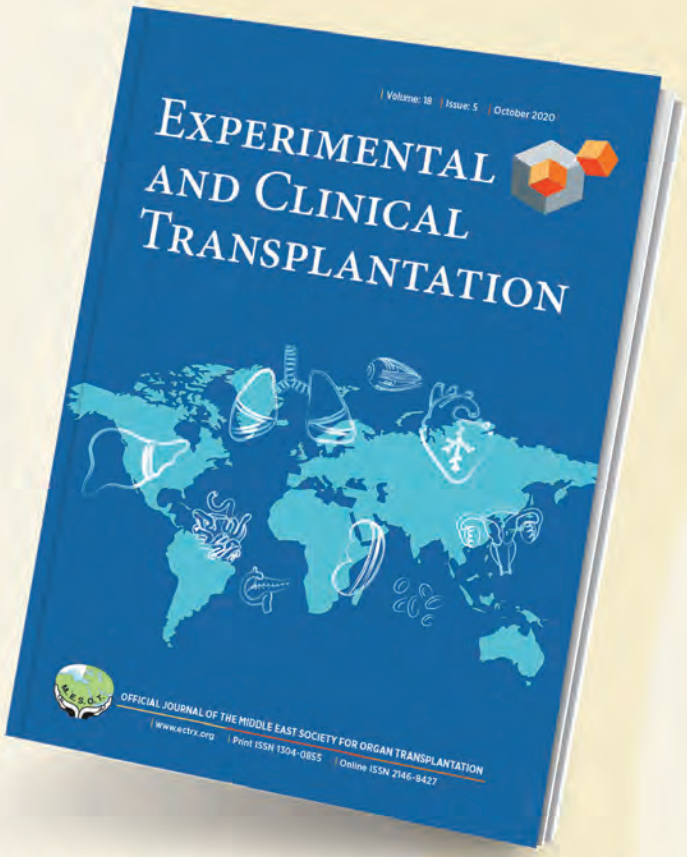

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EDITORIAL POLICY

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.

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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).

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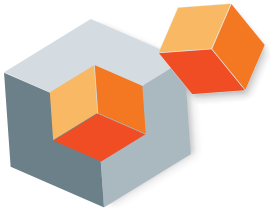

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Dear Colleagues,

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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**.

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

Sincerely,

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

Mustafa Al-Mousawi, MD, FRCS
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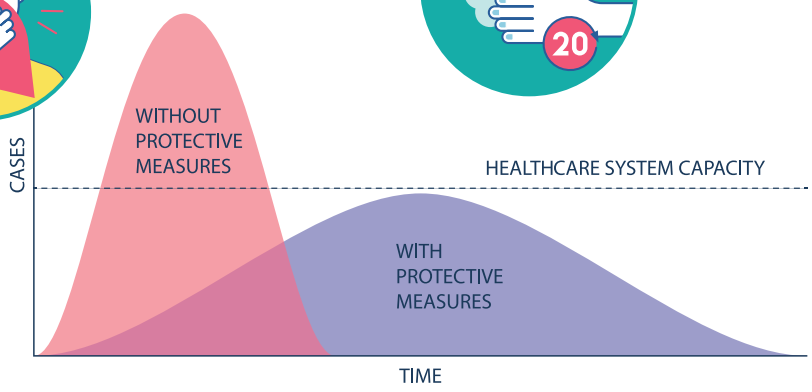

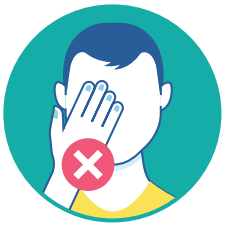

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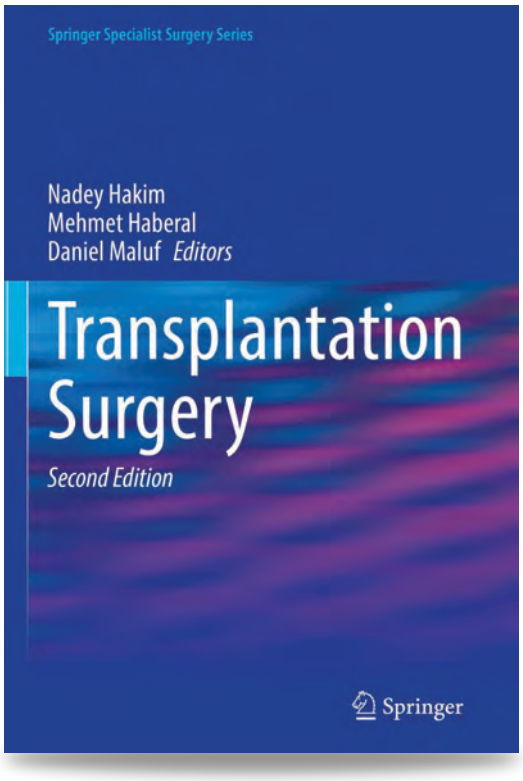

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- to promote ethical activities in transplantation
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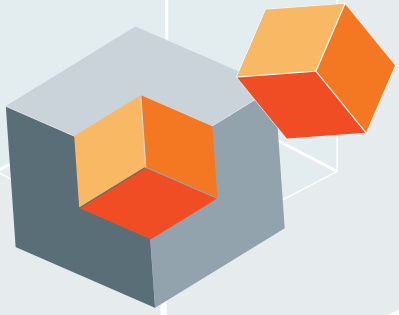

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Founder Professor Mehmet Haberal,
Founded in 1990.

Aims to promote and encourage research and education in the field of organ transplantation, to partake in national and international scientific activities and to ensure communication between organizations alike.

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- To collaborate with other organizations alike in Turkey and to organize meetings, symposiums and conferences
- To inform and educate the public at large on organ transplantation by means of publications and conferences
- To organize programs which will promote organ donation and its importance in saving lives
- To ensure the training of qualified personnel in the field of organ transplantation and encourage research by means of funds
- To collaborate with existing international organizations alike to promote and encourage scientific research
- To work on ethical and legal aspects of organ transplantation and related fields and to encourage social and medical collaboration of organizations alike.

Turkic World Transplantation Society (TDTD)

Founder Professor Mehmet Haberal,
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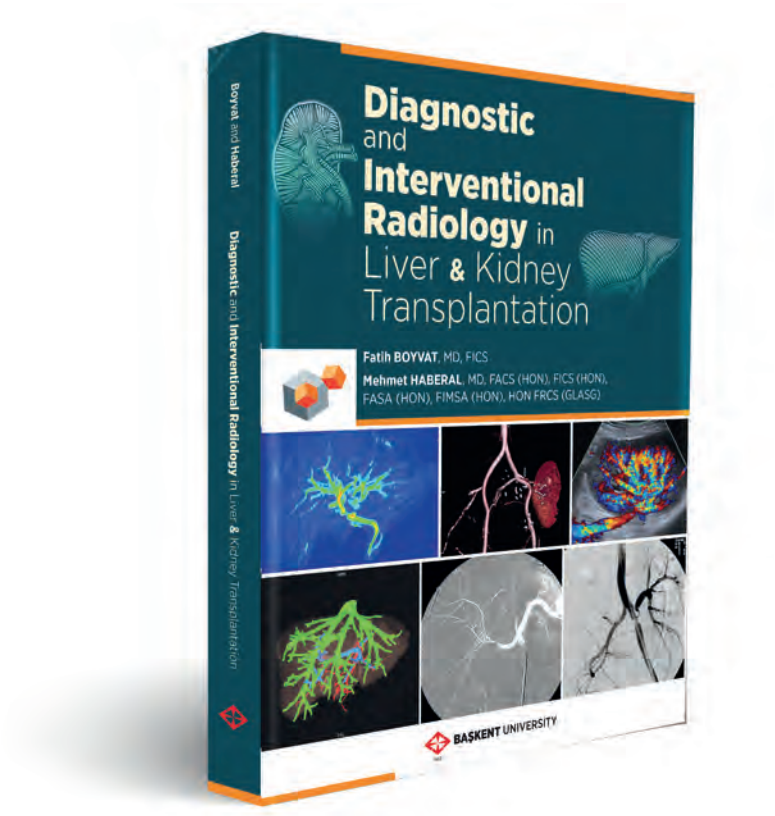
Aims to create an arena of communication and collaboration in the field of organ transplantation among the Turkic States of the world. Inclusive of Turkey, Azerbaijan, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, the society already has a total of 485 members from all member countries.

The primary goals of TDTD are:

- To promote and encourage education, research and cooperation in the field of organ transplantation for the purpose of advancing the art and science of transplantation, and to serve the patients of these states through the application of new knowledge and technical advances
- To create a scientific forum for the discussion of all problems related to the field of transplantation, including medical, social and legal aspects
- To collaborate with existing public and private organizations to promote and encourage research and clinical applications related to transplantation, and to participate and assist in the promotion of organ procurement and donation
- To encourage meetings, symposia and congresses to fulfill the above objectives



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.

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Drug-Induced Myelosuppression in Kidney Transplant Patients

Fedaey Abbas,[1,2] Mohsen El Kossi,[2,3] Ihab Sakr Shaheen,[2,4] Ajay Sharma,[2,5] Ahmed Halawa[2,6]

Abstract

Renal transplant is considered the best therapeutic option for suitable patients with end-stage kidney failure. Hematological complications that occur after kidney transplant include posttransplant anemia, leukopenia, neutropenia, and thrombocytopenia. Severely persistent leukopenia and neutropenia events predispose patients to infection, including opportunistic infections. The mainstay tactic for such complications is to reduce the burden of the immunosuppression by the offending agent, but this tactic is associated with increased risk of acute rejection. Given the absence of laboratory investigations to specifically identify the culprit, a complete withdrawal of these agents may be the ultimate diagnostic option. Future therapeutic strategies, however, should focus on reducing the immunosuppressive burden, the introduction of less myelotoxic agents, early recognition, and prompt treatment of infectious episodes. This will help in the optimization of the myelopoietic function and normalization of the hematological profile, resulting in better allograft and patient survival.

Key words: *Drug-induced cytopenia, Posttransplant leukopenia, Posttransplant neutropenia, Posttransplant thrombocytopenia, Renal transplantation*

From the [1]Jaber El Ahmed Military Hospital, Nephrology Department, Safat, Kuwait; the [2]Faculty of Health and Science, University of Liverpool, Institute of Learning and Teaching, School of Medicine, Liverpool, United Kingdom; the [3]Doncaster Royal Infirmary, Doncaster, United Kingdom; the [4]Royal Hospital for Children, Glasgow, Scotland, United Kingdom; the [5]Royal Liverpool University Hospitals, Liverpool, United Kingdom; and the [6]Sheffield Teaching Hospitals, Sheffield, United Kingdom

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Authors contributions: *F. Abbas designed the study, performed data collection, and wrote the manuscript; M. El Kossi, I. Shaheen, and A. Sharma reviewed and edited the manuscript; and A. Halawa conceptualized and designed the study, supervised data collection, and reviewed and edited the manuscript.*

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Introduction

Immunosuppressive drugs are crucial to allograft survival in transplant recipients. However, a number of these drugs are associated with hematological complications. Myelosuppression presenting as cytopenia is not uncommon in kidney transplant recipients (KTRs).[1] From 20% to 60% of KTRs experience at least 1 episode of cytopenia after transplant.[2] Most episodes of cytopenia are observed during the first 3 months.[2] The list of culprit agents includes mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium,[3-5] ganciclovir and valganciclovir,[3,6,7] antithymocyte globulin (ATG),[8] tacrolimus,[9] sirolimus, and trimethoprim-sulfamethoxazole.[10] Cautious reduction or complete withdrawal of the offending agent may be urgently warranted; however, as described here, potential risks should be expected and managed accordingly.

The higher risk of acute rejection after reduction of myelosuppressive immunosuppressive agents requires careful consideration. There is an increased risk of infection, including opportunistic infections, for example, cytomegalovirus (CMV), after cessation of valganciclovir or risk of *Pneumocystis jirovecii* after trimethoprim-sulfamethoxazole withdrawal.[11]

Cytopenia can be identified as follows: pancytopenia involves all 3 cells lines, that is, white blood cells (WBCs), red blood cells (RBCs), and platelets; bicytopenia involves 2 of 3 cell lines; thrombocytopenia involves low platelet count; and leukopenia can be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) into 4 levels.[12] These levels are 3000 cells/mm^3 (normal), 2000 to 3000 WBCs/mm^3, 1000 to 2000 WBCs/mm^3, and <1000 WBC/mm^3 (the latter 3 levels indicating abnormal with variable severities). Leukopenia is also termed alternatively with neutropenia, although these terms are not synonymous. Many laboratories indicate 4000 cells/mm^3 as the lower limit of normal. Other

laboratories have used neutropenia to classify severity of granulocytopenia.

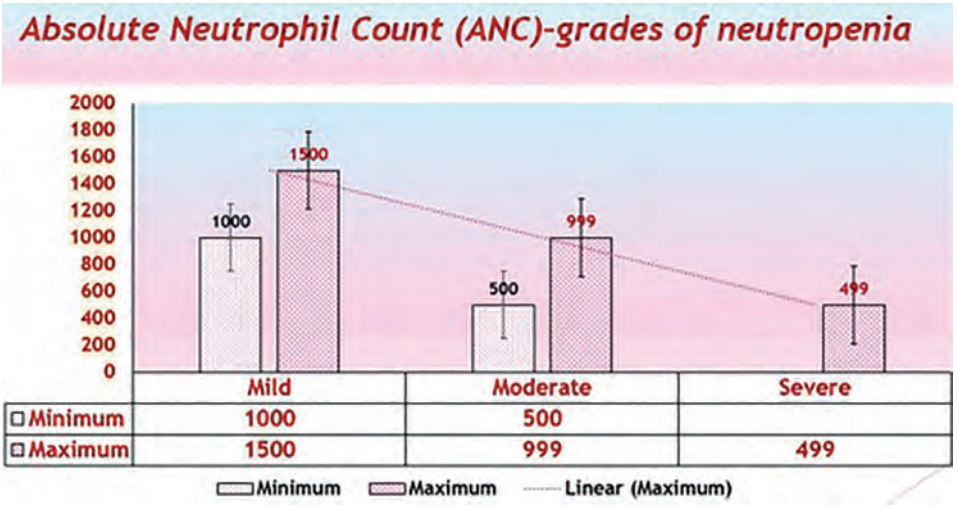
Absolute neutrophil count (ANC) is used to assess the magnitude of neutropenic severity as follows[13]: ANC = (WBCs/μL) × (percentage of polymorphonuclear cells + bands)/100. An ANC of <1500/μL or <1.5 × 10^9/L can be termed neutropenia and graded as mild, moderate, or severe. Mild neutropenia is ANC level of 1000 to 1500/μL or 1 to 1.5 × 10^9/L, moderate neutropenia is ANC level of 500 to 999/μL or 0.5 to 0.99 × 10^9/L, and severe neutropenia (agranulocytosis) is ANC level of <500/μL or <0.5 × 10^9/L (Figure 1).[11]

Platelet count of 150 000/mm^3 is considered the lower limit of normal level in many laboratories.[11] The CTCAE (Figure 2) has also graded thrombocytopenia into 4 levels.[12] These levels are grade I or subnormal (75 000-150,000 cells//mm^3), grade II or low (50000-75000 cells/mm^3, grade III or moderate (25 000-50 000 cells/mm^3), and grade IV or critical (<25 000/mm3) (Figure 2).

Consequences of Hematological Cytopenia

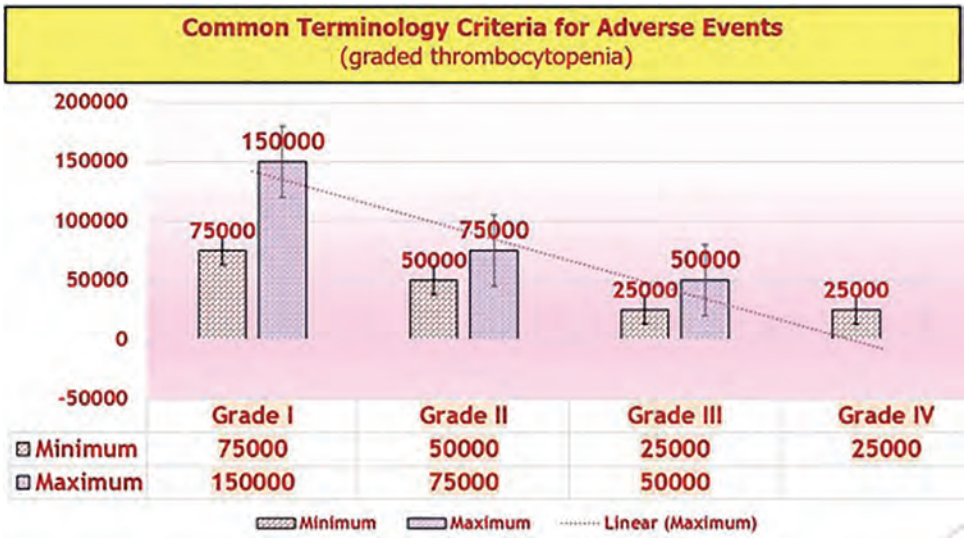
Neutrophils and lymphocytes have fundamental roles in prevention of infection. However, as detailed here, cytopenia can cause additional drawbacks.

Figure 1. Absolute Neutrophil Count Grades of Neutropenia



Severe neutropenia <500/μL.[11]

Figure 2. Common Terminology Criteria for Adverse Events, Graded Thrombocytopenia



Grade IV = <25 000.[11]

Leukopenic KTRs are vulnerable to the development of opportunistic infections. When ANC is <1000 cells/μL, susceptibility to infection increases. The predisposition to frequency and severity of infection is related to duration of neutropenia and magnitude of neutropenic decline. Infection with *Escherichia coli* is also more prevalent in neutropenic KTRs.[14,15]

Neutropenic KTRs commonly experience more intra-abdominal infections (22.5%) than those with normal neutrophil counts (7%-10%). Both tacrolimus and MMF therapy are commonly associated with neutropenia.[14] Withdrawal of prophylactic agents for CMV or *Pneumocystis jirovecii* opens the door to their spread. To summarize, leukopenia augments the risk of infection by disrupting immunogenic integrity and liability of ubiquitous and opportunistic infections.

In an attempt to reduce the severity of neutropenia, transplant physicians often reduce or withhold MMF. Despite the expected rise in WBC counts, risk of rejection increases, which is usually evident in the first year posttransplant. For example, Zafrani and associates[14] observed that increased mycophenolic acid (MPA)-free periods were considered a robust predictor of acute rejection. Vanhove and associates[16] reported a significantly high risk of acute allograft rejection with more than 50% reduction in MMF dosage. Therefore, physicians should be cautious in the treatment of patients with high immunological risk, especially those within 3 months after transplant.

Given the absence of robust evidence-based strategies to manage drug-induced cytopenia after kidney transplant, transplant clinicians should carefully analyze patient drug history and clinical experience (by trial and error) to find the offending medication. In addition to hematological cytopenia (eg, due to MMF, rituximab, and ATG), a number of clinical situations may also result in cytopenia.[11]

Drug-Induced Leukopenia and Neutropenia

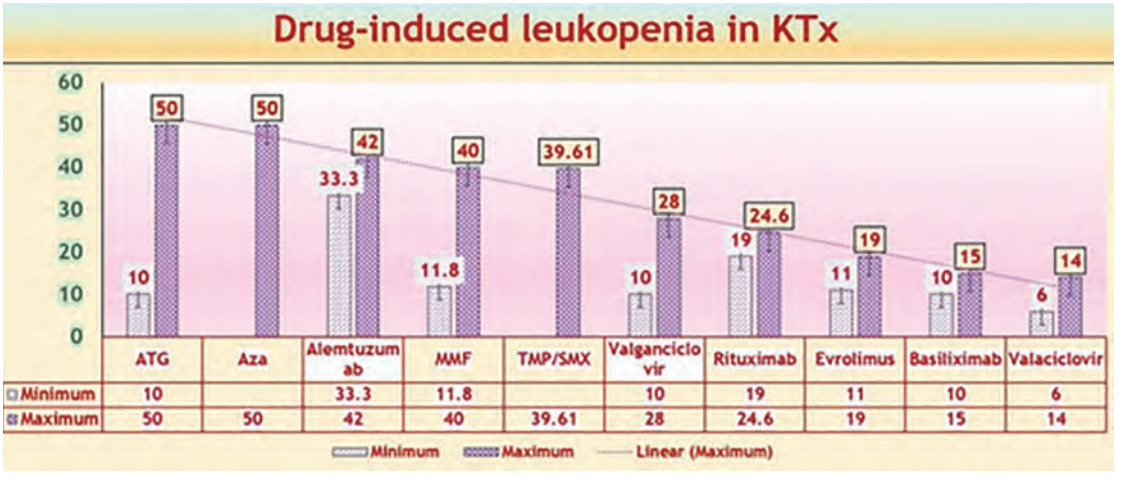
As detailed here, a number of agents have been implicated in posttransplant leukopenia and neutropenia development (Figure 3 and Figure 4).

Rituximab

Rituximab is a potent chimeric anti-CD20 monoclonal antibody that binds CD20 antigen, resulting in B-cell depletion and thus affecting phagocytosis by macrophages, complement-mediated cytotoxicity, and antibody-dependent cell-mediated toxicity by natural killer cells.[17] Rituximab is commonly used as a part of the induction agent in ABO-incompatible transplant procedures,[18,19] in the treatment of acute rejection episodes,[20] in attempted treatment of chronic antibody-mediated rejection,[21,22] and in resolution therapy of posttransplant lymphoproliferative disorder.[23]

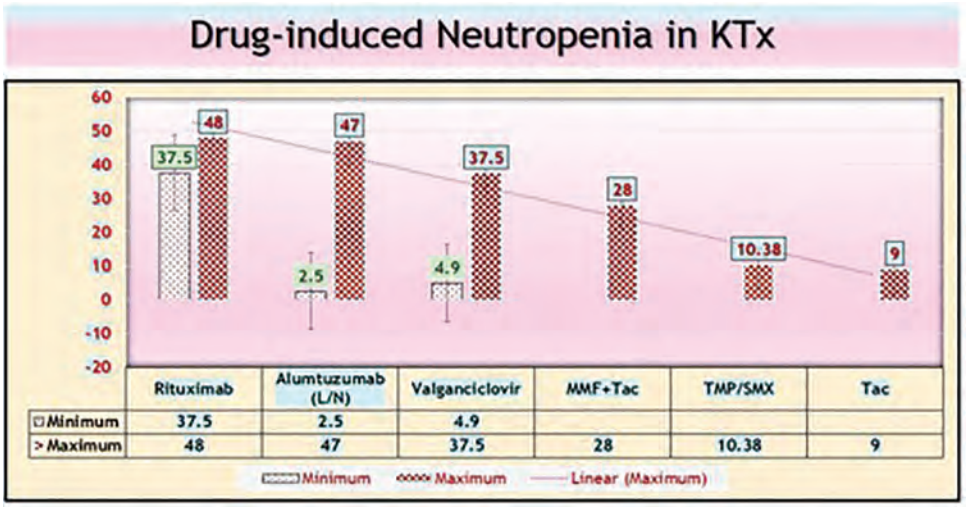
Rituximab-induced cytopenia (grade 3/4) has been reported in 48% of patients in 1 trial as follows: 40% lymphopenia, 6% neutropenia, and 4% leukopenia in patients with lymphoma.[24] Cytopenia can occur 4 weeks after start of rituximab therapy (late-onset neutropenia). Late-onset neutropenia can

Figure 3. Incidence of Drug-Induced Leukopenia in Kidney Transplant Recipients



Abbreviations: ATG, antithymocyte globulin; AZA, azathioprine; MMF, mycophenolate mofetil; TMP/SMX, trimethoprim-sulfamethoxazole

Figure 4. Incidence of Drug-Induced Neutropenia in Kidney Transplant Recipients



Abbreviations: L/N, leukopenia/neutropenia; MMF, mycophenolate mofetil; Tac, tacrolimus; TMP/SMX, trimethoprim-sulfamethoxazole

be defined as neutropenia that is observed 4 weeks after the last dose of rituximab after exclusion of other causes (ie, use of ganciclovir, valganciclovir, or MMF). The reported incidence of late-onset neutropenia in KTRs has approached 37.5% to 48%,[25,26] with a time gap of 38 to 175 days and duration of 5 to 77 days. Late-onset neutropenia is usually observed after the sixth rituximab dose. Mycophenolate mofetil, ganciclovir, and valganciclovir are frequently implicated in its evolution.[26-29] The reported incidence of leukopenia after rituximab therapy ranges from 19% to 24.6% with an average relative risk of leukopenia of about 8.8 if rituximab is administrated as induction therapy in an ABO-compatible nonsensitized KTR.[30]

With regard to rituximab management, the threshold of suspicion of rituximab-induced toxicity should be lowered, particularly 6 weeks after the sixth rituximab dose. Dose reduction or drug withdrawal is usually the ideal response for hematological recovery.[31]

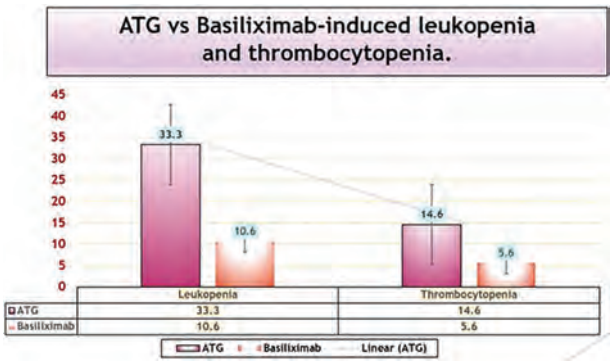
Antithymocyte globulin

Thymoglobulin activity is not confined to T cells; rather, a wide range of blood cells are vulnerable to the antibody effects of this agent, including T cells, B cells, natural killer cells, monocytes, neutrophils, platelets, and RBCs.[32,33] Moreover, cross-reaction of antibodies toward nonlymphoid tissue may result in the development of neutropenia.[34] Both higher doses of ATG and the nonspecific avidity to neutrophils and platelets may induce neutropenia.[35]

Leukopenia incidence with ATG is variable, with studies showing 10%,[36] 38%,[37] 33.5%,[38] and 50%,[39] as a result of differences in protocols and variabilities in periods of administration. The highest incidence (50%), however, as reported by Gaber and colleagues, may be explained by the concurrent use of azathioprine.[39] However, Brennan and associates reported etiologies leading to ATG cessation/dose reduction as leukopenia in 45.2% and as combined leukopenia and thrombocytopenia in 14.3% of studied KTRs[38] (Figure 5).

The dose of ATG should be halved when platelet count reaches 50 000 to 75 000 per mm^3 or WBC count reaches 2000 to 3000 per mm^3.[36,39] Treatment with ATG should be held when platelet count declines to less than 50 000 per mm^3 or when WBC count is less than 2000 per mm^3. $CD3^+$ T-cell count should be monitored when less than 0.05×10^9/L (<50/μL;

Figure 5. Incidence of Antithymocyte Globulin- Versus Basiliximab-Induced Leukopenia and Thrombocytopenia[11]



Abbreviations: ATG, antithymocyte globulin

normal range, 128-131/μL) to avoid unnecessary higher doses. This approach is successful in reducing the incidence of acute rejection episodes, infections, and cytopenia.[40] Total lymphocyte count should be maintained as less than 0.3×10^9/L, which is a suitable alternative if CD3 monitoring is not available.

Other medications that may cause cytopenia should also be monitored, including MMF (hold off MMF with concurrent ATG-induced cytopenia[37]) and steroids (loss of stimulatory effects on bone marrow in early steroid withdrawal regimens after ATG induction can be complicated with a higher incidence of leukopenia[41]).

Alemtuzumab

Alemtuzumab is an anti-CD52 humanized monoclonal immunoglobulin G1 antibody. The former is a glycoprotein expressed on mononuclear cells (eg, T and B lymphocytes), monocytes, and natural killer cells. Alemtuzumab can be administrated as an induction agent[42,43] or as antirejection medication.[44,45]

With alemtuzumab, the leukopenic incidence in KTRs ranges from 33.3% to 42% in various reports.[34,46] The incidence is higher (47%) if neutropenia is also present.[47] Compared with ATG, the myelotoxic effects of alemtuzumab are more severe,[34,46] with the lowest WBC counts observed 130 days after the last given dose.[47] However, infectious episodes are usually not life-threatening.[46,47] A dose reduction of MMF, in response to alemtuzumab-induced leukopenia, may reach 14 mg/kg, a dose that is much less than that required for ATG-induced leukopenia. Subsequently, a strict monitoring of allograft function at that time is mandated, particularly in high-risk KTRs.[48,49] Through B-cell dysregulation, alemtuzumab has been blamed in the evolution of many autoimmune disorders. With regard to management, dose modification of other drugs such as MMF or valganciclovir or cotrimoxazole is required.

Interleukin receptor antagonists

Two interleukin 2 receptor (IL-2R) antagonist induction agents are basiliximab, a monoclonal chimeric, and daclizumab, a humanized murine antibody against CD25 that can suppress IL-2-mediated T-cell activation and proliferation in KTRs.[50] The latter agent has been withdrawn from the market. Because anti-IL-2R activity is confined to activated T cells, their leukopenic and thrombocytopenic drawbacks are currently rare compared with drawbacks with ATG and alemtuzumab (10% to 15% vs 5% for basiliximab).[51] Moreover, leukopenia has been reported to be 3.6 times higher in KTRs with alemtuzumab induction compared with basiliximab.[52] Brennan and colleagues reported leukopenia in 33.3% of their patients who received ATG induction. In comparison, the incidence of leukopenia was 10.6% for KTRs who received basiliximab.[38] Another study reported a significantly higher incidence of leukopenia in KTRs who received thymoglobulin compared with those who received basiliximab (22.8% vs 11.8%; $P < .05$).[53] Considering all these observations, the anti-IL-2R agents would be an optimum therapeutic option for leukopenic KTRs with low/moderate risk of rejection.

Mycophenolic mofetil and enteric-coated mycophenolate sodium

These agents are inosine monophosphate dehydrogenase inhibitors that can inhibit both cell-mediated and humoral immune responses through suppression of guanosine nucleotide synthesis de novo pathways in T/B lymphocytes, arresting their differentiation.[11]

With regard to MMF hematotoxicity, 11.8% to 40% of KTRs can develop leukopenia related to MMF therapy.[54,55] Both ATG-related and alemtuzumab-related cytopenia may mask the diagnosis of MMF myelotoxicity, as they may require MMF dose reduction.[56] Single-nucleotide polymorphism has its role in the development of MMF-related cytopenia.[57] The hematological sequelae with myelosuppression are the most common cause requiring MMF dose reduction, with 46.5% of MMF-reducing events due to leukopenia, anemia, thrombocytopenia, and pancytopenia.[16]

The myelotoxic impact of MMF is dose dependent and is usually related to the trough levels of MPA.[2,58] Concomitant administration of valganciclovir,[3] valacyclovir, and fenofibrate[59] may exaggerate MMF-related leukopenia.

To manage cytopenia, a dose reduction or complete withdrawal seems to be a reasonable response to MMF-induced neutropenia and leukopenia.[58,60] However, this response would trigger the risk of acute rejection, with subsequent high risk of graft loss in many retrospective reports. Nevertheless, this risk has still not been substantiated for the following reasons: retrospective nature of

studies and intensity of the immunological risk and its role in predisposition of rejection. Dose reduction of MMF has been mostly attempted in the first year posttransplant, a time of highest risk of allograft rejection.

Several approaches could be added for the management of this type of leukopenia, including preemptive dose reduction of MMF after ATG and alemtuzumab induction, shifting to a suitable mammalian target of rapamycin inhibitor (eg, sirolimus or everolimus may reverse cytopenia[5,61]), and halving the CMV prophylactic dose of valganciclovir, which could also be another preventive measure. Efficacy of valganciclovir 450 mg daily has been proven to be equal to a dose of 900 mg daily for CMV prophylaxis.[62] For resistant cases, cessation of both MMF and valganciclovir may be the last resort for cytopenia reversal.[46]

Tacrolimus

Tacrolimus is the mainstay of clinical immunosuppression regimens.[63] Although much less common in renal transplant recipients, 16.92% of hematological alterations in cardiothoracic transplant recipients were related to tacrolimus therapy, including anemia, neutropenia, and combined anemia/neutropenia.[11] Tacrolimus may also intensify MMF myelotoxicity, and tacrolimus and MMF combinations have been shown to induce neutropenia in 28% of KTRs.[14]

Mechanisms of tacrolimus-induced neutropenia include direct suppression of myeloid cells, with bone marrow hypoplasia observed in hepatic transplant recipients,[64] altered cytokine production by T lymphocytes and monocytes, and production of antimyeloid precursors and anti-mature neutrophil antibodies. Tacrolimus has been shown to prevent MPA glucuronidation that results in intensification of blood levels.[65] In contrast to cyclosporine, tacrolimus does not interfere with MMF enterohepatic circulation, leading to augmented MPA levels.[66] A combination of tacrolimus and MMF expands the area under the curve for MMF within 3 months by approximately 20% to 30%.

However, tacrolimus-induced direct myeloid inhibition has not been observed in vitro.[67] A stunted myeloid maturation has not been proven in vivo.[9] Direct inhibition of myeloid precursors may not be a convincing mechanism for tacrolimus-induced neutropenia and leukopenia.

Tacrolimus-induced neutropenia can be observed within the first 3 months after transplant.[9] There is no particular test for diagnosis, except for leukocytic count normalization after the withdrawal of tacrolimus.[9] A dose reduction of MMF is suggested in patients on dual immunosuppression therapy.[68,69] In such patients, other alternatives include everolimus, belatacept, or eculizumab.[65,66]

Azathioprine

Azathioprine is a traditional antimetabolite that was introduced in 1960 and has been greatly replaced by the more potent MMF in immunosuppression after kidney transplant. Azathioprine may induce leukopenia and neutropenia in almost half of KTRs, particularly with doses greater than 1.99 mg/kg body weight/day. Most cases of azathioprine-induced leukopenia present in the first month after transplant. In this situation, a dose reduction or transient drug withdrawal is usually sufficient. Of note, a past history of drug-induced leukopenic events would increase the risk of leukopenia by 70%.[11]

An important factor to determine the magnitude of azathioprine-induced myelotoxicity is thiopurine S-methyl transferase (TPMT) activity. Moderately active TPMT may lead to higher risk of myelotoxicity with conventional doses of azathioprine. Patients with complete lack or low TPMT activity are vulnerable to developing severe, life-threatening myelotoxicity.

To ameliorate the risk of myelosuppression, 2 techniques have been proposed. The first is monitoring of 6-thioguanine nucleotide in RBCs, which is an efficacious and more beneficial method than monitoring of 6-mercaptopurine in plasma.[70,71] Genotyping and phenotyping of TPMT may also help to recognize KTRs at higher risk for myelotoxicity.[72-74]

Drugs that interact with azathioprine include allopurinol, which inhibits xanthine oxidase activity leading to decline in purine metabolism to uric acid. Therefore, concomitant administration with allopurinol necessitates dose reduction of azathioprine by 25% to 50%; otherwise, catastrophic myelosuppression will ensue. Of note, the need for azathioprine instead of MMF is frequently utilized in areas with low economic standards.[75]

Azathioprine levels should be monitored weekly, with full blood count monitoring in the first month,

then twice per month during the second and third months, and then monthly or less according to dose adjustment.[76]

Mammalian target of rapamycin inhibitors

The most common mammalian target of rapamycin inhibitors (mTORi), sirolimus and everolimus, have been involved in many myelotoxic side effects.[77,78] Leukopenia has been reported in a meta-analysis of 8 trials that involved conversion from calcineurin inhibitor (CNI) to mTORi.[79] Severity of myelotoxicity is dose dependent,[80] with involvement of about 20% of KTRs on sirolimus. A trough level of >12 ng/dL has been shown to be highly associated with development of leukopenia and thrombocytopenia,[81] although it can commonly occur even with lower drug levels.

Sirolimus and MMF combination therapy after alemtuzumab induction in steroid and CNI-free regimens may result in severe leukopenia.[82] Everolimus therapy, on the other hand, can be also complicated by leukopenia (11%-19%).[83] The development of cytopenia with mTORi agents can be usually observed within the first 4 to 8 weeks. For patients with sirolimus-induced cytopenia, 7% need dose reduction, 4% need drug withdrawal, and 89% resolve spontaneously.[11]

Mechanisms postulated for mTORi-induced myelotoxicity include disrupted signal transduction by mTORi through the gp130 β chain and platelet aggregation and degranulation triggered by sirolimus responding to adenosine monophosphate and thrombin effects in vitro. Variable cytokines (interleukin [11], granulocyte colony-stimulating factor [G-CSF], and erythropoietin) can stimulate production of RBCs, leukocytes, and platelets via signal transduction of the gp130 β chain. Therefore, mTORi may induce cytopenia via inhibition of signal transduction through gp130 β-chain inhibition.

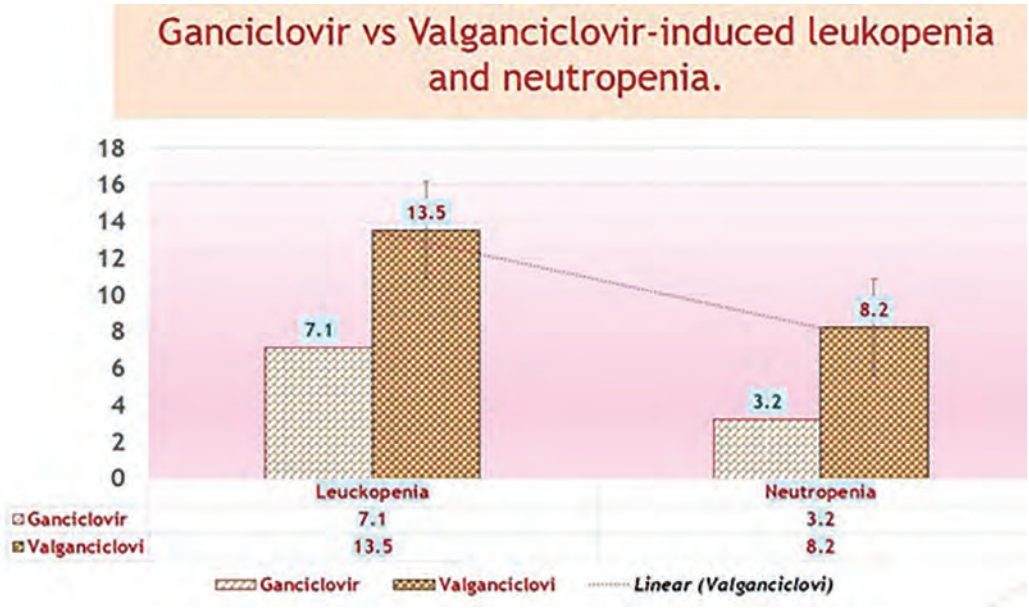
In most patients, mTORi-induced leukopenia resolves spontaneously. If it persists, then a reduction of the MMF dose with simultaneous reduction of mTORi to a lower therapeutic range is required.[84] However, drug cessation may be the last resort for resistant cases.[85]

Valganciclovir

The higher bioavailability of this agent (70% vs 7% for oral ganciclovir) has also affected its myelotoxicity profile[42,43] (Figure 6).

Although 10% to 28% of KTRs are vulnerable for leukopenia development,[3,38,41,42] 4.9% to 37.5% of patients may develop neutropenia.[3,38-40] The resultant cytopenia may be potentiated by several factors, including higher doses of the drug (900 mg or more) having a significant impact on leukopenia and neutropenia development,[43] low body mass index, which is a significant potentiating factor for leukopenia,[40] and concomitant MMF administration, which can also aggravate valganciclovir myelotoxicity.[3,86]

Figure 6. Incidence of Ganciclovir-Versus Valganciclovir-Induced Leukopenia and Neutropenia[11]



Although leukopenia can develop within 3 months,[3] resolution of leukopenia can occur spontaneously with or without treatment. Risk of infection is usually low.[3,42] The need for G-CSF administration may be required with prolonged periods of prophylaxis.[41]

Dose reduction to 450 mg/day or transient drug cessation may be sufficient for cytopenia reversal. This dose level, however, has been shown to be equally effective as 900 mg/day for CMV prophylaxis; consequently, the lower dose has been recommended.[62]

Ganciclovir

Ganciclovir is used for anti-CMV therapy and prophylaxis in KTRs. The bioavailability of this drug is rather poor when given orally; therefore, it is always given intravenously. Through its myelosuppressive effects, ganciclovir causes leukopenia, with rates of 7.1% to 23.1%.[42] Compared with valganciclovir, ganciclovir exerts modest myelosuppression. Considering the higher bioavailability of valganciclovir (10 times versus that with ganciclovir), the risk of neutropenia in the former agent exceeds 188%.[43] A lesser incidence of leukopenia (7.1% vs 13.5%) and neutropenia (3.2% vs 8.2%) was observed in ganciclovir-treated patients compared with those on valganciclovir therapy, as reported by Tan and associates.[42] Although patients (23%) on ganciclovir therapy respond to dose reduction, some (2.4%) require ganciclovir cessation.[42]

Valacyclovir

Valacyclovir is a remarkable agent for CMV prophylaxis and treatment of herpes simplex in KTRs. Compared with valganciclovir and ganciclovir, myelotoxicity with valacyclovir is relatively mild. Incidence of leukopenia ranges from 6% to 14% in randomized clinical trials. The risk of neutropenia with valganciclovir therapy is currently 730% higher than with valacyclovir.[43] However, combined MMF and valacyclovir therapy may aggravate drug-induced myelotoxicity.[87] Moreover, MMF may aggravate bone marrow toxicity by increasing the intracellular concentration of valacyclovir.[88] When compared with ganciclovir, dose modification is less frequently employed with valacyclovir. In addition, withdrawal of ganciclovir is more frequent (23.1% vs 8.3%) compared with valacyclovir.[87] However, the pill burden of valacyclovir is higher and the neurological complications are more frequent.[11]

Trimethoprim-sulfamethoxazole

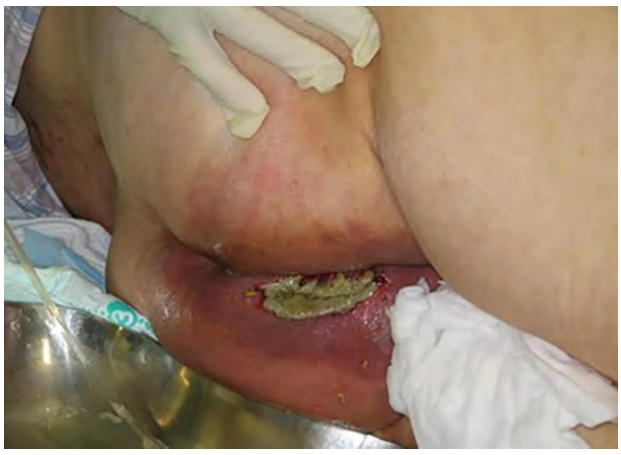
Trimethoprim-sulfamethoxazole is a commonly used drug for *Pneumocystis jirovecii* prophylaxis. Several types of cytopenia are associated with use of trimethoprim-sulfamethoxazole; these include neutropenia and leukopenia and megaloblastic anemia. Trimethoprim per se can cause dose-dependent inhibition of granulopoiesis in vitro. Folinic acid can reverse this side effect. Similarly, folate-depleted granulocyte precursors have been observed in another in vitro report.[11]

The use of trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis in KTRs may induce leukopenia in only 2% of recipients. However, combined azathioprine therapy with trimethoprim-sulfamethoxazole can aggravate drug-induced myelosuppression.[88]

Dapsone

Dapsone is an alternate agent for *Pneumocystis jirovecii* prevention that is associated with many hematological complications, including neutropenia.[69] Moreover, the neutropenic effects of dapsone may be aggravated by development of agranulocytosis (Figure 7).[44]

Figure 7. Dapsone-Induced Agranulocytosis Leading to Perianal Abscess and Death[70]



Drug-Induced Lymphopenia

In leukopenic KTRs, it is essential to recognize lymphopenia, which is different from leukopenia due to neutropenia. The latter is usually complicated by augmented risk of serious infection; on the other

hand, lymphopenia is usually the result of induction therapy with lymphocyte-depleting medication (eg, rabbit ATG).[89]

Posttransplant Drug-Induced Thrombocytopenia

A number of medications have been implicated in the evolution of posttransplant thrombocytopenia (Figure 8).

Rituximab

Rituximab-induced thrombocytopenia (grade 3/4) has been reported in 48% of patients in one trial, with rate of thrombocytopenia in 2% of lymphoma patients. Because rituximab-related thrombocytopenia rarely induces bleeding, platelet infusion is rarely indicated.[31]

Antithymocyte globulin

Cross-reaction of antibodies toward nonlymphoid tissue may result in the development of thrombotic events and thrombocytopenia.[34] Both higher doses of ATG and its nonspecific affinity to platelet cells may induce thrombocytopenia.[35] An incidence of thrombocytopenia ranging from 10% to 26.5% has been reported in KTRs.[11] Brennan and associates reported cessation or reduction in doses of ATG due to thrombocytopenia in 11.9% of KTRs.[38]

For treatment, medications that affect cytopenia prevalence should be monitored (eg, holding off or reducing MMF with concurrent ATG-induced cytopenia).[37] Thrombocytopenia can also be exacerbated with ATG and mTORi combination.[11]

Alemtuzumab

Autoimmune thrombocytopenia has been observed in multiple sclerosis and chronic lymphocytic leukemia with an incidence ranging from 1% to 2.5%.[90,91] Alemtuzumab-induced thrombocytopenia has been observed in 14% of KTRs. Bleeding requiring surgical intervention has been observed in 12% of KTRs.[90]

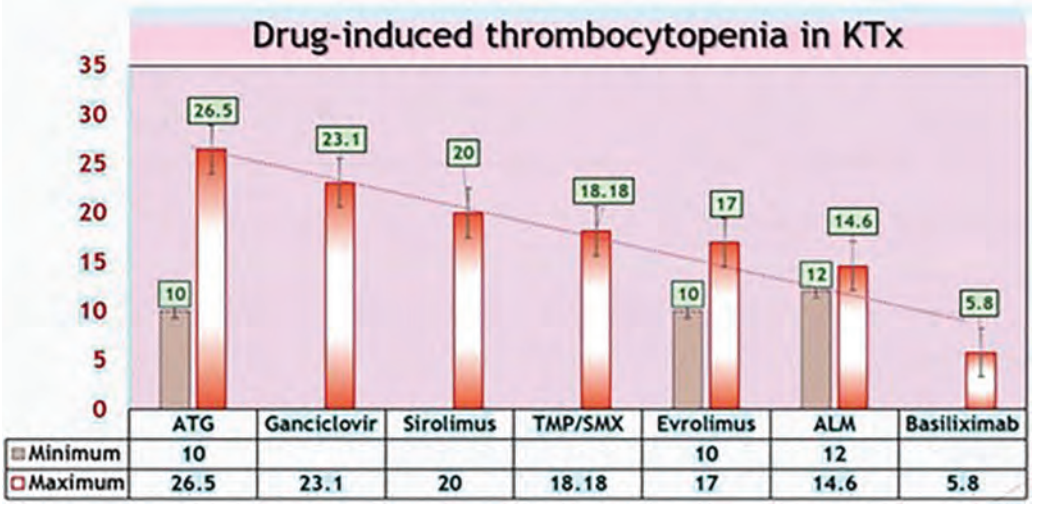
Interleukin receptor antagonists

Because anti-IL-2R activity is confined to activated T cells, their thrombocytopenic drawbacks are currently rare compared with that shown with ATG and alemtuzumab (5% for basiliximab).[46] With regard to ATG, Brennan and colleagues have reported thrombocytopenia in 14.6% of patients who received ATG, in contrast to thrombocytopenia in 5.8% of KTRs who received basiliximab therapy.[34] Another study reported a significantly higher incidence of thrombocytopenia in KTRs who received thymoglobulin compared with those who received basiliximab (8.1% vs 2.8%; $P < .05$).[48] Considering these observations, an anti-IL-2R agent would be an optimum therapeutic option for thrombocytopenic KTRs with low/moderate risk of rejection.

Mycophenolic mofetil and enteric-coated mycophenolate sodium

The hematological sequelae of myelosuppression are the most common reason for reducing MMF dose. Up to 46.5% of patients require a reduction in MMF dose for leukopenia, anemia, thrombocytopenia, and

Figure 8. Incidence of Drug-Induced Thrombocytopenia in Kidney Transplant Recipients



Abbreviations: ALM, alemtuzumab; ATG, antithymocyte globulin; TMP/SMX, trimethoprim-sulfamethoxazole

pancytopenia.[16] Myelotoxicity of MMF is dose dependent and usually related to the trough levels of MPA.[2,70]

Mammalian target of rapamycin inhibitors

The most common members of mTORi are sirolimus and everolimus, which cause a range of myelotoxic sequelae.[77,78] Thrombocytopenia has been reported in a meta-analysis of 8 trials that described shift from CNI to mTORi.[79] Severity of myelotoxicity is dose dependent,[80] with involvement in perhaps 20% of KTRs on sirolimus therapy. A trough level of >12 ng/dL is associated with thrombocytopenia.[81] Everolimus therapy is associated with thrombocytopenia in 10% to 17% patients.[82] A suggested mechanism of mTORi-induced myelotoxicity is the potential predisposition to thrombocytic microangiopathy with subsequent development of thrombocytopenia. Both everolimus[84-86] and sirolimus[87,88] have a potential capability to induce thrombocytic microangiopathy.

Ganciclovir

This poorly bioavailable agent can be given orally or through an intravenous route in a high dose (1 g at 3 times/day). Through its myelosuppressive effect, it can induce thrombocytopenia in 23.1% of KTRs.[42]

Trimethoprim-sulfamethoxazole

Several hematologic complications have been observed with this agent, including thrombocytopenia.

Differential Diagnosis of Drug-Induced Leukopenia and Thrombocytopenia

In addition to medication-induced myelotoxicity, a variety of etiologies can share in the development of these serious hematological events, including B12, folic acid, zinc, and copper deficiencies, as shown in a nontransplant cohort.[92]

Epstein-Barr virus-induced posttransplant proliferative disorders invade bone marrow of recipients, causing cytopenia.[11] Cytomegalovirus, parvovirus B19, human herpesvirus 6, influenza viruses, and ehrlichiosis (a tick-borne bacterial infection) can lead to myelosuppression-induced cytopenia.[11]

Hemophagocytic Syndrome

Hemophagocytic syndrome can be associated with cytopenia. Several viral infections (CMV, adenovirus, Epstein-Barr virus, human herpesvirus 8, human herpesvirus 6, parvovirus B19, and BK polyomavirus) have been incriminated in hemophagocytic syndrome evolution.[93]

Thrombocytic microangiopathy with consequent thrombocytopenia can develop in the following situations: renal ischemic events, antibody-mediated rejection,[2] and viral infection (CMV, human immunodeficiency virus, and parvovirus B19).

Therapy for Drug-Induced Hematological Cytopenia

In addition to the aforementioned protocols, there are a number of other specific interventions, as detailed below.

Specific treatment of neutropenia

In medical emergencies, such as severe pneumonia and septic shock, measurement of ANC can be used to evaluate the severity of neutropenia, with severe neutropenia indicated by ANC <500/μL or $<0.5 \times 10^9$/L.[94] A further decline in WBC count (ANC <100 cells/mm^3) persisting more than 7 days constitutes an extremely high risk of opportunistic infection.[95] Consequently, the first step is determining the patient's full detailed history to unravel possible culprit(s). With no suitable diagnostic tools to recognize culprit medications, a dose reduction or complete withdrawal of the suspected agent with correction of WBC count may serve as the only available technique to find a diagnosis. The next therapeutic step for WBC count correction would be administration of "colony-stimulating" factors if there is no accepted response to the previous maneuvers. Of note, an increased expression of inflammatory cytokines may lead to activation of the innate immunity that, in turn, would activate the adaptive immune system leading to acute rejection as a side effect.[96]

Colony-stimulating factors have been introduced to manage severe leukopenia in KTRs.[46] Granulocyte colony-stimulating factor has 3 major effects: neutrophil proliferation, reduction of inflammatory cytokines (eg, tumor necrosis factor, interleukin 1 [IL-1], IL-12, and interferon), and production of anti-inflammatory soluble tumor necrosis factor receptors p55 and p75, in addition to IL-l receptor antagonist and prostaglandin E2.[97-99] Granulocyte colony-stimulating factor has minimal effects on lymphocytes as they are devoid of specific G-CSF receptors.[97] Some evidence has suggested that G-CSF

may decrease rejection episodes.[99,100] Several studies have reported improved WBCs counts, fewer infection episodes, and absence of related rejection episodes.[101]

Granulocyte-monocyte colony-stimulating factor (GM-CSF) is a stimulating agent that can activate neutrophils, monocytes, macrophages, and dendritic cells. Unlike G-CSF, GM-CSF involves a proinflammatory criterion. However, safety of this agent has been documented in solid-organ transplant patients, with improved WBC count and fewer infectious episodes. The safety and efficacy of this agent, however, warrant more randomized clinical trials.[62]

Summary

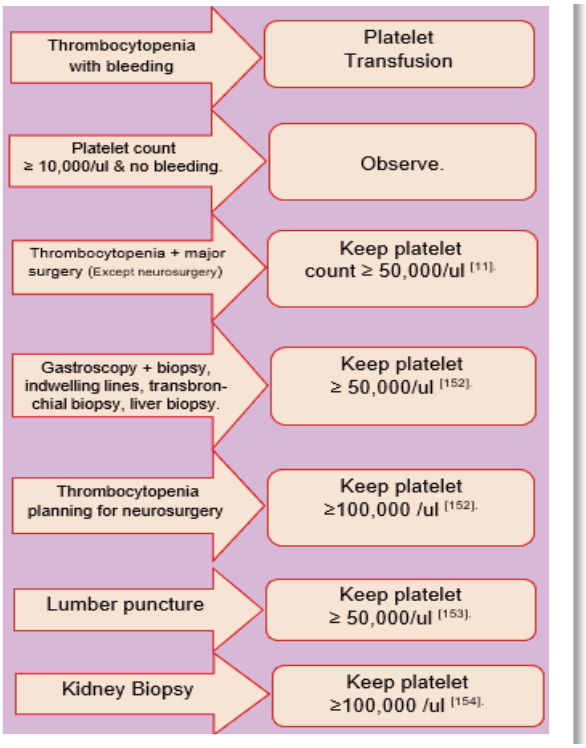
Through clinical assessments, culprit medications in cytopenia have been identified, including MMF, trimethoprim-sulfamethoxazole, valganciclovir, ganciclovir, alemtuzumab, and ATG. The first therapeutic step is reduction or complete withdrawal of the suspected agent. Until correction of cytopenia is accomplished, the treating clinician should remain alert to look for (1) an opportunistic infection and (2) early signs of acute rejection as a result of reduction of immunosuppression. Based on findings from G-CSF or GM-CSF use in oncology, these agents can be utilized in solid-organ transplant, but there is no consensus. The prophylactic administration of these agents is suggested in patients with febrile neutropenia,[103] those with diminished bone marrow reserves (eg, ANC $<1.5 \times 10^9$/L) due to extensive radiotherapy, patients with AIDS/human immunodeficiency virus infection, and patients older than 65 years. On the other hand, therapeutic indications include sepsis, hypotension, neutropenic pyrexia of >7 days, pneumonia or fungal infections, and adjunctive therapy along with antibiotics in the aforementioned indications.[103] Some conditions constitute medical emergencies of worse outcome that necessitate prophylactic administration of these agents, including prolonged pyrexia, severe neutropenia with ANC <500/μL,[94] and prolonged neutropenia of more than 7 days.[95]

Thrombocytopenia in kidney transplant can be attributed to either bone marrow suppression or to an idiosyncratic drug reaction.[104] The following steps in care are suggested: if thrombocytopenia is due to idiosyncratic reaction (eg, due to trimethoprim-sulfamethoxazole), then immediate withdrawal of the suspected agent is required[105,106]; if bone marrow suppression is the underlying mechanism, dose reduction or complete drug cessation is required for correction of platelet decline.

Occasionally, platelet transfusion may be required, such as in life-threatening bleeding risk, serious decline of platelet count (<20000/mm^3), or before an invasive procedure (eg, organ biopsy; Figure 9).[107] The recommended cut-off therapeutic level for platelet transfusion should be >50000/mm^3 with commencement of invasive maneuvers (eg, allograft biopsy, gastroscopic studies, indwelling catheter application, transbronchial biopsy, and laparotomy[108]).

Before ocular and neurosurgical invasive procedures, a minimum platelet count of ≥100000/mm^3 is usually advised.[108] For lumbar puncture procedures, a platelet count of ≥50000/ mm^3 is recommended.[109] Because of the high vascularity of renal tissue, a minimum level of 100000/mm^3 is usually recommended for renal invasive procedures.[110] The treating clinician should be aware that anemia associated with bleeding may lead to a major bleeding event, and platelet transfusion at that time may be required even if the platelet count is >100000/μL.[111,112] The risk of CMV-transmitted infection via platelet transfusion is considered a rare

Figure 9. Suggested Guidelines for Platelet Transfusion[11]



event; however, presence of leukocytes may occasionally permit this transmission.

In summary, in view of the paucity of data on immunosuppressive medication-induced cytopenia and the few randomized controlled trials, our present article serves to focus on the most recent evidence-based information.

Further Developments

Thrombopoietin receptor agonists have been efficacious in thrombocytopenia management. These agents include romiplostim and eltrombopag, which have been used with better results in "idiopathic thrombocytopenic purpura" therapy.[113,114] Eltrombopag has also been successful in the management of hepatitis C virus infection and aplastic anemia-associated thrombocytopenia,[115,116] and both romiplostim and eltrombopag have shown good results in treatment of chemotherapy-related thrombocytopenia.[117-120] Several trials have reported no response to romiplostim therapy to correct platelet counts in patients with tacrolimus-associated thrombocytopenia.[121] However, another case report documented a marvelous response to eltrombopag therapy in plasmapheresis in KTRs with transplant-related immune thrombocytopenia.[122] Further studies are warranted to elucidate both the safety and efficacy of these agents in drug-induced thrombocytopenia in KTRs.

Conclusions

Kidney transplant recipients often present with hematological cytopenia. The risk of infection, including opportunistic, is life-threatening in the presence of leukopenia and neutropenia. Moreover, risk of bleeding can be also triggered once thrombocytopenia ensues. Drug withdrawal or dose reduction may be the only technique to recognize the suspected medication or medications. Severe neutropenia (ie, agranulocytosis) may have a grave outcome, particularly in patients with neutropenic fever. A number of new agents have been introduced to manage serious cytopenia (eg, G-CSF, GM-CSF, and thrombopoietic agents); however, there is dearth of safety and efficacy profiles for these novel agents.

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Rankings From US *News and World Report* Have Minimal Correlation With Kidney and Liver Transplant Recipient Survival Results From Retrospective Data

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Abstract

Objectives: **Increased demand for quality health care has led to lay-press ranking systems, such as the ranking from *US News and World Report* (US News). Their "Best Hospitals" publication advertises itself as the go-to resource for patients seeking care in a number of specialty areas. We sought to test the relationship between US News rankings and transplant outcomes.**

Materials and Methods: **Using data from 2014 to 2018, we compared outcomes from the Scientific Registry of Transplant Recipients database for liver and kidney transplants against US News-ranked centers using the categories "Nephrology" and "GI Surgery and Gastroenterology" as substitutes, as US News does not rank transplant centers specifically. *P* < .05 was set as significant.**

Results: **Using hazard ratio data, we found that kidney transplant center rank had only a small impact on postoperative outcomes in terms of patient survival (hazard ratio = 0.996, *P* = .049) but had no impact on graft survival (hazard ratio = 0.997, *P* = .077). In addition, liver transplant center rank had no impact on liver graft survival (hazard ratio = 1.003, *P* = .304). The impact of hospital ranking on survival was minimal compared with other variables.**

Conclusions: **The US News rankings for "Nephrology" and "GI Surgery and Gastroenterology" have minimal values as a measure of liver and kidney transplant outcomes, highlighting that these lay press rankings are not useful to the unique transplant patient population and that providers should help guide patients to transplant-specific resources.**

Key words: *Kidney transplantation, Liver transplantation, Outcomes, Quality of health care*

Introduction

Increased national attention toward the cost and quality of health care, as well as the advent of value-based purchasing by the Centers for Medicare and Medicaid Services (CMS), has led to an increase in the availability of hospital rankings and outcomes data. The *US News and World Report* (US News) is one of the most recognized and publicly available sources for consumer hospital ratings and has been noted to be more responsive to changes than other public ranking platforms.[1-4] Testing these relationships reveals mixed results, although they generally favor a positive correlation between quality and rank.[1,4-9] Despite this, examination of the various nationally published hierarchies has demonstrated little agreement pertaining to which institutions are "best." Each agency measures centers on differing criteria, a fact that has gained the national spotlight with a popular *New York Times* article.[5,8,10,11]

For some specialty services, such as liver and kidney transplantation, the lay literature may not correlate with measured outcomes. Although there is no direct source for transplant center rankings, US News is commonly used by physicians to guide patients. The field of transplantation,

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with its long-recognized focus on ethical and efficient use of precious and limited resources, is ripe for review.

Quality measurements for reimbursement from the CMS are flawed. Jackson and colleagues revealed that regional referral centers are unfairly biased due to nonmedical-related factors, such as travel distance.[12] This is of particular importance in transplantation, where a majority of care is handled by relatively few specialty centers.[12] Previous reports have evaluated the relationship between US News rankings and patient outcomes for other areas of complex surgery and revealed strong correlations. However, none specifically addressed liver and kidney transplantation, and no press ranking systems have established correlations in transplant outcomes data.[1]

Materials and Methods

The Scientific Registry of Transplant Recipients (SRTR) database was queried to identify kidney and liver transplant outcomes and hazard ratios (HR) for patient and graft survival at 1 year, grouped into rolling 2.5-year cohorts. This time period best matches temporally with the US News 3-year data evaluation period. In addition, 1-year survival is a common outcome measure evaluated by 2 major oversight agencies.[13] The annual US News "Best Hospitals" issues were obtained from the publisher. Due to the nature of this analysis, because we only collected secondary data without identifiers, there was no need for informed consent. These data were merged using transplant center codes. Data covered transplant information between 2014 and 2018.

The US News rankings are determined by several factors. The hospitals must meet one of the following criteria to be included: it is a teaching hospital, it is affiliated with a medical school, it has at least 200 beds, or it has at least 100 beds in addition to offering at least 4 medical technologies deemed significant by US News. If a hospital has a specialty ranked, additional criteria are required. Hospitals must meet a volume or discharge threshold that varies by specialty. The underlying methodology of these rankings involves a composite score based on structure, process, and outcomes. Structure refers to hospital resources related directly to patient care. Examples include intensity of nursing staff and availability of certain technologies and services. Process is the way in which a hospital delivers care, treatment, and education. The most obvious measure from the outcomes data is death, which is measured by risk-adjusted mortality. This accounts for the complexity of the individual and their current condition. As part of its ratings, US News also considers patient survival and safety for a 3-year period ending 2 years prior to each publication. For this reason, US News rankings from 2014 to 2018 were paired with SRTR data from June 2008 to December 2015, so that each agency was evaluating 1-year survival averaged over the same 3-year period. From these data, our outcome variables were patient and graft survival after kidney and liver transplantation.

US News only provides numerical rankings for the top 50 programs. US News Hospital rankings for "GI Surgery and Gastroenterology" and "Nephrology" were substituted for liver and kidney transplant programs, respectively, as US News does not specifically score or rank hospitals for transplantation.

Statistical analyses

The basic patient, donor, and transplant characteristics were compared across high-, medium-, and low-ranking transplant centers using *t* or Wilcoxon-Mann-Whitney tests and chi-square or Fisher exact tests, depending on the sample size and the distribution of the variables used. Patient and graft survival rates (1- and 2-years posttransplant) were analyzed. Survival curves and the estimates for transplant outcomes were obtained using the Kaplan-Meier product limit method. A series of log-rank tests were performed to evaluate whether the graft survival rate and the patient mortality rate varied depending on the transplant center's ranking. In the survival analysis of transplant outcomes, patient death and graft failure were the endpoints, thereby generating death-censored survival estimates. Death data are measured by risk-adjusted mortality. This accounts for the complexity of the individual and their current condition. To control for patient- and donor-level risk factors, we performed multivariate Cox regression to determine statistically significant associations between rankings of transplant center and graft survival as well as patient mortality for both kidney and liver transplant. Statistical significance was defined as $P < .05$ in the analysis unless noted otherwise. Stata version 15 (StataCorp LLC) was used for the analysis.

Results

Kidney transplant outcomes according to nephrology rankings

Rankings of transplant centers were coded, with higher numbers indicating a better position in the ranking (ie, the rank of 50 is the top-ranked program). We grouped the transplant centers into 3 cohorts: high rank (>35), medium rank (35 to 20), and low rank (<20). Table 1 compares the demographic, clinical, and donor-level factors across the 3 groups for the kidney transplant cohort.

Kidney transplant: patient survival

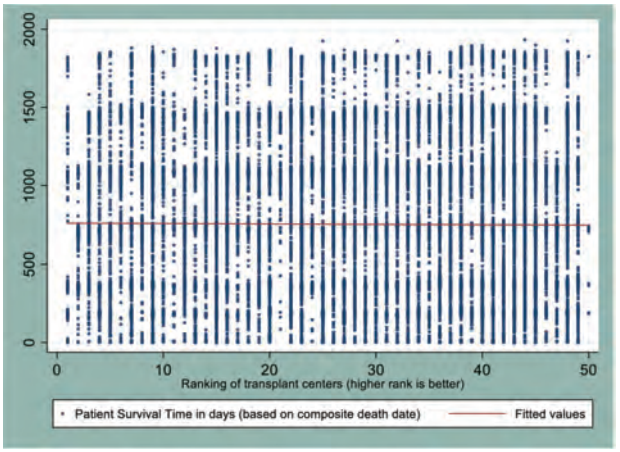
Figure 1 demonstrates that there was no clear relationship between hospital ranking and patient survival. This did not control for baseline differences between hospital demographics. We estimated the Spearman correlation coefficient between ranking and most recent patient status (based on composite death date) (n = 34 946, Spearman rho = -0.015). The Spearman correlation coefficients for ranking and patient death status were negative and significant, indicating that higher-ranking transplant centers have lower probability of patient death at the time of the observation. However, this correlation did not account for other demographic, clinical, and donor-level factors that may determine the transplant outcomes.

Table 1. Descriptive Statistics: Kidney Transplant

	High Rank (>35)	Medium Rank	Low Rank (<20)	*P* Value
Recipient demographics				
Mean age (SD), y	51.28 (14.08)	51.75 (13.84)	51.56 (13.75)	.003
Male, No. (%)	7518 (61%)	7533 (60%)	6153 (61%)	.718
White, No. (%)	6230 (50%)	6450 (52%)	4576 (45%)	<.001
Black, No. (%)	2925 (24%)	3057 (25%)	3183 (32%)	<.001
Hispanic, No. (%)	1985 (16%)	1796 (14%)	1519 (15%)	.027
Mean days on wait list (SD)	772 (823)	742 (789)	761 (793)	
Recipient clinical factors				
Prior transplant, No. (%)	1695 (14%)	1574 (13%)	1244 (12%)	.002
Dialysis at time of transplant, No. (%)	8569 (77%)	9326 (75%)	7729 (70%)	<.001
Mean cPRA at time of transplant (SD)	18.71 (33.99)	20.89 (34.64)	21.67 (34.93)	
Mean HLA mismatch level (0-6) (SD)	3.83 (1.60)	3.92 (1.55)	3.96 (1.55)	<.001
Mean BMI (SD), kg/m^2	27.93 (5.46)	28.40 (5.43)	28.46 (5.45)	
Donor/organ characteristic				
Mean KDPI (SD)	0.45 (26)	0.46 (26)	0.47 (0.26)	<.001
Locally shared, No. (%)	9938 (80%)	9891 (79%)	8077 (80%)	.617
Regionally shared, No. (%)	848 (7%)	1178 (9%)	806 (8%)	<.001

Abbreviations: BMI, body mass index; cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index

Figure 1. Scatterplot Between Ranking and Kidney Patient Survival



There was no clear relationship, as average survival time was similar across all ranks. This did not control for the baseline differences between the cohorts.

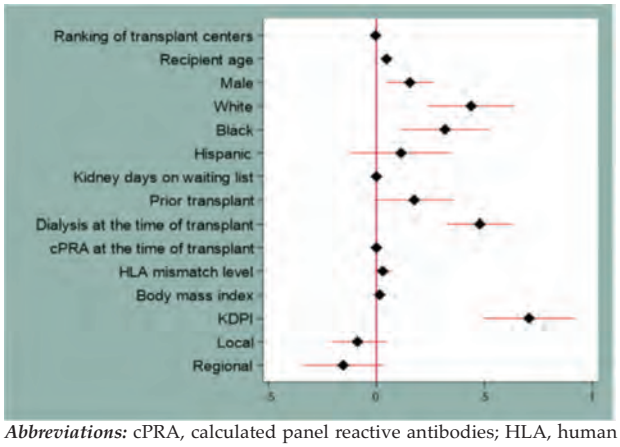
Table 2 presents the Cox regression results, where the dependent variable was risk of patient mortality (based on composite death date) and the primary independent variable was the ranking of transplant centers. Our results indicated that higher-ranking transplant centers were associated with lower risk of patient mortality, which was shown to be a significant association (HR = 0.996, P = .49). This was further illustrated in Figure 2, which shows the impact that each factor had on patient mortality risk. As shown in Figure 2, the higher ranking of a transplant center was negatively associated with patient mortality, although the impact was small (<0.01% reduction in risk as the ranking increased by 1). When all other variables were kept constant, older age, male sex, White ethnicity, Black ethnicity, Hispanic ethnicity, prior transplant, being on dialysis, higher HLA mismatch, Kidney Donor Profile Index (KDPI), and having higher body mass index (BMI) were all risk factors for patient mortality, whereas having a local and regional donor reduced the risk of patient mortality compared with having a national donor. Figure 3 presents the Kaplan-Meier patient survival estimates; as shown, a significantly higher number of patients had better survival in the high- and medium-ranking centers compared with the low-ranking centers.

Table 2. Cox Regression Results: Kidney Transplant Patient Survival

	HR	*P* Value	CI	
			Lower	Upper
Ranking of the transplant center	0.996*	.049	0.992	1.000
Patient factor				
Age	1.048**	<.001	1.043	1.053
Male	1.167**	.006	1.045	1.304
White	1.550**	<.001	1.269	1.894
Black	1.373**	.002	1.121	1.683
Hispanic	1.120	.333	0.890	1.411
Days on waitlist	1.000**	<.001	1.000	1.000
Prior transplant	1.191	.063	0.991	1.432
Dialysis at time of transplant	1.613**	<.001	1.386	1.878
cPRA at time of transplant	1.000	.819	0.998	1.002
HLA mismatch level (0-6)	1.029	.122	0.992	1.068
BMI	1.015**	.004	1.005	1.026
Donor factor				
KDPI	2.027**	<.001	1.642	2.502
Local	0.916	.201	0.801	1.048
Regional	0.858	.101	0.714	1.030

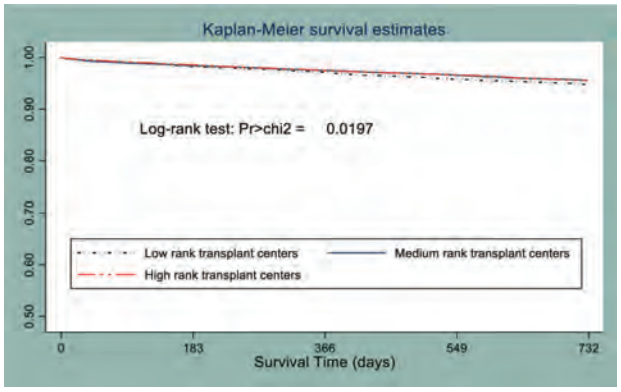
Abbreviations: BMI, body mass index; cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index
Note: Standard errors are robust. *$P < .05$; **$P < .01$. Ranking was recoded as higher number means a better ranking. Number of observations = 22210.

Figure 2. Coefficient Plot of Cox Regression: Kidney Patient Survival



Abbreviations: cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index
Graph presents the coefficient plot of the Cox regression results from Table 3, showing that the magnitude of the role of transplant center ranking was nominal compared with other determinants of patient survival at 1 year.

Figure 3. Kaplan-Meier Survival Analysis: Kidney Transplant Patient Survival



Kidney patient survival rate was higher in high- and medium-ranking centers compared with low-ranking centers.

Kidney transplant: graft survival

We estimated the Spearman correlation coefficient between ranking and kidney graft status (n = 34946, Spearman rho = -0.024). The Spearman correlation coefficients for ranking and kidney graft status were negative and significant, indicating that higher-ranking transplant centers had lower probability of graft failures. However, this correlation did not account for other demographic, clinical, and donor-level factors that may determine transplant outcomes. Similar to Figure 1, there was no clear relationship demonstrated between ranking and graft survival time.

As shown in Table 3 and Figure 4, ranking of the transplant center had no significant effect on graft survival status (HR = 0.997, $P = .077$). Similar to patient survival results, having a local or regional donor increased graft survival. As shown in the Kaplan-Meier results in Figure 5, higher survival was shown in high-ranking centers compared with medium- and low-ranking centers.

Table 3. Cox Regression Results: Kidney Graft Survival

	HR	*P* Value	CI	
			Lower	Upper
Ranking of the transplant center	0.997	.077	0.994	1.000
Patient factor				
Age	1.010**	<.001	1.006	1.014
Male	1.062	.184	0.972	1.160
White	1.505**	<.001	1.276	1.775
Black	1.508**	<.001	1.279	1.778
Hispanic	1.129	.204	0.936	1.361
Days on wait list	1.000**	<.001	1.000	1.000
Prior transplant	1.121	.108	0.975	1.290
Dialysis at time of transplant	1.460**	<.001	1.287	1.656
cPRA at time of transplant	1.000	.540	0.999	1.002
HLA mismatch level (0-6)	1.049**	.002	1.018	1.081
BMI	1.011**	.010	1.003	1.019
Donor factor				
KDPI	2.771**	<.001	2.331	3.293
Local	0.871*	.012	0.781	0.970
Regional	0.909	.202	0.785	1.052

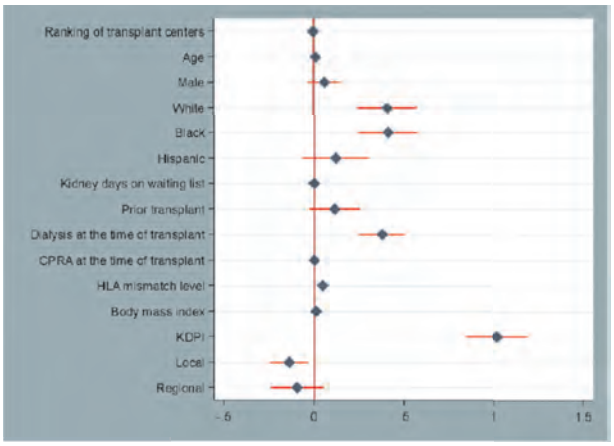
Abbreviations: BMI, body mass index; cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index
Note: Standard errors are robust. *$P < .05$; **$P < .01$. Ranking was recoded as higher number means a better ranking. Number of observations = 22210.

Liver transplant according to gastrointestinal rankings

In the ranking of liver transplant centers, those coded with higher numbers indicated a better position in the ranking (ie, rank of 50 is the top-ranked program), similar to the analyses of kidney transplant centers. We again grouped the transplant centers into 3 cohorts: high rank (>35), medium rank (35 to 20), and low rank (<20). Table 4 compares the demographic,

clinical, and donor-level factors across the 3 groups for the liver transplant cohort.

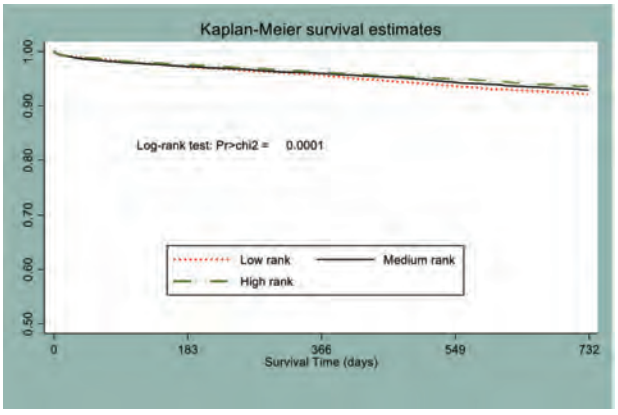
Figure 4. Coefficient Plot of Cox Regression: Kidney Graft Survival



Abbreviations: cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index

Graph presents the coefficient plot of the Cox regression results from Table 3, showing that the magnitude of the role of transplant center ranking was nominal compared with other determinants of graft failure at 1 year.

Figure 5. Kaplan-Meier Survival Analysis: Kidney Graft Survival



Graft survival rate was higher in high-ranking centers compared with medium- and low-ranking centers.

Again, no clear relationship was shown between hospital ranking and graft survival. This did not control for baseline differences between hospital demographics. We estimated the Spearman correlation coefficient between ranking and liver graft status (n = 14657, Spearman rho = -0.001). The Spearman correlation coefficient for ranking and liver graft status was negative and significant, indicating that higher-ranking transplant centers had lower probability of graft failure. However, this correlation did not account for other demographic, clinical, or donor-level factors that may determine transplant outcomes.

Table 4. Descriptive Statistics: Liver Transplant

	High Rank (>35)	Medium Rank	Low Rank (<20)	*P* Value
Recipient demographics				
Mean age (SD), y	55.83 (11.26)	55.42 (10.97)	55.72 (10.98)	.26
Male, No. (%)	3567 (68%)	3002 (66%)	3281 (67%)	.28
White, No. (%)	3771 (72%)	3423 (76%)	3464 (71%)	.24
Black, No. (%)	412 (8%)	318 (7%)	514 (11%)	<.001
Hispanic, No. (%)	734 (16%)	522 (14%)	653 (15%)	.29
Mean days on wait list (SD)	283 (538)	231 (420)	238 (438)	
Recipient clinical factors				
Prior transplant, No. (%)	244 (5%)	222 (5%)	202 (4%)	.21
Dialysis prior week to transplant, No. (%)	713 (14%)	532 (12%)	579 (12%)	.006
Mean HLA mismatch level (0-6) (SD)	4.68 (1.08)	4.61 (1.07)	4.68 (1.07)	.191
Mean BMI (SD)	29.23 (5.78)	29.27 (5.80)	29.49 (5.90)	
Donor/organ characteristics				
Mean age (SD), y	42.51 (16.01)	41.96 (15.30)	42.59 (15.47)	.013
Male, No. (%)	3076 (59%)	2722 (60%)	2909 (59%)	.415
White, No. (%)	3554 (68%)	3067 (69)	3071 (63%)	<.001
Black, No. (%)	877 (17%)	749 (17%)	974 (20%)	<.001
Hispanic, No. (%)	622 (12%)	507 (11%)	662 (14%)	.012
Mean BMI (SD)	28.19 (6.89)	28.41 (6.77)	28.47 (6.71)	
DCD, No. (%)	309 (6%)	384 (9%)	363 (7%)	.002
ECD, No. (%)	1327 (25%)	1070 (24%)	1271 (26%)	.472
Locally shared, No. (%)	3563 (68%)	2979 (67%)	3184 (65%)	.002
Regionally shared, No. (%)	1440 (28%)	1371 (30%)	1504 (31%)	<.001

Abbreviations: BMI, body mass index; cPRA, calculated panel reactive antibodies; DCD, donor after cardiac death; ECD, extended criteria donor; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index

As shown in Table 5, there was no significant effect of hospital ranking on liver graft survival (HR = 1.003, *P* = .304) at 1 year. Age, time on wait list, retransplantation, being on dialysis before transplant, donor age, and donor sex had significant impacts on graft survival at 1 year. These impacts are further demonstrated in Figure 6, which visually interprets the magnitude of the results from Table 5. As shown in the Kaplan-Meier liver graft survival results in Figure 7, medium-ranking centers had better survival compared with low- and high-ranking centers. When we conducted the same analyses with liver patient outcomes, the results were the same; therefore, we decided to show only graft survival for the sake of clarity and to reduce redundancy.

Discussion

The relationship between lay press ratings and objective clinical outcomes is complex. When we considered both liver and kidney transplant

outcomes after analyzing hazard ratios, a better US News ranking was only slightly correlated with decreased survival of kidney transplant patients, but not with survival of kidney grafts. Similar analyses showed no correlations with liver graft survival or with survival of liver transplant recipients. For this reason, US News rank appears to be a poor tool for a consumer concerned about duration of life and transplanted organs.

It is important that this information be explicitly conveyed to patients, as it has been shown to greatly impact their decision-making process.[16] The methodology of US News, which evaluates and scores many aspects of care besides outcomes, may remain a useful tool for consumers who are more concerned about other areas of their care, including the hospital environment, support services, and the prestige of their transplant center.[3,8,17] Superior patient and graft survival alone, compared with national average survival rates, would make for excellent hospital press and appear convincing to the public. Health professionals, however, understand that population characteristics play a critical role in outcomes.[3,8,18,19] This is even more significant among transplant populations, as their unique risk factors are a huge determinant in organ eligibility and transplant center availability.

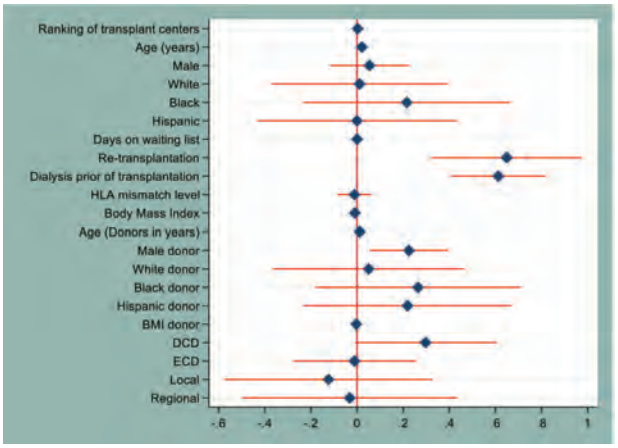
Table 5. Cox Regression Results: Liver Graft Survival

	HR	*P* Value	95% CI	
			Lower	Upper
Ranking of the transplant center	1.003	.304	0.997	1.008
Patient factor				
Age	1.021	<.001	1.012	1.029
Male	1.055	.543	0.887	1.255
White	1.011	.956	0.689	1.482
Black	1.241	.346	0.792	1.943
Hispanic	0.999	.999	0.648	1.541
Days on wait list	1.000	.033	1.000	1.000
Retransplantation	1.914	<.001	1.383	2.648
Dialysis prior of transplant	1.845	<.001	1.504	2.263
HLA mismatch level (0-6)	0.989	.773	0.918	1.066
BMI	0.991	.269	0.975	1.007
Donor factor				
Age	1.012	.003	1.004	1.019
Male	1.252	.01	1.056	1.484
White	1.052	.812	0.694	1.593
Black	1.303	.244	0.834	2.033
Hispanic	1.245	.343	0.791	1.957
BMI	0.997	.635	0.984	1.010
DCD	1.346	.057	0.991	1.828
ECD	0.989	.936	0.758	1.290
Local	0.883	.59	0.562	1.387
Regional	0.969	.894	0.607	1.546

Abbreviations: BMI, body mass index; DCD, donor after cardiac death; ECD, extended criteria donor; HLA, human leukocyte antigen

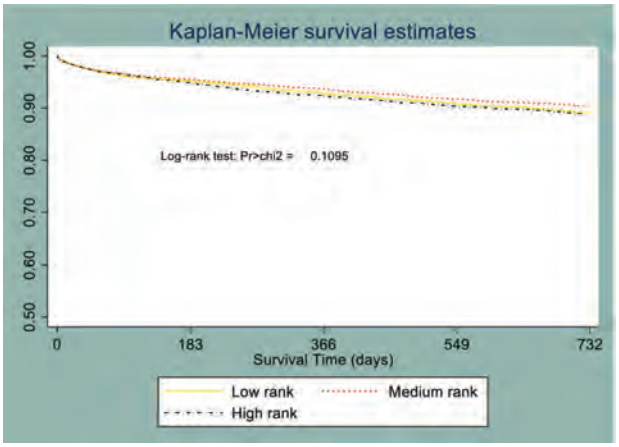
Note: Standard errors are robust. Ranking was recoded as higher number means a better ranking. Number of observations = 5385.

Figure 6. Coefficient Plot of Cox Regression: Liver Graft Survival



Graph presents the coefficient plot of Cox regression results from Table 5, showing that the magnitude of role of transplant center ranking was nominal compared with other determinants of liver graft failure at 1 year.

Figure 7. Kaplan-Meier Survival Analysis: Liver Graft Survival



Liver graft survival rate was higher in medium-ranking centers compared with low- and high-ranking centers.

The US News and World Report states that the purpose of their annual rankings is to help consumers make a choice about where to find "especially skilled inpatient care" when faced with operations or care that poses "unusual technical challenges or a significantly increased risk."[20] The increasing digitalization of ranking information has given individuals easier access to data.[21] Although there are concerns about unintended negative consequences from hospital report cards, they appear at face value to provide transparency and public accountability.[3,4,9,18,22,23] How one chooses a transplant center is not perfectly understood and likely differs to some extent from person to person.[16,24] Consumers, when presented with quality information in an easy-to-understand format, such as rankings, will consider quality along with cost as major decision-

making factors, including choice of surgeon and center.[16,25-28] However, the transplant population is not as straightforward as the general elective surgical patient population, as their choices are not simply their choice but depend on organ and transplant center availability. In a study that analyzed how patients choose a kidney transplant center, one of the major themes patients used to make decisions was their perceived reputation of the center, along with reliance on opinions from providers, family, and friends, valued relationships, convenience, and insurance coverage.[16] The choice of center may be made entirely by the referring physician with little input from the patient, although the referring physician may consult rankings before making a recommendation.[4,24,29] According to one survey given to those choosing a transplant center, the wait list was given the highest priority.[30] The wait list was almost twice as likely as outcomes data to be the most important factor.[30] The survey also found that many relied on information from physicians to help with their decision, whereas a minority cited transplant-specific organizations.[30] Data have also indicated that, while both patients and providers often state that they would like to make the decision based primarily on quality, few actually do.[17]

Outcomes of transplant centers are more dependent on their referral pool than other factors.[31] In kidney transplantation, a high graft failure rate is associated with a decline in wait-list registrations, although it is unclear whether individual choice, education level, referring physician choice, or insurance companies drive this decision.[32,33] Hospitals clearly believe that the pool can be influenced by attracting customers with the use of high US News rankings, as evidenced by their use in advertising.[8,18,34] To make matters more compelling, both transplant outcomes and "patient perceptions of care" are tied to CMS reimbursement and public funding.[18,19,35] Hospitals have a strong financial incentive to recruit the healthy and wealthy, influence their referral pools, and work to increase their perceived reputation. There is a growing concern within the field of transplantation that increased scrutiny may cause centers to avoid high risks and unnecessarily discard organs.[3,13,19] If a definitive ranking system were to be created, this could potentially have disastrous negative consequences that could lead to transplant centers rejecting more organs and limiting access in an already limited field.

Our analysis indicated that more research is needed concerning public hospital rankings and how patients are able to interpret these and other outcome databases such as the SRTR. Ratings agencies consider many factors in ranking a hospital beyond treatment outcomes. These include, but are not limited to, reputation, clinical and basic science research, the availability of ancillary services, overall hospital safety measurements, and services designed to increase patient comfort, which have variable levels of impact on the treatment and care actually provided.

One limitation of our study was the lack of US News hospital categories specific to kidney and liver transplantation. This weakness demonstrates the need for dedicated transplant resources that are easy for patients to understand. Our use of "GI Surgery and Gastroenterology" and "Nephrology" subspecialties from the US News report was the best substitute for actual transplant rankings but assumes consumers will arrive at a similar conclusion. We feel this is valid for 2 reasons. First, most referrals for transplantation, almost all pretransplant care, and a large amount of posttransplant care are provided by hepatologists and nephrologists. Second, a plurality of top 50-ranked programs (99.33% for kidney and 78.28% for liver) are transplant centers. However, for a center to be analyzed by US News, it needs to meet specific criteria, including being a teaching hospital, being affiliated with a medical school, having at least 200 beds, or having at least 100 beds in addition to offering at least 4 medical technologies deemed significant by US News. Because not every transplant center meets these requirements, some may have been missed by our analyses, which further demonstrates the need to produce transplant-specific resources for patients. An additional weakness of this study was the recent changes to allocation for liver and kidney transplantation, which may not have been captured in this analysis; these may, in the future, alter outcomes and rankings.

The goal of the data provided by SRTR is to help consumers gain a broad understanding of a transplant program's general outcomes and trends, which is key for the transplant patient population. It is also useful for comparisons of different programs. The SRTR provides timely and accurate information from programs for various types of transplants. However, the metrics presented in SRTR are calculated for all patients at the program that is

searched for and may not represent an individual's particular need. Each transplant patient has a unique situation and history that needs to be considered holistically to find the "best" fit for them, but this does not lend itself to a simple ranking system.

A strength of this study is that the SRTR 1-year evaluation periods aligned perfectly with the US News evaluation periods. Although longer-term data, for instance 3-year or 5-year survival rates, would be interesting to investigate, it would not have aligned temporally with the US News evaluation period. In addition, the SRTR 1-year survival data are used by the CMS and the Organ Procurement and Transplantation Network Membership and Professional Standards Committee for regulatory review of transplant programs.[13] Another strength is the use of hazard ratios, which account for patient mix and are rigorously validated by the SRTR.[14,15]

Conclusions

The field of transplantation treats a population that is particularly desperate and for whom care is exceedingly complex. Practitioners in the field are responsible for the ethical distribution of a precious and limited resource, donated organs. Transplant center survival results are tied to outcomes and federal funding. It is critical for the public to have an accurate assessment of the quality of their transplant center while also understanding how their unique situation fits with each center. The US News advertises its rankings as the go-to resource for advice on seeking specialized care. However, because we showed these to be poor representations of objective transplant outcomes, such as graft survival, it may be most prudent for providers to guide transplant patients away from simple ranking data and to more detailed resources such as the SRTR, which can be evaluated with the patient and provider together to determine what factors are most critical to each patient. The SRTR is an incredibly valuable tool unique to transplant surgery that is very transparent and meticulous in its methods, which is crucial in such a delicate field with constant vigilance for upholding ethical standards of organ use.

The field of transplantation is complex. With its duty to ethically and efficiently use its donated organs and the rigorous standards that are constantly evaluated and reviewed, along with results from our study that current ranking systems are of little to no use to the consumer, we believe it would be prudent for providers to encourage transplant patients to not rely on a "one size fits all" approach to their care. In this age of readily available information, it is easy for consumers to find a "top 50" list without understanding the details of how these conclusions are formed. Because of this, it is more important now than ever before as physicians to help patients process the data they are finding in a meaningful way. This is even more important in transplantation, as the unique situation of each patient must be accounted for and there is no one best center; rather, it is more important to find the best center for each patient, looking at transplant center volume, risk tolerance, and the patient's risk factors.

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A Multicenter Cohort Study of Indian Centers on Reoccurring SARS-CoV-2 Infections in Kidney Transplant Recipients

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Abstract

Objectives: **There is scarcity of data on reoccurrence of SARS-CoV-2 infections in kidney transplant recipients.**
Materials and Methods: **We conducted a retrospective multicenter cohort study and identified 13 kidney transplant recipients (10 living and 3 deceased donors) with recurrent COVID-19, and here we report demographics, immunosuppression regimens, clinical profiles, treatments, and outcomes.**
Results: **COVID-19 second infection rate was 0.9% (13/1350) in kidney transplant recipients with a median age of 46 years; median time interval from transplant to first episode of COVID-19 diagnosis was 9.2 months (interquartile range, 2.2-46.5 months). The most common comorbidities were hypertension (84%) and diabetes (23%). Fever was significantly less common with recurrent COVID-19. COVID-19 severity ranged from asymptomatic (23%), mild (31%), and moderate (46%) during the first infection and asymptomatic (8%), mild (46%), and severe (46%) in the second infection. All 6 kidney transplant recipients with severe second infections died. The median interval between the 2 episodes based upon reverse transcriptase polymerase chain reaction COVID-19-positive tests was 135 days (interquartile range, 71-274 days) without symptoms. Statistically significant risk factors for mortality were dyspnea (*P* = .04), disease severity (*P* = .004), allograft dysfunction (*P* < .05), higher levels of neutrophil-to-lymphocyte ratio (*P* = .05), and intensive care unit/ventilator requirement (*P* = .004). Although our limited resources did not allow for molecular diagnostics and typing, we suggest that these second episodes were reinfections with SARS-CoV-2.**
Conclusions: **To our knowledge, this is the largest study of kidney transplant recipients with reoccurring SARS-CoV-2 infection, and we observed 46% mortality.**

Key words: *COVID-19, Immunosuppression, Infectious agents, Living kidney donor, Viral reinfection*

Introduction

India is being ravaged by a second wave of the COVID-19 pandemic. India overtook Brazil as the country with the second highest number of recorded cases of COVID-19 after the United States. The health infrastructure in-country has collapsed, with acute shortages of hospital beds and oxygen supply.[1] We recognize that many decisions are made based on the practical limitations that transplant programs face in settings of scarce resources, such as the highest daily COVID-19 cases in the world amid an oxygen shortage and health care shortage in India.[2,3]

The first cases of SARS-CoV-2 reinfection were confirmed by whole genome sequencing in the general population and in a liver transplant recipient by To and colleagues[4] and Tomkins-Tinch and colleagues,[5] respectively. Ye and colleagues reported a reactivation rate of 9% in adults with SARS-CoV-2 infection.[6] Reactivation and reinfection of previously

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recovered COVID-19 patients may rarely occur but can result in devastating complications for transplant outcomes.[5,7-11] Reinfections in patients recovered from COVID-19 could create a serious challenge in tackling the COVID-19 pandemic because these patients could be a source of virus spread in the community.[10,12-18] In organ transplant recipients, risk of SARS-CoV-2 reactivation may be related to immunosuppression, age, sex, and underlying comorbidities, including diabetes, heart disease, obesity, cancer, and virologic factors. Immunocompromised transplant recipients may be at higher risk for reactivation and reinfections with SARS-CoV-2. The degree of protective immunity conferred by infection with SARS-CoV-2 is currently unknown.

Reoccurring SARS-CoV-2 infections in transplant recipients are not well understood. To date, few reports of reoccurring COVID-19 infection in the posttransplant setting have been published.[5,6,8-10] Reoccurring SARS-CoV-2 infections raise several unanswered questions. Can SARS-CoV-2 infection have a second episode in kidney transplant recipients (KTR) who had previously recovered from COVID-19? Is the mortality in immunosuppressed patients higher in the second episode of COVID-19? Clearly, there is a need to evaluate the clinical significance of reoccurring COVID-19 in KTR. Here, we have addressed these questions and have reported information gathered through a multicenter cohort study from India of reoccurring SARS-CoV-2 infections in 13 KTR.

Materials and Methods

We conducted a retrospective observational cohort study of 8 Indian transplant centers and identified 13 KTR (10 living and 3 deceased donor) with real-time reverse transcription polymerase chain reaction (RT-PCR)-confirmed reoccurring SARS-CoV-2 infections from April 2020 to May 2021. Ethical approval for this study was obtained from the Ethics Committee of Institute of Kidney Diseases and Research Center, Dr. H. L. Trivedi Institute of Transplantation Sciences. All transplants were performed according to local laws and regulations (Transplantation of Human Organs and Tissues Act, India) and the Declaration of Helsinki and Declaration of Istanbul. We recorded details of demographics, immunosuppression regimens, clinical profiles, treatments, and outcomes.

Definition of second episode of COVID-19

The diagnosis of COVID-19 was confirmed by SARS-CoV-2-positive RT-PCR from nasopharyngeal (nasal) and oropharyngeal (throat) swabs in the first and second infections. The COVID-19 severity was graded according to Government of India criteria as asymptomatic or mild, moderate, or severe disease.[19] None of the patients was symptomatic at the time of discharge subsequent to the primary COVID-19 infection. Two successive SARS-CoV-2 RT-PCR tests >48 hours apart were negative before the second episode parallel with clinical convalescence. The methodology of swab collection was properly performed, hence excluding chances of false-negative results due to sampling errors.

Immunosuppression regimen for kidney transplant recipients with COVID-19

The mainstay of treatment consisted of reduction of immunosuppression therapy based on disease severity and a case-by-case evaluation. In patients who were asymptomatic or had mild disease, mycophenolate/azathioprine doses were reduced/-stopped and no change was made in steroids and calcineurin inhibitors. For patients with moderate or severe disease, mycophenolate/azathioprine were discontinued and calcineurin inhibitors were reduced or discontinued and steroids were increased. We have previously reported our treatment details.[20,21]

Institutional protocol for transplant surgery and COVID-19 treatment

COVID-19-specific treatment algorithms were created by a hospital-based multidisciplinary team, and treatment protocols were updated regularly with available evidence and resources. Patients in the intensive care unit (ICU) were managed by the same transplant teams, infectious disease consultants, and intensivists. However, there was no standard consensus on the treatment of COVID-19; therefore, treatments were applied according to guidelines for COVID-19 established by the National Organ and Tissue Transplant Organization (NOTTO), Government of India.[20] The investigational therapies used for patients who were in this study included favipiravir (in mild and moderate cases), remdesivir, COVID-19 convalescent plasma, tocilizumab, and intravenous immunoglobin (in moderate to severe cases).

We prepared donors and recipients for transplant surgery according to transplant-specific guidelines from NOTTO.[20] All transplant recipients and donors provided fully documented written informed consent. We ensured adequate availability of personal protective equipment and ensured that health care workers were properly trained regarding COVID-19 and "COVID free safe transplant pathways" (per NOTTO) to reduce the risk of transmission. We performed routine clinical and epidemiological screening for COVID-19 in donors, recipients, health care workers, and caretakers; routine laboratory screening with COVID-19 RT-PCR tests on nasopharyngeal and oropharyngeal swabs and chest computed tomography scans or chest radiography were performed 24 to 72 hours before surgery, for all living and deceased donors. We ensured social distancing and COVID-19 preventive measures before surgery for living donors, recipients, and health care workers. We used induction and other immunosuppressive drugs based on each recipient's immune risk stratification as practiced before COVID-19.

Statistical analyses

Statistical analyses were performed with the SPSS software (version 17.0). Continuous data are presented as median values and interquartile ranges (IQR); *t* tests were used to compare 2 groups. Categorical data were compared with the chi-square test or the Fisher exact test. $P < .05$ was accepted as statistically significant.

Results

Study population

The second infections of 13 KTR with COVID-19 were included from (1) the Muljibhai Patel Urological Hospital, Nadiad (n = 3); (2) the Institute of Kidney Diseases and Research Center and Dr. H. L. Trivedi Institute of Transplantation Sciences, Ahmedabad (n = 2); (3) the Postgraduate Institute of Medical Education and Research, Chandigarh (n = 2); (4) the Rabindranath Tagore International Institute of Cardiac Sciences, Kolkata (n = 2); (5) the Jaslok Hospital and Research Centre, Mumbai (n = 1); (6) the Indraprastha Apollo Hospitals, New Delhi (n = 1); (7) the Primus Hospital, New Delhi (n = 1); and (8) the King Edward Memorial Hospital, Mumbai (n = 1).

Demographics

We identified 13 KTR (10 living related donors and 3 deceased donors) with second COVID-19 infections in our analysis. The second infection rate was 0.9% (13/1350 KTR with first episode of COVID-19) in our study. Eleven patients had both (first and second) COVID-19 episodes after transplant; 2 KTR had their first COVID-19 episode before transplant while on dialysis and a second episode after transplant. The overall median age of our cohort was 46 years (IQR, 28-50 years); most of the patients were men (62%, n = 8). None of the recipients was obese, and all underwent ABO-compatible transplants without desensitization. We divided patients into subgroups by age, including 21 to 30 years (4 patients), 31 to 40 years (2 patients), 41 to 50 years (4 patients), 51 to 60 years (2 patients), and 61 to 70 years (1 patient). Patients had a median time interval from transplant to first episode of COVID-19 diagnosis of 9.2 months (IQR, 2.2-46 months). In detail, the time after transplant surgery was <12 months in 8 patients (62%), from 1 to 5 years after transplant in 3 patients (23%), and more than 5 years after transplant in 2 patients (15%). None of the KTR had received COVID-19 vaccination before or after transplant.

For living donors, recipients were close relatives (mother, 38.5%; sister, 15.4%; wife, 15.4%; husband, 7.7%); 23% of recipients had a graft from a deceased donor. Median human leukocyte antigen matching (HLA A, B, and DR) was 1.5 (IQR, 0-3). All donors who donated kidneys after March 2020 had a SARS-CoV-2-negative RT-PCR test at time of surgery. Baseline demographics, clinical symptoms, and laboratory results of COVID-19 second infections in KTR are summarized in Table 1. Clinical symptoms and laboratory results during first and second COVID-19 infections are summarized in Table 2.

Comorbidities

Comorbidities were present in 11 patients (84.6%) and included arterial hypertension (84.6%; n = 11), diabetes (23%; n = 3), allograft dysfunction (84.6%; n = 11 episodes), hypothyroid (15.4%; n = 2), heart disease (15.4%; n = 2), hepatitis C virus (7.7%, n = 1), and retransplant (15.4%; n = 2). Multiple comorbidities were present in 8 patients (61.5%), with hypertension and diabetes being the most common. Two patients (15.3%) were on an angiotensin-converting enzyme

Table 1. Baseline Demographics, Clinical Symptoms, and Laboratory Results of COVID-19 Second Infections in Kidney Transplant Recipients

Parameter	Outcome		*P*
	Survival (n = 7)	Death (n = 6)	
Age, median (IQR), y	47 (39-48)	30.5 (27.5-44.7)	.16
Sex	4 M; 3 F	4 M; 1 F	1
BMI, median (IQR)	23 (20.3-23.6)	24 (24.2-24.9)	1
Blood group, No.	1 O; 3 A; 3 B	3 O; 2 A; 1 B	.26
ESKD etiology, No.	2 CGN; 2 DM; 1 CKDu; 1 CIN; 1 ADPKD	2 HTN; 2 CGN; 1 CKDu; 1 IgA N	1
Dialysis vintage, median (IQR), y	6 (3-8.5)	7.5 (3.7-11.2)	.69
Comorbidities, No.	7 HTN; 3 DM; 2 CAD; 1 LVH; 1 HyT	4 HTN; 1 HyT; 1 HCV; 1 TB; 1 RT	1
ACEi/ARB, No. (%)	1 (14)	1 (16)	1
H/O pneumococcal vaccine, No. (%)	4 (57)	4 (66)	1
Donor relation, No.	3 spouse; 2 parent; 1 sibling; 1 DD	3 parent; 2 DD; 1 sibling	1
HLA match, median (IQR)	3 (2.5-3)	3 (1.5-3)	1
Induction, No.	5 ATG; 2 None	3 ATG; 1 G; 1 B; 1 None	1
Tacrolimus level, median (IQR), ng/mL	5.6 (5.1-7.8)	5.4 (4.6-6.6)	.59
Hospital stay for transplant, median (IQR), d	8 (7-8)	14.5 (9.5-19.5)	.09
Antirejection treatment before infection, No. (%)	3 (43)	4 (66)	1
COVID-19 timeline, median (IQR), d			
Duration from first to second episode	102 (61-156)	103 (63-239)	1
RT-PCR positive to negative	17 (9-20)	19.5 (11.2-24.7)	.36
Ct value in RT-PCR, median (IQR)	25 (23.5-28.5)	22 (18-24)	.47
Cumulative symptoms combined in 2 episodes, No. (%)			
Fever	6 (42)	9 (75)	.13
Dry cough	8 (57)	9 (75)	.4
Breathing difficulty	3 (21)	8 (66)	.04[a]
Diarrhea	2 (14)	2 (16)	1
Anosmia	3 (21)	1 (8)	.59
Ageusia	4 (28)	0 (0)	.1
Headache	3 (21)	3 (25)	1
Myalgia	3 (21)	4 (33)	.6
Fatigue	5 (35)	5 (41)	1
Laboratory parameters, median (IQR)			
Peak LDH, IU/L	467 (287-680)	500 (352-521)	.67
Peak CRP, mg/dL	25 (8-64)	55.5 (14.6-101)	.11
Peak D-dimer, ng/mL	860 (187-1800)	1270 (718-1937)	.5
Peak ferritin, ng/mL	602 (168-1000)	550 (170-850)	.84
Peak IL-6, pg/mL	19.16*	47 (16-82)	NA
Peak NLR,%	5.2 (3.58-6.5)	12.3 (8-16.4)	.04[a]
Peak RDW,%	17 (16-18.6)	16 (15-18)	.19
Nadir lymphocyte count, $\times 10^3$ mm^3	1150 (500-2000)	300 (230-1500)	.9
Nadir platelet count, $\times 10^3$ mm^3	257 (111-315)	213 (165-230)	.18
Hemoglobin, g/dL	10.2 (12.5-14.9)	10.7 (9.9-11.4)	1
Baseline SCr before COVID-19, mg/dL	0.9 (1.25-2.6)	1.4 (1.2-2.15)	.015[a]
SCr before treatment, mg/dL	1.58 (1.2-1.8)	2.8 (2.17-3.9)	.001[a]
SCr after treatment, mg/dL	1.3 (0.9-2)	2 (1.9-3.17)	.002[a]
COVID-19 severity combined in 2 episodes, No. (%)			
Asymptomatic	3 (21)	1 (8)	.59
Mild	7 (50)	3 (26)	.24
Moderate	4 (29)	2 (16)	.65
Severe	0 (0)	6 (50)	.004[a]
Radiological abnormalities	2 (14)	6 (50)	.08
ICU/ventilator requirement	0 (0)	6 (50)	.004[a]

Abbreviations: ACEi/ARB, angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker; ADPKD, autosomal dominant polycystic kidney disease; ATG, antithymocyte globulin; B, basiliximab; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAD, coronary artery disease; CGN, chronic glomerulonephritis; CIN, chronic interstitial nephritis; CKDu, chronic kidney disease of unknown etiology; DD, deceased donor; DM, diabetes; ESKD, end-stage kidney disease; F, female; G, Grafalon; GN, glomerulonephritis; HCV, hepatitis C virus; HLA, human leukocyte antigen (A, B, DR match); H/O, history of; HTN, hypertension; HyT, hypothyroid; ICU, intensive care unit; IgA N, IgA nephropathy; IQR, interquartile range; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; M, male; NA, not available; NLR, neutrophil-to-lymphocyte ratio; RDW, red cell distribution width; RT, retransplant; TB, tuberculosis

*There were only 3 values for peak IL-6, so IQR was not possible. [a]$P < .05$, statistically significant.

inhibitor or an angiotensin receptor blocker at the time of COVID-19 diagnosis, and 8 patients (61.5%) had received pneumococcal vaccine before transplant. No KTR had received COVID-19 vaccine before the first or second COVID-19 infection.

Immunosuppression regimens

Induction regimen consisted of antithymocyte globulin (median dose of 3 mg/kg; IQR, 1.5-3 mg/kg; Sanofi-Aventis) for 8 patients (61.5%), basiliximab (Simulect, Novartis) for 1 patient (7.7%), and Grafalon

(6 mg/kg rabbit anti-human T-lymphocyte globulin [previously marketed as ATG-Fresenius], Neovii) for 1 patient (7.7%); 3 patients (23%) did not receive an induction treatment. The most common maintenance immunosuppression regimen included a triple regimen consisting of prednisolone, tacrolimus, and mycophenolate. Forty-six percent of patients (n = 6) had a history of rejection treatments, including steroid pulse (n = 6), antithymocyte globulin (n = 3), and rituximab (n = 1).

Table 2. Clinical Symptoms and Laboratory Results During First and Second COVID-19 Episodes

	First Episode (N = 13)	Second Episode (N = 13)	*P*
Duration from first to second episode, median (IQR), d	NA	102 (61-162)	
Ct value in RT-PCR, median (IQR)	24 (24-26)	24 (18-26)	1
RT-PCR positive to negative, median (IQR), d	20 (15-27)	9 (8-13.5)	.01[a]
RT-PCR positive to death, median (IQR), d	NA	10.5 (8.5-13.2)	
Antirejection treatment before infection, No. (%)	4 (30)	6 (46)	.68
Clinical symptoms, No. (%)			
Fever	11 (84)	4 (30)	.01[a]
Dry cough	9 (69)	8 (61)	1
Breathing difficulty	5 (38)	6 (46)	1
Diarrhea	2 (15)	2 (15)	1
Anosmia	2 (15)	2 (15)	1
Ageusia	2 (15)	2 (15)	1
Headache	5 (38)	1 (8)	.16
Myalgia	5 (38)	2 (15)	.37
Fatigue	5 (38)	5 (38)	1
Graft dysfunction	3 (23)	3 (23)	1
COVID-19 severity, No. (%)			
Asymptomatic	3 (23)	1 (8)	.59
Mild	4 (31)	6 (46)	.68
Moderate	6 (46)	0 (0)	.01[a]
Severe	0 (0)	6 (46)	.01[a]
IPD/OPD, No. (%)	3/10 (23/77)	4/9 (30/70)	.9
Radiological abnormalities, No. (%)	10 (77)	7 (54)	.4
Laboratory parameters, median (IQR)			
Peak LDH, IU/L	432 (311-544)	512 (392-647)	.38
Peak CRP, mg/L	48 (14-66)	44 (13-101)	.82
Peak D-dimer, ng/mL	636 (270-1400)	1589 (830-1937)	.036[a]
Peak ferritin, ng/mL	188 (135-589)	924 (436-999)	.07
Peak IL-6, pg/mL	17 (12-208)	22 (20-23)	.93
Peak NLR, %	5.5 (3.9-10.2)	9.1 (6.4-26.4)	.57
Peak RDW, %	16.2 (15.7-17.7)	16.2 (15.7-18.6)	1
Nadir lymphocyte counts, $\times 10^3$ mm^3	1000 (337-1416)	500 (294-1300)	.244
Nadir platelet counts, $\times 10^3$ mm^3	191 (100-213)	242 (218-315)	.14
Hemoglobin, g/dL	11.4 (10.2-13.7)	10.5 (10-13.9)	.6
Baseline SCr before COVID-19, mg/dL	1.08 (0.8-1.35)	1.46 (1.15-1.72)	.04[a]
SCr before treatment, mg/dL	1.56 (1.4-3.4)	.72 (1.46-2.55)	.78
SCr after treatment, mg/dL	1.7 (1.18-2.05)	1.4 (0.97-3.3)	.59

Abbreviations: CRP, C-reactive protein; Ct, cycle threshold; IL-6, interleukin 6; IPD, inpatient department; LDH, lactate dehydrogenase; Mod, moderate; NA, not available; NLR, neutrophil-to-lymphocyte ratio; OPD, outpatient department; RT-PCR, real-time reverse transcriptase polymerase chain reaction; RDW, red cell distribution width; SCr, serum creatinine

[a]$P < .05$, statistically significant.

Clinical presentation

Table 2 shows clinical symptoms and laboratory parameters for the first and second COVID-19 episodes. The most common symptoms included fever (84%), cough (69%), and dyspnea (38%) during the first episode of COVID-19. Fever was significantly less common with the second episode of COVID-19 (84% vs 30% in the first vs second episode; $P = .01$). Clinical severity during the first episode of COVID-19 ranged from asymptomatic in 3 patients (23%), mild in 4 patients (31%), and moderate in 6 patients (46%); during the second episode, 1 patient was asymptomatic (8%), 6 patients had mild COVID-19 symptoms (46%), and 6 patients had severe symptoms (46%).

Outcomes

The median cycle threshold (Ct) value for the SARS-CoV-2 RT-PCR tests was 24 (IQR, 24-25) in the first episode and 24 (IQR, 20-27) in the second episode. The median time interval between the first episode and the second episode based on COVID-19-positive RT-PCR tests was 135 days (IQR, 71-274 days) without symptoms. The median time interval from RT-PCR-confirmed COVID-19-positive results to negative results for surviving patients during the first episode was 20 days (IQR, 15-25 days) and for the second episode was 9 days (IQR, 8-13.5 days). There were 7 patients (54%) who survived, but 6 patients (46%) with severe disease died. One patient lost his graft function, and 1 patient died while on dialysis for acute kidney injury. Median time from RT-PCR-confirmed COVID-19-positive test results to death was 10 days (IQR, 8-21 days). The median follow-up duration was 64 days (IQR, 47-133 days) with reoccurring COVID-19 infection in KTR who survived. Overall patient mortality was 46% (6/13) but was 100% (6/6) for patients who required ICU treatment and mechanical ventilation. In 6 KTR with asymptomatic, mild, or moderate symptoms during the first COVID-19 episode, disease severity advanced to death during reinfection. In 7 of the 13 KTR, the second COVID-19 infection was severe. Statistically significant risk factors for mortality in the second episode were dyspnea ($P = .04$), disease severity ($P = .004$), allograft dysfunction ($P < .015$), higher levels of neutrophil-to-lymphocyte ratio ($P = .05$), and ICU/ventilator requirement ($P = .004$).

Discussion

We present 13 KTR with 2 distinct episodes of SARS-CoV-2 infections, separated by a median of 135 days (IQR, 71-274 days) of clinical quiescence and 2 negative RT-PCR test results for SARS-CoV-2 infection between the first and the second episodes. Our results showed that the second episode of COVID-19 was more severe. Our careful clinical assessment indicated a second, new COVID-19 infection. The published literature suggests that reactivation of an RNA virus as a consequence of immunosuppression is unlikely. Therefore, such a case is most likely a reinfection, but unfortunately our resources were restricted and we were unable to apply molecular testing and typing; we were also not able to provide binding antibodies to mitigate convalescence. Hence, alternative scenarios may explain the outcomes, such as (1) a false-positive initial SARS-CoV-2 RT-PCR test, (2) a false-negative RT-PCR result at discharge subsequent to the primary infection, (3) intermittent/prolonged viral shedding, known to occur among chronic hemodialysis and transplant patients, (4) nosocomial transmission of infection, (5) false-positive RT-PCR retests, and (6) reactivation/reinfection. Most of our KTR in the first COVID-19 episode were symptomatic (77%), and RT-PCR tests with low Ct values were consistent with active primary infection, which ruled out the possibility of an initial false-positive test. Two negative nasopharyngeal RT-PCR results before the second COVID-19 infection with clinical convalescence ruled out the possibility of a previous false-negative RT-PCR result at discharge. Moreover, we observed increased disease severity and higher mortality during the second episode; therefore, the possibility of prolonged viral shedding was unlikely.

Notably, reoccurring COVID-19 infections in KTR demonstrated an increased disease severity with an augmented oxygen requirement. A history of COVID-19 exposure, increased inflammatory markers, abnormal chest imaging, and the subsequent low-Ct RT-PCR tests were all consistent with active infection; therefore, residual effects from the first infection were unlikely. In this scenario, a false-positive RT-PCR test result for SARS-CoV-2 during the reoccurring episode is unlikely. Positive follow-up RT-PCR test results may derive from remnant virus material transferred from the lower respiratory tract to the throat and nose with coughing. In COVID-19-positive RT-PCR retests, before reinfection/reactivation is suggested, it is necessary to consider (1) the possibility of prolonged viral shedding during the convalescence period, (2) the methods of specimen collection, and (3) possible sampling/technical errors associated with each component of the swab testing procedure, including details related to potential operator technical error, the method for discharging patients, and possibility of infection by other variants of SARS-CoV-2. Thus, with a careful clinical assessment, characteristic symptoms, a RT-PCR-confirmed COVID-19-positive test after a long period of clinical remission, and 2 negative tests of nasopharyngeal swabs in 13 KTR cases, it is reasonable to conclude that the second episode is the result of a SARS-CoV-2 reinfection that is different from the original infection.

The median duration of the first episode after kidney transplant was 9.2 months, which suggested that KTR patients were vulnerable in the early period after transplant. On the other hand, the KTR patients with COVID-19 second infections who were not admitted to a transplant center may be overlooked because such patients are more often admitted to transplant centers in the early period after transplant rather than at later periods.

Notably, second episodes of COVID-19 occurred in 8 patients (62%) during the first year of transplant. Thus, a higher immunosuppressive load including lymphocyte-depleting and antibody-depleting therapy during the first year after transplant may be associated with a higher risk of a second COVID-19 episode. Certainly, more detailed studies are required to document whether COVID-19 reoccurrence is associated with certain immunosuppression doses. Whether we should use immunosuppression with lower doses after COVID-19 recovery and shorter duration of the immunosuppression regimen remains an open question. With the exception of fever, no other clinical symptom distinguished the first episode from a second episode. The absence of fever might indicate a more immunocompromised state induced by the previous episode of COVID-19 infection. Mild to moderate disease courses might predict future severity of COVID-19 episodes. This association rules out the possibility of virus persistence after discharge.

The immune response of transplant patients to COVID-19 compared with the response of the general population is expected to be different, and hence the

incidence of reoccurring infection is expected to be higher, although data are still evolving.[7] Moreover, evidence that antibody response to COVID-19 vaccine in transplant patients is much lower compared with the general population has been recently published.[22-24]

Recent studies have reported that some patients have tested positive for COVID−19 by RT−PCR days or even weeks after disease recovery, even after previous negative results.[25,26] A COVID-19-positive RT−PCR result does not prove SARS-CoV-2 viability with certainty, even if genome sequencing is performed.[27] In fact, RT−PCR is not able to differentiate infectious virus from noninfectious RNA.[28] Li and colleagues reported that 36 of 378 patients had COVID-19 RNA shedding longer than 30 days.[29] However, in our study, COVID−19-positive tests combined with recurrent clinical symptoms and death in 46% of patients (6/13) suggest a viable infection is in play. For example, the virus may persist in a latent state in the lysogenic stage (viral reproduction), inactive or hidden in cells, without causing disease symptoms for a substantial period of time and then reactivate to cause the second episode.[9-18] A recent study reported COVID-19 reinfections in 2 liver transplant recipients in Africa and the Middle East, 96 and 55 days after the primary infection, respectively, without patient death or graft loss.[8] Another study reported a case of a liver transplant recipient with 2 distinct episodes of SARS-CoV-2 infections, separated by 111 days without symptoms and 2 negative test results for SARS-CoV-2 infection.[5] Our recent study reported the first case of a KTR with some confirmatory features of a lethal COVID-19 reinfection.[9]

In our previous study on COVID-19 in 250 KTR from India, mortality was 14.5% in hospitalized patients.[21] In the first episode in our present study, none of the patients had severe disease. However, in the second episode, 46% of patients developed severe COVID-19 that led to death, similar to findings of a recent study from Ecuador.[30] An increase in disease severity and mortality during the second episode could be related to a different strain of the SARS-CoV-2. The SARS-CoV-2 B.1.617.2 (Delta) variant, designated as a Variant of Concern by the World Health Organization, has been described as the main variant in some parts of India during the second wave and may demonstrate resistance to previously formed antibodies.[31,32] The Indian SARS-CoV-2 Genomics Consortium has so far processed more than 13 000 samples for genome sequencing. The RT-PCR tests in use in India do not miss the UK (Alpha, B.1.1.7), South Africa (Beta, B.1.351), Brazil (Gamma, P.1), and so-called double mutation India (Delta, B.1.617.2) variants, because the tests in use in India do target more than 2 genes. Sensitivity and specificity of the present RT-PCR tests remain as rigorous as earlier tests.[33] Other possible causes of severe disease in the second COVID-19 episode are residual lung function abnormalities due to previous SARS-CoV-2 infection, as well as host susceptibility with recent use of antirejection treatment. Surveillance of pulmonary testing can be done after discharge to detect patients who are at higher risk for an adverse outcome in case of reinfection.

Implications for further work

We have witnessed an interesting panorama of COVID-19 before vaccinations were fully implemented in India, which has the second largest population in the world after China. There is a need for caution for KTR in future surges of COVID-19 in India, as the mortality rate in the second infection was high (46%). Our study suggests that a second episode of SARS-CoV-2 with a high morbidity and mortality can occur in KTR. Thus, previous exposure to SARS-CoV-2 might not result in a sufficient immunity in all KTR. This observation may be of relevance not only for vaccine development and application but all also for immunosuppression in transplant recipients recovering from COVID-19.[34] All KTR, regardless of previous infection, must take identical precautions to prevent infection with SARS-CoV-2. The new SARS-CoV-2 B.1.617.2 (Delta) variant is associated with immune-escape properties, which may render the pathogen partially or fully resistant to the body's immune response and antibody therapies.

Limitations

The lack of the molecular confirmation is the primary limitation to our study, as it has been stated that solid-organ transplant recipients can maintain positivity for months and in an intermittent fashion.[12,14,16] In some of the patients, the interval between infections was as short as 37 to 44 days. Thus, these details raise some concern of whether these are examples of true reinfection or are simply cases of positive test results attributable to the previous (first) infection. However, we suggest that

these infections are second episodes of COVID-19. After the first episode, frequent RT-PCR tests were performed, but we did not follow the patients after a subsequent COVID-19-negative RT-PCR test; therefore, we could not exclude the possibility that a new (second) episode was a true reinfection or simply the result of intermittent persistence of the original (first) infection episode. There was no dedicated database to include and follow up all KTR with COVID-19 after the first infection, and we chose only those recipients who tested positive for COVID-19 at a certain point in time. Patient populations are not homogeneous. In fact, 2 recipients did develop the first episode of COVID-9 infection before transplant.

Conclusions

We report results from 13 KTR with a mortality rate of 46% subsequent to reoccurrence of SARS-CoV-2 infection. Our results showed that recurrent episodes of COVID-19 were symptomatically more severe than the first episode. These cases stress that caution should be exercised during follow-up of patients previously infected with COVID-19, especially in vulnerable KTR, even after they appear to have overcome the first infection. Physicians and patients should be apprised of the possibility of reoccurring SARS-CoV-2 infections in KTR. Close monitoring on an outpatient basis is crucial. Further research into the possibility of reoccurring SARS-CoV-2 infection in KTR is required. Moreover, research from different transplant centers worldwide will provide more evidence to understand the true burden of reoccurring SARS-CoV-2 infections in the KTR setting and the clinical spectrum and outcomes of these cases.

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Desensitization Regimen Consisting of High-Dose Intravenous Immunoglobulin, Plasmapheresis, and Rituximab (an Anti-CD20 Antibody), Without Eculizumab and/or Bortezomib, in 41 Highly Sensitized Kidney Transplant Recipients

Hideki Ishida, Kohei Unagami, Kazuya Omoto, Taichi Kanzawa, Kazunari Tanabe

Abstract

Objectives: **Antibody-mediated rejection in patients with positive crossmatches can be severe and result in sudden onset of oliguria, leading to graft loss. In an attempt to prevent posttransplant oliguria, we adopted a preoperative desensitization protocol involving the use of high-dose intravenous immunoglobulin/plasmapheresis and the anti-CD20 antibody, rituximab, in 41 transplant recipients with positive crossmatch test results.**

Materials and Methods: **We retrospectively examined the clinical courses of the 41 kidney transplant recipients, paying special attention to renal graft function, urine volume, and changes in the titers of donor-specific antibodies.**

Results: **Four grafts were lost during an average of 4.5-year follow-up. Average graft function was excellent, with a serum creatinine level of 1.3 ± 0.4 mg/dL. Sufficient urine output, with no oliguria or anuria, was achieved postoperatively in 40 of the 41 patients. However, among the 34 patients who underwent graft biopsies, the biopsies revealed acute antibody-mediated rejection in 21 patients (62%), and chronic antibody-mediated rejection in 10 patients (30%).**

Conclusions: **The high-dose intravenous immunoglobulin treatment included in our desensitization protocol was shown to be safe and effective for achieving successful transplant outcomes and allowed the avoidance of more aggressive B-cell-targeted treatments, such as C5 inhibitors and/or proteosome inhibitors, for preventing posttransplant oliguria and anuria.**

Key words: *Antibody-mediated rejection, Anuria, Graft dysfunction, Human leukocyte antigen, Oliguria, Plasma exchange, Positive crossmatch tests*

Introduction

Sensitization to human leukocyte antigens (HLA antigens) is one of the most important hurdles to overcome for successful kidney transplant.[1] Severe antibody-mediated rejection (AMR) caused by donor-specific antibodies (DSAs) in sensitized recipients is characterized by acute onset of insufficient urine output, oliguria or anuria, and renal dysfunction in the posttransplant period. Without suitable treatment for this critical situation, most cases eventually result in graft loss after a rapid rise in the titers of DSAs and formation of fibrin thrombi and cortical necrosis.[2-6] To overcome the critical posttransplant situation of oliguria/anuria, a John Hopkins' team reported splenectomy and/or eculizumab administration as effective salvage therapies in addition to preoperative desensitization induction with low-dose intravenous immunoglobulin (L-IVIg)/plasmapheresis and rituximab.[7-9]

In practical clinical settings, it is often difficult to reliably assess the underlying critical condition in the presence of insufficient urine output and to provide appropriate treatment to recipients because these patients may have already received various kinds of desensitization treatments (like plasmapheresis) before transplant. Fluid imbalance in recipients after desensitization may make it difficult to precisely

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assess the water balance before administration of any salvage treatment.[10-12] Thus, suitable therapies to prevent the development of oliguria/anuria in the posttransplant period are desirable.

Since 2011, we have used a desensitization protocol consisting of high-dose IVIg (H-IVIg)/plasmapheresis and high-dose rituximab for kidney transplant recipients with positive crossmatch test results.[13] In this retrospective study, we examined the clinical courses of 41 highly sensitized recipients who received desensitization according to this latest protocol, with special attention paid to the onset of oliguria/anuria and renal graft function.

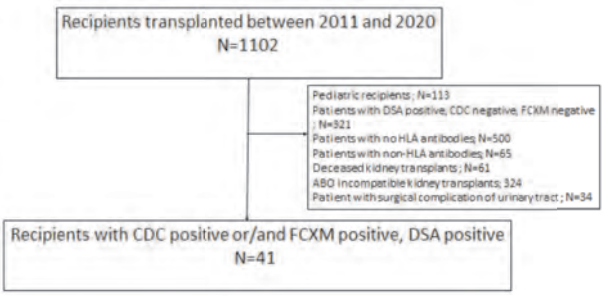
Materials and Methods

Data were extracted from the Japan Academic Consortium of Kidney Transplantation (UMIN Clinical Trials Registry number UMIN 000018327). This study was approved by the ethical committee at the Tokyo Women's Medical University (approval number 3366R) and was consistent with the 2000 Declaration of Helsinki as well as the 2008 Declaration of Istanbul. All patients provided consent for use of their anonymized data before registration in the Japan Academic Consortium. Details that could disclose the identity of the study patients were omitted.

Patients

Figure 1 presents a flowchart showing patient exclusion and inclusion criteria. Between 2011 and 2020, we performed a total of 1102 kidney transplant procedures at the Tokyo Women's Medical University Hospital. Patients with only DSAs (n = 321) detected by Luminex were not enrolled in this study. In addition, pediatric recipients (n = 113), recipients with no HLA antibodies (n = 500), ABO-incompatible kidney transplants (n = 324), deceased donor kidney transplants (n = 61), and recipients with non-HLA antibodies (n = 65) were excluded from this study. Thirty-four recipients with surgical complications of the urinary tract followed by reoperation or insertion of double J stents were also excluded because these complication would preclude accurate assessment of urine output. There were 41 remaining patients with positive crossmatch tests (positive with either the flow cytometric crossmatch [FCXM] test or the complement-dependent crossmatch [CDCXM] test). A 100% positive result in the CDCXM test is considered as a contraindication for kidney transplant at our institution, similar to protocols in other institutions around the world.

Figure 1. Patient Flowchart



Abbreviations: CDC, complement-dependent crossmatch; DSA, donor-specific antibody; FCXM, flow cytometric crossmatch

Between 2011 and 2020, of 1102 kidney transplant procedures performed at the Tokyo Women's Medical University Hospital, we excluded patients with only DSAs detected by Luminex, pediatric recipients, recipients with no HLA antibodies, ABO-incompatible kidney transplants, deceased donor kidney transplants, recipients with non-HLA antibodies, and recipients with surgical complications of the urinary tract followed by reoperation or insertion of double J stents. Thus, the remaining 41 kidney transplant recipients with positive crossmatch tests were included in this study.

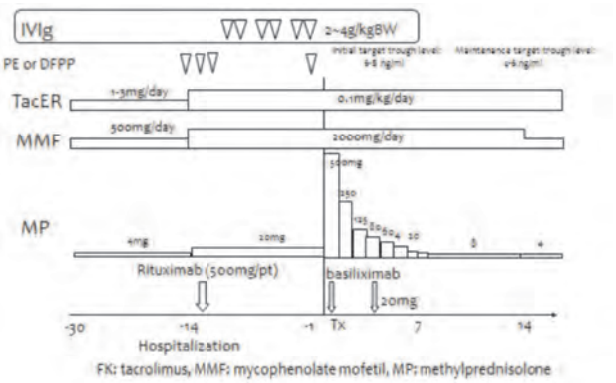
Immunosuppressive regimen

Figure 2 shows the latest immunosuppressive regimen used at our center; this regimen was established in 2011 and includes use of H-IVIg/plasmapheresis and rituximab. In brief, triple immunosuppression with tacrolimus, mycophenolate mofetil, and methyl-prednisolone was started 1 month before transplant. Figure 2 also shows the preoperative doses of tacrolimus, mycophenolate mofetil, and methyl-prednisone. After transplant, the dose of tacrolimus was adjusted according to target trough levels,[14] as shown in Figure 2.

High-dose intravenous immunoglobulin was administered to the highly sensitized recipients in 4 or 5 divided doses, because H-IVIg has too much fluid and is too viscous to administer to patients undergoing maintenance hemodialysis. The total IVIg dose is 2 g/kg/body weight (body wt) for patients with a positive FCXM test and 4 g/kg/body wt for patients with a positive FCXM and positive CDCXM test. Headache, which can be observed as an adverse event during intravenous infusion of IVIg, can be resolved by lowering the speed of infusion. The number of double-filtration plasmapheresis (DFPP) sessions for these patients is normally 3 or 4; however, this number could vary depending on the antibody titers. Plasmapheresis is performed on alternate days rather than every day, from the point of view of the

efficacy of antibody removal.[10,12] If crossmatch test positivity persists until immediately before transplant, the last plasmapheresis session is changed from DFPP to whole plasma exchange (PEX). The exchange volume at the time of PEX is calculated according to a previously described formula.[10] Rituximab is given to all highly sensitized recipients at a total dose of 500 mg/body, provided on 2 separate days at 200 mg and 300 mg.

Figure 2. Immunosuppressive Regimens for Patients With Strong Sensitization at Tokyo Women's Medical University Hospital Between 2011 and 2020



Abbreviations: BW, body weight; DFPP, double-filtration plasmapheresis; CDCXM, complement-dependent cytotoxicity crossmatch; F, female; FCXM, flow cytometric crossmatch; FK, tacrolimus; IVIg, intravenous immunoglobulin; M, male; MMF, mycophenolate mofetil; MP, methylprednisolone; PE, plasma exchange; TacER, tacrolimus; Tx, transplant

The immunosuppressive regimen, established in 2011, includes use of high-dose IVIg/plasmapheresis and rituximab. Triple immunosuppression with tacrolimus, MMF, and methylprednisolone was started 1 month before transplant. After transplant, tacrolimus dose was adjusted according to target trough levels.

Postoperative patient management after transplant surgery

The protocol for postoperative management after transplant surgery is as follows: adjust infusion speed of saline every 2 hours according to urine output over the previous 2 hours in the intensive care unit and then administer the same amount of saline as the urine output in the previous 2 hours by intravenous infusion over the next 2 hours. However, maximum infusion speed should be set at 200 mL/hour to prevent pulmonary edema, and the minimum speed should be set at 50 mL/hour. If there are no problems on posttransplant day 1, the patient is moved from the intensive care unit to the general ward. After the move to the general ward, the amount of fluid administered by intravenous infusion is normally 2000 to 2500 mL/day, followed by tapering to 500 mL/day and/or oral intake of water and foods within 4 days after transplant. On posttransplant day 2, the recipient is required to stand up at bedside to check body weight. In sensitized recipients, body weight is often higher due to insufficient urine output compared with dry weight at the time of the previous dialysis. This overweight state is controlled by the administration of diuretics, with dose adjusted so as to lower the body weight to the previously measured dry weight maintained during the period of hemodialysis. Doppler ultrasonography is performed at the bedside daily by a nephrologist to confirm the renal blood flow. A double J stent is not routinely inserted into the site of the bladder anastomosis. On posttransplant day 4, the balloon urethra catheter is removed, except for patients who have small bladder capacity of less than 50 mL.

Complement-dependent cytotoxicity test, flow cytometric crossmatch test, and solid-phase assay using Luminex single antigen beads

The crossmatch test using FCXM is superior to conventional CDCXM in terms of its sensitivity for detecting DSAs. Since 1983, when this finding was first reported, we have conducted crossmatch examinations using both methods.[15] For the CDCXM test, the recipient's serum potentially containing anti-HLA DSAs is added to the donor lymphocytes, along with complement. The proportion of lysed cells is assessed, and the crossmatch is graded as being mildly, moderately, or strongly positive.

For the FCXM test, donor lymphocytes are added to the recipient's serum, followed by incubation for 30 minutes at room temperature. After 2 washes, phycoerythrin-labeled CD19 (Pharmingen) and cytochrome-labeled CD3 (Pharmingen) are added, and the reaction is allowed to occur for 30 minutes at 4 °C. After 3 washes, fluorescein isothiocyanate-labeled anti-human immunoglobulin G antibody (Pharmingen) is added as the secondary antibody, and the cells are fixed in 2% formalin-phosphate buffered saline. The FCXM test is performed using a fluorescein-activated cell sorter (Becton Dickinson), and a positive FCXM is defined by a channel shift of >10.

For the solid-phase assay (Luminex single antigen bead assay), manufacturer's instructions are followed. The positive range at our institution is defined as a mean fluorescence intensity of over 800.

Graft biopsies

Patients undergo 2 or 3 biopsies with a 16-gauge needle. All recipients undergo posttransplant protocol biopsies (at 0 hour, at <6 months, and at >6 months), as well as episode biopsies. All biopsies are evaluated by light microscopy and immunofluorescence staining for C4d. In brief, the specimens are fixed with 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and cut into 2-μm sections. The sections are stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and periodic acid methenamine silver stains for light microscopy.

For immunohistochemistry, paraffin sections on glass slides coated with saline are stained with a peroxidase-labeled streptavidin-biotin staining kit (DAKO). The primary antibodies used are rabbit polyclonal antibodies against immunoglobulin G, immunoglobulin A, C3, and immunoglobulin M (Hoechst, Behringwerke). Pathological findings are then classified according to the Banff 2007 working classification and the Banff 2005 updated edition and comparatively evaluated among the recipients classified according to sensitization status.[16,17] Briefly, AMR was defined as (1) C4d and/or (rarely) immunoglobulin deposition in the peritubular capillaries, (2) serologic evidence of circulating antibodies to donor HLA antigens, and (3) morphologic evidence of acute tissue injuries. Chronic AMR was also defined using the Banff criteria. At our institution, a single pathologist made the diagnoses based on examination of the graft specimens.

Statistical analyses

Data are expressed as means ± SD. Statistical analysis was performed with SAS version 9.4 TS1M5 (SAS Institute). One-way analysis of variance was used to compare normally distributed continuous variables, and the Kruskal-Wallis H test was used to evaluate skewed or discrete ordinal variables. The chi-square test was used to compare nominal scale variables. A 2-tailed P value < .05 was considered statistically significant by the biostatistics datacenter (STATZ Institute, Tokyo Japan).[13]

Results

Patient and donor background and clinical course after transplant

As shown in Table 1, the total number of recipients enrolled in this study was 41, consisting of 19 men and 22 women. The immunological test status was CDCXM positive and FCXM positive in 7 recipients and CDCXM negative and FCXM positive in the remaining 34 recipients. The cause of sensitization was a history of kidney transplant or transplant of other organs (ie, heart and liver) in 21 recipients and sensitization by pregnancy, such as in a spousal pair (husband to wife), in 15 recipients. The remaining 5 recipients were sensitized by blood transfusion or the reason was indeterminate. The average dose of IVIg in these recipients was 2.5 ± 2.0 g/kg/body wt. The average number of DFPP in the posttransplant period was 4.5 ± 1.2. Table 2 shows the background of donors in this study.

Table 1. Background of Recipients Before Transplant

Characteristic	Finding
Number of patients (M/F)	41 (19/22)
Age	52 ± 13 years
Original disease	
Diabetes mellitus	23
Renal sclerosis	10
Glomerular disease	7
Polycystic kidney	1
Hemodialysis duration	4.3 ± 2.1 years
Living/deceased donor	41/0
Relationship to recipient	
Husband to wife	25
Children to parents	4
Father to mother	12
Number of HLA mismatches	3.4 ± 1.2
Immunological test status	
CDCXM positive, FCXM positive	7
CDCXM negative, FCXM positive	34
Cause of sensitization	
Transplant history	21
Pregnancy (eg, husband to wife/child to mother)	15
Blood transfusion, unknown	5
Average dose of rituximab	450 ± 200 mg
Average No. of plasmapheresis	4.5 ± 1.2 times
FK/MMF/MP/CD25 antibody	41
Average dose of IVIg	2.5 ± 2.0 g/kg

Abbreviations: CDCXM, complement-dependent cytotoxicity crossmatch; F, female; FCXM, flow cytometric crossmatch; FK, tacrolimus; IVIg, intravenous immunoglobulin; M, male; MMF, mycophenolate mofetil; MP, methylprednisolone

Table 2. Background of Donors Before Transplant

Characteristic	Finding
No. of donors (M/F)	41 (25/16)
Age	56 ± 9 years
Average BMI	24 ± 5
Kidney weight	160 ± 110 g
Average serum creatinine	0.73 ± 0.21 mg/dL
eGFR	70 ± 21 mL/min/1.73 m^2
Warm ischemia time	4.3 ± 4 min
Total ischemia time	60.1 ± 30.4 min
Immediate function/delayed graft function/primary nonfunction	40/1/0

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); eGFR, estimated glomerular filtration rate; F, female; M, male

As shown in Table 3, the follow-up period was 4.5 ± 3.5 years. Four of the grafts were lost (4/41, 10%) during this period. Cause of graft loss was suicide and chronic AMR in 1 recipient each and cardiovascular events in the remaining 2 recipients. Average serum creatinine level and eGFR were 1.3 ± 0.4 mg/dL and 49 ± 15 mL/min, respectively, except in the 4 patients with graft loss.

Table 3. Results of Recipients After Transplant

Characteristic	Finding
Number of patients (M/F)	41 (19/22)
Graft loss, No. (%)	4 (10%)
Suicide, No.	1
Cardiovascular event, No.	2 (pre- and posttransplant)
Chronic AMR, No.	1
Anuria, No.	0
Oliguria, No.	1
Serum creatinine (except for patient with graft loss)	1.3 ± 0.4 mg/dL
eGFR (except for patient with graft loss)	49 ± 15 mL/min
Pathology	
Acute AMR, No. (%)	21/34 (62%)
ATMR, No. (%)	14/34 (41%)
Chronic AMR, No. (%)	10/34 (30%)
IFTA, No. (%)	6/34 (18%)

Abbreviations: AMR, antibody-mediated rejection; ATMR, acute T-cell mediated rejection; eGFR, estimated glomerular filtration rate; F, female; IFTA, interstitial fibrosis and tubular atrophy; M, male

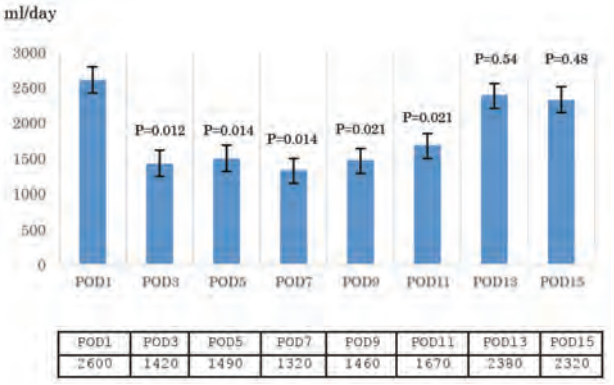
Urine output and peripheral blood platelet count in the 41 desensitized recipients in the early phase posttransplant

Figure 3 and Figure 4 show the average daily urine output and the average daily peripheral blood platelet count, respectively, in the 41 recipients of this study during the first 15 days posttransplant. All recipients showed a gradual decrease of urine output accompanied by a decrease in platelet count immediately posttransplant.

As shown in Figure 3, the maximum urine output was 2600 ± 1100 mL/day on posttransplant day 1, whereas the minimum urine output was 1320 ± 620 mL/day on day 7.0 ± 4.5 after transplant surgery. The urine outputs on posttransplant days 3, 5, 7, 9, and 11 were significantly less compared with the urine output on posttransplant day 1 ($P < .05$). As shown in Figure 4, the minimum platelet count of $9.5 \times 10^4 \pm 3.2 \times 10^4/mm^3$, on average, was observed 3.0 ± 2.1 days posttransplant. Platelet counts on posttransplant days 1, 3, 5, 7, and 9 were significantly lower than the count before transplant ($P < .05$). The changes in the peripheral blood platelet count and urine output ran almost parallel to each other; that is, the daily urine output decreased and increased in accordance with decrease and increase of the peripheral blood platelet count.

Of the 41 recipients, only 1 recipient showed oliguria with a urine output of less than 400 mL/day posttransplant. This patient was the only patient in this study who temporarily needed 2 sessions each of hemodialysis and extracorporeal ultrafiltration method to maintain body weight and to remove uremic toxins. Other than this patient, none of the patients needed hemodialysis or any other rescue treatments, including additional rituximab, plasmapheresis, or IVIg. None of the 41 recipients who received desensitization according to the latest protocol showed anuria with a urine output of less than 100 mL/day.

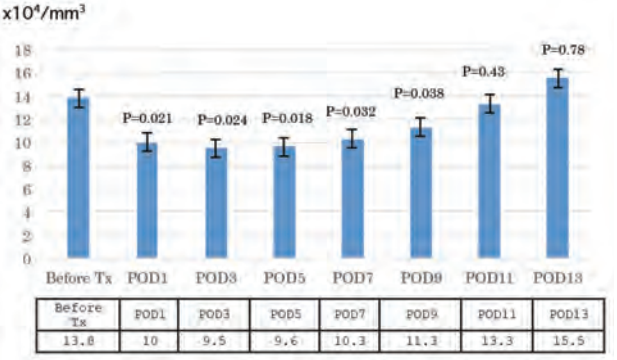
Figure 3. Urine Output in the 41 Highly Sensitized Recipients Treated With the Latest Protocol



Abbreviations: POD, postoperative day

On POD1, the maximum urine output was 2600 ± 1100 mL/day; on POD7 (± 4.7), output was 1320 ± 620 mL/day. The urine outputs on POD3, 5, 7, 9, and 11 were significantly less than on POD1 ($P < .05$).

Figure 4. Peripheral Blood Platelet Count in the 41 Highly Sensitized Recipients Treated With the Latest Protocol Before and After Transplant



Abbreviations: POD, postoperative day

Minimum platelet count was observed on POD3. Platelet counts on POD1, 3, 5, 7, and 9 were significantly lower than before transplant ($P < .05$). Changes in peripheral blood platelet count and urine output were almost parallel with each other (ie, daily urine output decreased and increased in accordance with decrease and increase of peripheral blood platelet count).

Titer changes of donor-specific antibodies after the desensitization protocol

Figure 5 shows the titer change of DSAs after the desensitization protocol and at last follow-up, compared with baseline titers, in 15 highly sensitized recipients. The monitoring period from before to after transplant was almost 4 weeks. Last follow-up monitoring refers to monitoring for DSAs at the latest outpatient visit. Among the 41 recipients, 15 recipients whose serum samples had been refrigerated and stored were investigated by Luminex assay. As shown in Figure 5, in general, class I antibodies (patients 1, 4, 5, 7, 9, 10) as well as DQ antibodies (patients 2, 3, 9, 11, 15), regardless of the titers, were responsive to this latest desensitization protocol. DR antibodies were not very responsive to the desensitization protocol in the early phase; however, low titers of DR antibodies (patients 9, 13, 14) responded to the desensitization regimen in the early phase, but high titers of DR antibodies (patient 8) proved quite resistant. Patient 3 had high titers of DR4 and its cross-reactive group DR53. The titers of these antibodies decreased transiently after desensitization, only to increase again, eventually resulting in chronic AMR. Patient 6 showed markedly high titers of DSAs (DR12), which were responsive to the sensitization regimen because this DR12 alone may be produced without the related cross-reactive group. Patient 6 has remained stable with very mild AMR; in patient 3, the graft was lost with the development of severe AMR.

Figure 5. Titer Changes of Donor-Specific Antibodies Before and After Desensitization Protocol

Case	DSA	Pre-desensitization (MFI)	Post-desensitization (MFI)	Last follow-up (MFI)	
1	B51, B52, DR51	11186/10808/2320	1692/2009/0	0/0/0	
2	DQ6	2095	0	1480	
3	DR4, DR53, DQ8	11445/20819/20094	0/1252/1942	3238/14847/0	Loss
4	A2	13558	3602	2030	
5	B35, DR12	10062/4414	2851/288	6345/623	
6	DR12	19437	4327	0	
7	A24	13517	1832	0	
8	DR15	9016	300	0	
9	B44, DR13, DQ5, DQ6	2371/4063/7169/3344	0/0/3880/5897	0/4068/0/0	
10	A24, B7, DR1	7745/8277/6821	0/0/1824	0/0/2654	
11	DQ6	15656	1622	0	
12	DR7	7972	0	0	
13	DR15	1710	0	0	
14	DR4	1246	0	0	
15	DQ4	8685	991	0	

Abbreviations: DSA, donor-specific antibody; MFI, mean fluorescence intensity

Acute rejection

As shown in Table 3, among the 41 recipients, 34 recipients underwent graft biopsies, which revealed acute AMR in 21 patients (62%), acute T-cell-mediated rejection in 14 patients (41%), chronic AMR in 10 patients (30%), and interstitial fibrosis-tubular atrophy in 6 patients (18%). Graft biopsies among the 34 recipients showed no evidence of rejection.

Discussion

Despite the numerous efforts made to increase the availability of organs from deceased donors by education and by improvements in public awareness, organ donation still remains modest globally. In Japan, more than 90% of kidney transplant procedures during the past 2 decades have been from living donors.[18] No donor exchange program has been developed yet in Japan, most likely because of ethical and religious barriers. In contrast, the number of repeat transplant candidates, who constitute an immunologically high-risk population, on deceased- and living-donor transplant wait lists have been increasing rapidly year by year because of the high level of panel reactive antibody assays.[18]

Immunologically high-risk recipients sometimes experience unusual clinical manifestations, such as oliguria/anuria,[2] low-grade fever, spiky systolic blood flow with low diastolic blood flow, indicative of high resistance of the peripheral renal blood flow, on Doppler ultrasonography, and graft dysfunction in the posttransplant period. Graft biopsies can reveal evidence of typical AMR, including peritubular capillaritis, glomerulitis, and deposition of C4d according to Banff criteria.[16,17] Although most of these immunologically high-risk recipients are likely to respond to the usual treatment of a short course of L-IVIg/plasmapheresis and low-dose rituximab (200 mg/body; anti-CD20 antibody) with recovery of sufficient urine volume, some recipients can have severe AMR accompanied by oliguria/anuria, which can lead to graft dysfunction or graft loss. Insufficient urine volume at the onset of oliguria/anuria immediately after kidney transplant makes it difficult to treat these patients because sensitized recipients often receive aggressive desensitization treatments prior to transplant, including high-dose globulin administration and many sessions of plasmapheresis. These courses of treatment often result in fluid imbalance inside versus outside the

blood vessels in the recipient's body, thus making it difficult to administer appropriate treatment in recipients with insufficient urine volume in the immediate posttransplant period.

For candidates of HLA-incompatible kidney transplant procedures, we adopted a desensitization protocol in 2005 consisting of plasmapheresis and low-dose rituximab (200 mg/body); this protocol is almost similar to the ABO-incompatible kidney transplant protocol that was already in place since 2000.[19-22] Unfortunately, a protocol that includes only plasmapheresis and low-dose rituximab can be ineffective in recipients with high titers of anti-HLA antibodies and strongly positive crossmatch tests, regardless of whether the FCXM or CDCXM test was used. Most of these recipients can show insufficient urine volume and graft dysfunction. Although rescue therapy of additional use of B-cell-targeted treatments could be used postoperatively, not all patients respond, resulting in graft loss.

To prevent oliguria/anuria and inhibit humoral immunity in the posttransplant period, in 2011, we adopted a protocol consisting of H-IVIg (2-4 g/kg), in addition to plasmapheresis and high-dose rituximab (500 mg/body), with increase of rituximab from 200 to 500 mg/body in line with other studies.[23-26]

In our retrospective study of 41 immunologically high-risk recipients who underwent this new desensitization protocol between 2011 and 2020 at a single center study, only 1 recipient (patient 3; see Table 3) developed oliguria posttransplant. He had three DSAs (DR4, DR53, DQ8), including a cross-reactive group antibody. The titers of DR4 and DR53 decreased after a series of desensitization treatments, only to increase again at the last follow-up, with eventual graft loss by 2 years posttransplant. The titer of DQ8 continued to be suppressed after the desensitization. Graft biopsy showed chronic severe active AMR, as well as mixed-type rejection. In contrast, the single appearance of class II DR12 in patient 6, of DR15 in patient 8, and of DR7 in patient 12 continued to be suppressed, even though the titers were quite high before the desensitization treatment. These class II antibodies may be produced alone without related production of a cross-reactive group antibody. These changes in DSAs according to the DSA titers and DSA class after desensitization are almost similar to those described in previous reports.[23-26]

Of note, we found that changes in urine output were almost parallel to those of the peripheral blood platelet count in the posttransplant period. The decreases in urine output and peripheral blood platelet count may be caused by immunological responses of AMR and/or a preoperative aggressive desensitization protocol for DSAs (plasmapheresis and PEX), although these changes were not observed in any ABO-incompatible transplants under the almost similar desensitization protocol. The difference in the clinical courses between HLA-incompatible and ABO-incompatible settings may be because of difference in physiological characteristics of each antibody, anti-HLA, and anti-blood type antibodies.[14] No rescue therapy for acute AMR was adopted for any of the patients in our study, except for patient 3, because of excellent and stable graft function accompanied by good blood flow to the graft shown on Doppler ultrasonography. Of 41 patients in our study, all but patient 3 showed stable urine output of at least 1000 mL/day without any additional treatment. Postoperative splenectomy and/or administration of the C5 inhibitor eculizumab has been reported as an effective treatment for posttransplant oliguria with a urine output of 400 mL/day or less to counter severe AMR.[1-3] However, although we have used these treatments as rescue for patients with severe AMR at our center, we believe that emergency operations, such as splenectomy, immediately after transplant[27] and also several administrations of eculizumab[28] and/or bortezomib[29] in severely desensitized recipients who have already received desensitization therapy may increase not only the surgical risk but also the infectious risk.[27-29] Thus, we believe the combination of H-IVIg/plasmapheresis and high-level rituximab may be an effective noninvasive treatment to prevent posttransplant oliguria/anuria.

Both H-IVIg and L-IVIg are used in the transplant setting to reduce sensitization and to treat steroid-resistant rejections. The effectiveness of IVIg has been reported mainly for high-dose H-IVIg given monthly.[30,31] In contrast, the effectiveness of lower doses of IVIg, such as 100 to 500 mg/kg, remains difficult to assess. The uncertainty about the numerous mechanisms involved in the control of humoral activities in the body definitely contributes to the uncertainty of how best IVIg should be used in the field of transplantation. In other fields, for example, in the neurological field, H-IVIg has become established over the past few decades as an important component, exerting dose-dependent effects in the management of autoimmune disease.[32,33]

Among the limitations of this study were its retrospective nature, lack of a comparator group, and the small sample size. Prospective multicenter comparative studies of various desensitized protocols used to avoid posttransplant oliguria/anuria are needed. Also, studies that include a longer follow-up duration for determining the graft survival rate are also needed because of the high incidence of AMR after transplant previously reported by our team in patients treated with H-IVIg.[10] Aubert and colleagues reported that AMR with preexisting DSA was associated with an acceptable and better allograft survival compared with AMR with de novo DSAs during a similar 4-year follow-up, supporting the transplant of highly sensitized patients and the early detection of AMR.[34] Although since December 2019 H-IVIg has been covered by the National Health Insurance in Japan, another limitation is its high cost. With the consideration of medical economics and the scarcity of medical resources like H-IVIg, H-IVIg should be limited to patients with a strongly positive crossmatch test, and not patients with only DSA but a negative crossmatch test, prior to transplant.

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Monocyte-to-High-Density Lipoprotein Cholesterol Ratio Is Independently Associated With All-Cause Mortality in Deceased Donor Kidney Transplant Recipients

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Abstract

Objectives: **The primary objective of this study was to evaluate the impact of monocyte-to-high-density lipoprotein cholesterol ratio on all-cause mortality in deceased donor kidney transplant recipients.**

Materials and Methods: **This was a retrospective observational study in which all deceased donor kidney transplant recipients were included. Relevant data for analyses included clinical and demographic features, laboratory values, number of HLA matches, occurrence of delayed graft function, cold ischemia time, and survival status. Kaplan-Meier survival analysis and Cox proportional hazards analysis were performed to determine the effects of monocyte-to-high-density lipoprotein cholesterol ratio on all-cause mortality.**

Results: **Our study included 325 deceased donor kidney transplant recipients (43.1% females, mean age of 44.5 ± 11.2 years). Median value of monocyte-to-high-density lipoprotein cholesterol ratio was 14.0 (interquartile range, 9.94-21.03). The total median observation time was 227 weeks (range, 115-345 weeks). Twenty deaths (12.3%) occurred during the follow-up period in recipients with monocyte-to-high-density lipoprotein cholesterol ratio below median value, whereas 47 deaths (29%) occurred in recipients with ratio above the median ($P < .001$). Log-rank test showed significantly higher mortality in the group with monocyte-to high density lipoprotein cholesterol ratio higher than median ($P = .001$). In the multivariate Cox model, delayed graft function, duration of dialysis, cold ischemia time, and monocyte-to-high-density lipoprotein cholesterol ratio group appeared as independent predictors of all-cause mortality.**

Conclusions: **Monocyte-to-high-density lipoprotein cholesterol ratio before kidney transplant seems to affect survival independently in deceased donor kidney transplant recipients.**

Key words: *Cardiovascular disease, Dyslipidemia, Renal transplant*

Introduction

Kidney transplantation provides far better survival for patients than hemodialysis or peritoneal dialysis. However, compared with the general population, survival rates are still unacceptably low despite recent amelioration of mortality rates. According to the United States Renal Data System 2019 report,[1] adjusted mortality after kidney transplant decreased by 41% from 2001 to 2017. Recipients of deceased donor kidney transplant (DDKT) have been shown to have significantly higher average mortality rates than recipients of living related donor kidney transplant.[2]

Cardiovascular disease is the main culprit of mortality in kidney transplant recipients.[3] A cardiovascular cause of death can be identified in up to 40% of DDKT recipients.[4] Although many cardiovascular risk factors can be corrected or ameliorated to some extent by kidney transplant, prevailing traditional and newly emerging cardiovascular risk factors related to transplant have prevented further decreases in cardiovascular risk to levels shown in the general population. The incidence of cardiovascular death has been reported to be 4 to 5 times greater than in the general population.[5] Traditional risk factors such as hypertension, diabetes mellitus, dyslipidemia, and left ventricular hypertrophy are prevalent in renal transplant recipients. When

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additional factors are added after kidney transplant, such as new onset diabetes, hypertension, and dyslipidemia, the burden of cardiovascular risk factors reaches new heights.

Traditionally, much of the academic and clinical attention has been directed to posttransplant graft function, which plays a secondary role in the management of cardiovascular disease risk factors. Death of a transplant patient with a functioning graft still constitutes the foremost cause of renal allograft loss.[6] Thus, cardiovascular disease also emerges as an important reason for graft loss. Considering the shortages of donated kidneys, determining and working to protect patients against cardiovascular risk factors can provide doubled benefits, enabling survival of kidney transplant recipients and functioning kidneys.

This central place of cardiovascular morbidity and mortality among kidney transplant recipients makes prognostication of cardiovascular risk ever more important. Discovery of novel risk factors that can predict patients at high risk of cardiovascular morbidity and mortality starting from the pretransplant period is of paramount importance.

Monocyte-to-high-density lipoprotein (HDL) cholesterol ratio (MHR) has emerged as an easily calculated and readily available novel inflammation marker.[7] Moreover, MHR has been shown to be able to independently predict mortality and major adverse cardiac events in various patient populations, including, but not limited to, chronic kidney disease, acute stroke, acute coronary syndrome, and coronary artery disease.[8-10]

Despite the undeniable impact of cardiovascular disease in survival of kidney transplant recipients, the predictive role of MHR has not yet been evaluated in these patients. Given that mortality rates are higher in DDKT recipients than in recipients of living related kidney donors, we aimed to evaluate the ability of MHR to predict all-cause mortality in DDKT recipients.

Materials and Methods

Setting, design, and patients

This was a retrospective observational study in which 325 patients who underwent DDKT between January 2006 and November 2018 at Uludag University School of Medicine Department of Urology, in Bursa, Turkey, were analyzed. The primary aim of the study was to evaluate the independent predictors of all-cause mortality among the DDKT patients, including MHR. The last visit was taken as December 2019 for evaluation of survival status of study participants. The study protocol was approved by the Uludag University Clinical Research Ethics Committee (2020-21/711.25.2020).

Transplant recipients who were below 18 years old and those who underwent second or more transplant procedures were excluded. We also did not include transplant recipients who had missing data and were lost to follow-up. Kidney transplant recipients who were transplanted from related or nonrelated living donors were also excluded.

Data collection

Relevant data were retrieved from a prospectively maintained transplant database of the transplant unit. We collected age, sex, body mass index, modality of and time in dialysis before transplant, comorbid conditions, type of primary kidney disease, the number of HLA mismatches, type of induction regimen, occurrence of delayed graft function, graft function at several time points posttransplant, graft rejection episodes, survival status, and level of the latest renal function. With regard to data of deceased donors, we obtained information on age, sex, body mass index, chronic medical conditions, smoking status, use of inotropic agents, and cause of brain death.

Monocyte-to-HDL cholesterol ratio was calculated as the peripheral blood monocyte count divided by blood HDL cholesterol concentration. For this calculation, the most recent values before DDKT were used.

Delayed graft function was defined as kidney dysfunction that required dialysis after the first week posttransplant.[11]

We assessed histocompatibility between the deceased donor and the potential kidney recipient by 6-antigen (HLA-A, B, and DR) tissue typing. From this, we calculated total matched allele numbers between the donor and the recipient.

Statistical analyses

To check the normality assumptions of the data, the Shapiro-Wilk test and Q-Q plots were used. Normally distributed variables are presented as means ± standard deviations, whereas nonnormally distributed variables are given as medians and

interquartile ranges (IQR). For comparisons between 2 groups, the independent sample *t* test and the Mann-Whitney U test were used according to the distribution of variables. Categorical variables are presented as numbers and percentages. The chi-square test and the Fisher exact test were used to compare the categorical variables between 2 groups.

We divided the study cohort according to the median value of MHR. Kaplan-Meier curves were constructed to illustrate differences in survival between patients with MHR below and above the median value. The log-rank test was used to compare survival times of the groups.

To determine independent predictors of all-cause mortality of the DDKT recipients, we conducted univariate and multivariate Cox proportional hazards analyses. We calculated hazard ratios, which are presented along with 95% confidence intervals. Variables with $P < .05$ in univariate analysis were included in the multivariate analysis. Clinical judgment was also used when selecting variables for the regression model. Finally, age, delayed graft function, duration of dialysis, cold ischemia time, and MHR groups (MHR above median vs MHR below median) were included in the multivariate model. We used the IBM SPSS version 25.0 software package to analyze study data. $P < .05$ was accepted as statistically significant.

Results

Baseline clinical and demographic features and laboratory values

Initially, we obtained data of 396 patients who had undergone DDKT at our institution. Seventy-one patients were excluded because they lacked MHR values or had been lost to follow-up. In total, 325 DDKT recipients (43.1% females; mean age of 44.5 ± 11.2 years; range, 18-75 years) were included in the study. The most common chronic medical condition among the recipients was hypertension (42.5%). Of all recipients, 68.9% underwent hemodialysis, whereas 15% received transplants while on peritoneal dialysis. The median duration of dialysis was 8 years (IQR, 5-11 y). The median number of HLA antigen matches was 2 (IQR, 2-3). The median value of MHR was 14.0 (IQR, 9.94-21.03). Baseline clinical and demographic features and laboratory values are shown in Table 1.

Table 1. Clinical and Demographic Characteristics and Laboratory Values of Transplant Recipients (N = 325)

	Result
Age, y	44.5 ± 11.2
Female/male, No. (%)	140 (43.1%)/185 (56.9%)
Body mass index, kg/m^2	24.3 (22.5-26.4)
Comorbidities (n = 320), No. (%)	
Coronary artery disease	24 (7.5%)
Diabetes mellitus	43 (13.4%)
Hypertension	136 (42.5%)
COPD	6 (1.9%)
Type of dialysis (n = 321), No. (%)	
Hemodialysis	221 (68.8%)
Peritoneal dialysis	48 (15.0%)
Both (alternately)	52 (16.2%)
Duration of dialysis (n = 314) (IQR), y	8 (5-11)
Laboratory parameter	
White blood cell count (range), 10^3/μL	7420 (6030-9295)
Monocyte count (range), 10^3/μL	0.533 (0.400-0.699)
Lymphocyte count (range), 10^3/μL	1.76 (1.27-2.26)
Neutrophil count (range), 10^3/μL	6.12 (4.34-3700)
Platelet count (range), 10^3/μL	191 (158.5-254)
HDL cholesterol (range), mg/dL	36 (29-45)
MHR (IQR)	14.0 (9.94-21.03)
CRP (range), mg/dL	1.02 (0.33-4.13)
Month 1 creatinine (range), mg/dL	1.1 (0.91-1.2)
Month 3 creatinine, mg/dL	1.12 ± 0.33
Month 24 creatinine (n = 229), mg/dL	1.0 ± 0.22
Month 120 creatinine (n = 13), mg/dL	1.0 ± 0.43
Transplant feature	
No. of HLA antigen matches (IQR)	2 (2-3)
DGF (n = 321), No. (%)	59 (18.4%)
Induction regimen (n = 284), No. (%)	
Antithymocyte globulin	10 (3.5%)
Basiliximab	255 (89.8%)
IVIG	1 (0.4%)
IVIG + antithymocyte globulin	1 (0.4%)
Basiliximab + IVIG	17 (6.0%)
Total rejection, No. (%)	16 (4.9%)
Total nephrectomy, No. (%)	19 (5.8%)
Cold ischemia time (range), h	14 (12-15)
Total follow-up time (range), weeks	227 (115-345)

Abbreviations: COPD, chronic obstructive pulmonary disease; Cr, creatinine; CRP, C-reactive protein; DGV, delayed graft function; IQR, interquartile range; IVIG, intravenous immunoglobulin; MHR, monocyte-to-high-density lipoprotein cholesterol ratio

Total follow-up time was based on time to death or time to last follow-up visit.

The mean age of deceased donors was 46.1 ± 16.5 years. Males comprised a majority of the donors (64.9%). One-fourth of all donors had hypertension. Clinical and demographic features and laboratory values of deceased donors are shown in Table 2.

Comparison of kidney transplant recipients with below versus above the median MHR value

Kidney transplant recipients with higher MHR were significantly older than patients with lower MHR (45.3 ± 10.5 vs 43.7 ± 11.9 y; $P = .007$). Distribution of males versus females also showed a significant difference in that there were more male recipients with MHR above the median value. There was no difference between the groups in terms of chronic comorbid conditions. Number of matched HLA

antigens and rates of delayed graft function were similar between groups. Table 3 summarizes the comparison of clinical and laboratory values between the groups.

Table 2. Demographic Features and Laboratory Values of Deceased Donors (N = 325)

	Result
Age, y	46.1 ± 16.5
Female/male (n = 322), No. (%)	111 (34.2%)/211 (64.9%)
Body mass index (range), kg/m^2	25.7 (23.9-27.8)
Smoking (n = 156), No. (%)	67 (42.9%)
History of trauma (n = 154), No. (%)	61 (39.6%)
Comorbidity (n = 156), No. (%)	
Hypertension	40 (25.6%)
Diabetes mellitus	15 (9.6%)
Laboratory parameter (IQR)	
White blood cell count, 10^3/μL	15 750 (11 200-22 375)
Monocyte count, 10^3/μL	0.84 (0.50-1.63)
Lymphocyte count, 10^3/μL	1.10 (0.80-2.23)
Neutrophil count, 10^3/μL	14.8 (9.68-23.86)
Platelet count, 10^3/μL	184 ± 89
Median serum creatinine, mg/dL	1.2 (0.9-1.7)
Inotropic agent use (n = 324), No. (%)	272 (84%)

Abbreviations: IQR, interquartile range

Survival analyses

The total median observation time was 227 weeks (range, 115-345 weeks). Duration of posttransplant observation period was calculated based on time to death or time to last follow-up visit. During the entire follow-up period, 67 patients (20.6%) died. Of these deaths, 20 (12.3%) occurred in recipients with MHR below median value, whereas 47 deaths (29%) occurred in recipients with MHR above the median ($P < .001$) (Table 3). We constructed Kaplan-Meier curves to demonstrate the survival difference between the groups (Figure 1). Survival difference was significant when compared with the log-rank test ($P = .001$) (Table 4).

The median monocyte count was significantly higher in the deceased recipients than in those who survived (0.58 10^3/μL [IQR, 0.46-0.73] vs 0.51 10^3/μL [IQR, 0.39-0.69]; $P = .028$) (Figure 2). The median concentration of HDL cholesterol was significantly lower in the deceased recipients versus those who survived (32.5 mg/dL [IQR, 27.0-38.3] vs 37.0 mg/dL [IQR, 30.0-46.0]; $P = .005$).

Predictors of all-cause mortality

We performed univariate and multivariate Cox regression models for determination of independent predictors of all-cause mortality in DDKT recipients. Age, delayed graft function, duration of dialysis, cold ischemia time, and MHR emerged as significant associates of all-cause mortality. When these

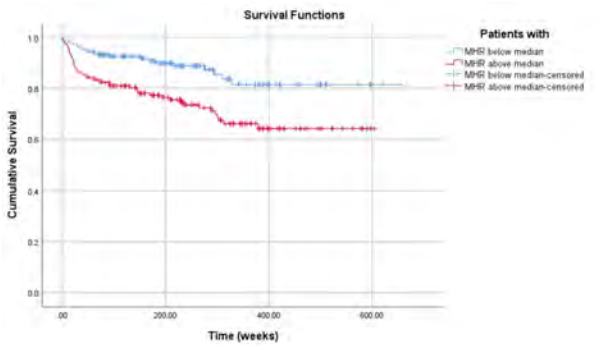
Table 3. Comparison of Patients With Monocyte-to-High-Density Lipoprotein Ratio Below and Above the Median Value in Terms of Clinical and Demographic Characteristics, Laboratory Values, and Graft Features

	Total Patients (N = 325)		
	Patients With MHR Below Median Value (n = 163)	Patients With MHR Above Median Value (n = 162)	*P* Value
Age, years	43.7 ± 11.9	45.3 ± 10.5	.007[a]
Female/male, No. (%)	82(50.3%)/81(49.7%)	58 (35.8%)/104 (64.2%)	.010[b]
Body mass index (range), kg/m^2	24.4 (22.0-26.5)	24.2 (22.7-26.4)	.750[d]
Comorbidity (n = 320), No. (%)			
Coronary artery disease	8 (5.0%)	16 (10.0%)	.136[b]
Diabetes mellitus	18 (11.3%)	25 (15.6%)	.325[b]
Hypertension	67 (41.9%)	69 (43.1%)	.910[b]
COPD	3 (1.9%)	3 (1.9%)	1.000[c]
Type of dialysis (n = 321), No. (%)			
Hemodialysis	111 (68.5%)	110 (69.2%)	
Peritoneal dialysis	25 (15.4%)	23 (14.5%)	.970[b]
Both (alternately)	26 (16.0%)	26 (16.4%)	
Duration of dialysis (n = 314) (range), y	7 (4-11)	8 (5-11)	.084[d]
Laboratory parameter (IQR)			
White blood cell count, 10^3/μL	7090 (5575-84909	8360 (6680-10350)	<.001[d]
Monocyte count, 10^3/μL	0.41 (0.31-0.51)	0.65 (0.55-0.80)	<.001[d]
Lymphocyte count, 10^3/μL	1.7 (1.21-2.16)	1.89 (1.44-2.48)	.001[d]
Neutrophil count, 10^3/μL	5.22 (3.73-3323)	6.98 (4.39-4120)	.001[d]
Platelet count, 10^3/μL	182 (153-221)	208 (167-266)	.006[d]
HDL cholesterol, mg/dL	42 (35-51)	31 (26-36.5)	<.001[d]
MHR	10 (8.06-11.8)	21.03 (17.01-26.69)	<.001[d]
CRP, mg/dL	1.00 (0.22-3.00)	1.20 (0.40-5.09)	.068[d]
Month 1 Cr, mg/dL	1.1 (0.91-1.23)	1.1 (0.86-1.3)	.002[d]
Month 3 Cr, mg/dL	1.07 ± 0.34	1.16 ± 0.35	.167[a]
Month 24 Cr (n = 229), mg/dL	0.91 ± 0.16	1.09 ± 0.25	.220[a]
Month 120 Cr (n = 13), mg/dL	1.00 ± 0.56	0.99 ± 0.34	.950[a]
Transplant features (IQR)			
No. of HLA antigen matches	2 (2-3)	2 (2-3)	.571[d]
DGF (n = 321), No. (%)	24 (15.1%)	35 (21.6%)	.150[b]
Induction regimen (n = 284), No. (%)			
Antithymocyte globulin	6 (4.1%)	4 (2.9%)	
Basiliximab	133 (89.9%)	122 (89.7%)	
IVIG	0	1 (0.7%)	.839[c]
IVIG + antithymocyte globulin	0	1 (0.7%)	
Basiliximab + IVIG	9 (6.1%)	8 (5.9%)	
Total rejection, No. (%)	7 (4.3%)	9 (5.6%)	.599[b]
Total nephrectomy, No. (%)	8 (4.9%)	11 (6.8%)	.490[b]
Cold ischemia time (range), h	14 (12-15)	14 (12-11)	.414[d]
Total follow-up time (range), weeks	224 (120-324)	228 (98.0-355)	.786[d]
Mortality, No. (%)	20 (12.3%)	47 (29%)	<.001[b]

Abbreviations: COPD, chronic obstructive pulmonary disease; Cr, creatinine; CRP, C-reactive protein; DGV, delayed graft function; IQR, interquartile range; IVIG, intravenous immunoglobulin; MHR, monocyte-to-high-density lipoprotein cholesterol ratio

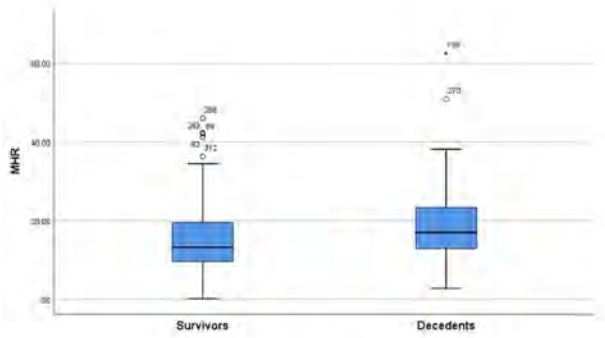
[a]Independent sample *t* test; [b]chi-square test; [c]Fisher exact test; [d]Mann-Whitney U test. Total follow-up time was based on time to death or time to last follow-up visit.

Figure 1. Kaplan-Meier Survival Curve of Patients With Monocyte-to-High-Density Lipoprotein Cholesterol Ratio Above Versus Below Median



Abbreviations: MHR, monocyte-to-high-density lipoprotein cholesterol ratio

Figure 2. Box Plot Showing Monocyte-to-High-Density Lipoprotein Cholesterol Ratio in Deceased and Surviving Recipients of Deceased Donor Kidney Transplant



Abbreviations: MHR, monocyte-to-high-density lipoprotein cholesterol ratio

parameters were assessed in the multivariate Cox model, delayed graft function, duration of dialysis, cold ischemia time, and MHR appeared as independent predictors of all-cause mortality. The hazard ratio was 2.444 (95% CI, 1.408-4.245) for MHR. Table 5 shows the Cox models.

Discussion

The salient findings of this study were as follows: (1) DDKT recipients with MHR above median value had a higher mortality rate than recipients with MHR below the median; and (2) level of MHR group (MHR above median vs MHR below median) emerged as an independent predictor of all-cause mortality in DDKT recipients, in addition to delayed graft function, duration of dialysis, and cold ischemia time. To the best of our knowledge, this is the first study that reported the predictive ability of MHR for mortality in renal transplant recipients.

It is well-established that monocytes and their tissue-resident offspring, macrophages, are causally involved in cardiovascular disease initiation and progression. These cells also play a major role in the development of unstable atherosclerotic plaques,[12] which itself is a chronic inflammatory lesion. Allen and colleagues speculated that circulating monocyte-platelet aggregates might be one of the missing links

Table 4. Log-Rank Test Showing Differences in Survival Times Between Patients with Monocyte-to-High-Density Lipoprotein Cholesterol Ratio Below and Above Median Value

	Number of Events/ Number of Patients (%)	Mean Survival Time, days	95% CI	*P* Value
Patients with MHR below median value	20/163 (12.3%)	566.3 ± 19.0	529.1-603.6	.001*
Patients with MHR above median value	47/162 (29.0%)	436.3 ± 20.4	396.4-476.2	

Abbreviations: HR, monocyte-to-high-density lipoprotein cholesterol ratio
*Log-rank test.

Table 5. Univariate and Multivariate Cox Proportional Hazard Models Showing Independent Predictors of All-Cause Mortality in Deceased Donor Kidney Transplant Recipients

Parameter	Univariate Cox Regression			Multivariate Cox Regression		
	Hazard Ratio	95% CI	*P* Value	Hazard Ratio	95% CI	*P* Value
Age	1.030	1.007-1.054	.010	1.023	0.997-1.050	.086
Sex	1.514	0.914-2.508	.107			
CRP	1.001	0.997-1.004	.672			
Delayed graft function	2.988	1.809-4.937	<.001	0.461	0.268-0.794	.005
Diabetes mellitus	1.147	0.585-2.249	.690			
Hypertension	0.954	0.584-1.559	.852			
Coronary artery disease	1.353	0.584-3.133	.480			
Duration of dialysis	1.064	1.012-1.117	.014	1.060	1.007-1.116	.025
Number of matched HLA antigens	0.990	0.783-1.250	.930			
Donor creatinine	1.036	0.988-1.086	.142			
Cold ischemia time	0.889	0.814-0.972	.010	0.892	0.810-0.981	.019
MHR groups (MHR above median vs MHR below median)	2.410	1.428-4.070	.001	2.444	1.408-4.245	.002

Abbreviations: CRP, C-reactive protein; MHR, monocyte-to-high density lipoprotein cholesterol ratio

between the atherothrombosis and monocytic inflammation.[13] The authors concluded that monocyte-platelet aggregates were strong surrogate markers of platelet activity and were elevated in patients with cardiovascular disease. The present study showed that median monocyte count was significantly higher and median HDL cholesterol concentration was significantly lower in deceased DDKT recipients compared with DDKT recipients who survived.

Interestingly, recent evidence has depicted that dyslipidemia induces monocyte production from the bone marrow. Of note, triglycerides and low-density lipoprotein cholesterol led to alterations in the activation status of the monocyte subsets.[12] High-density lipoprotein cholesterol conducts reverse cholesterol transport and is also known to have anti-inflammatory properties.[14] In an experimental study by Iqbal and colleagues, apolipoprotein A1, the major lipoprotein fraction in HDL particles, was shown to inhibit macrophage chemotaxis and monocyte recruitment.[15]

The dyslipidemia in chronic kidney disease has a specific pattern, in which serum triglycerides are elevated and HDL cholesterol level is reduced.[16] In addition, chronic kidney disease is now considered a chronic inflammatory disease.[17] This prevalent dyslipidemia and heightened inflammatory milieu to some extent account for the increased atherosclerotic burden and high cardiovascular mortality observed in this patient population. Chronic kidney disease is also unique in terms of monocyte alterations. Several studies have reported that patients with chronic kidney disease had elevated numbers of intermediate and $CD40^+$ monocytes corresponding to severity of kidney dysfunction.[18,19] Moreover, peripheral blood monocyte counts were found to be independently predictive of adverse major cardiovascular events.[20] In another study, monocyte chemoattractant protein 1, a marker reflecting inflammation and monocyte recruitment to atherosclerotic plaques, was found to be independently associated with cardiovascular events and death.[21]

Kanbay and colleagues, in the only study that investigated the role of MHR in patients with chronic kidney disease, found that MHR increased as the glomerular filtration rate decreased. In addition, both fatal and nonfatal major adverse cardiovascular events were more common in patients who had higher MHR compared with patients who had lower MHR values.[8] Unfortunately, no study to date has evaluated the values and predictive ability of MHR in kidney transplant recipients.

Vereyken and associates found that the number of proinflammatory (intermediate) monocytes expressing $CD16^+$ and $CD14^{++}$ were increased at the time of transplant, and this was maintained for at least 6 months after transplant.[22] Rogacev and associates found that steroid intake was associated with higher total counts of $CD14^{++}$-$CD16^+$ monocytes in kidney transplant recipients.[23] In another study, van den Bosch and colleagues demonstrated that kidney transplant recipients who developed acute rejection had significantly higher numbers of $CD16^+$ monocyte counts relative to kidney transplant recipients who did not experience rejection and control subjects. Interestingly, a higher number of $CD16^+$ monocytes before transplant was significantly associated with a higher risk of acute rejection episodes.[24] Combining experimental and clinical data, we can safely say that monocytes, along with macrophages into which they are transformed, play pivotal roles in acute rejection episodes.[25]

Monocytes with proinflammatory phenotype have also been found to be associated with subclinical atherosclerosis in kidney transplant recipients.[26] A sizeable portion of kidney transplant recipients have impaired kidney function and thus labeled as having chronic kidney disease starting early after transplant. Thus, we can assume that monocytic changes observed in patients with chronic kidney disease continue after kidney transplant. In addition, prednisone affects the monocytes in the direction of increasing proinflammatory monocyte subsets in transplant recipients. Thus, it is plausible to hypothesize that monocyte counts, particularly the proinflammatory intermediate type, may be an independent predictor of acute rejection episodes and cardiovascular events and mortality.

Some limitations of the present study deserve mention. First, we assessed all-cause mortality instead of cardiovascular mortality because we lacked the causes of death in all study participants with sufficient certainty. Because MHR is principally related to cardiovascular mortality, we assumed that the association and hazard ratio for mortality would have been higher in that case. Second, we did not account for long-term drug use, including maintenance immunosuppressive drugs, which may have affected survival.

Conclusions

Our results showed for the first time that higher MHR values seemed to be independently associated with all-cause death in DDKT recipients. Transplant recipients with higher MHR values had significantly lower survival rates than transplant recipients with lower MHR values. These preliminary observations should be evaluated in large samples with cardiovascular mortality as the outcome measure.

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Clinical Analysis and Proteomic Screening Biomarkers for Graft-Versus-Host Disease After Liver Transplant

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Abstract

Objectives: **Graft-versus-host disease is a serious, fatal complication following liver transplantation. The diagnosis is challenging, owing to nonspecific clinical features and invasive procedures. High-throughput proteomics could provide an effective approach to identifying potential serum biomarkers for graft-versus-host disease.**

Materials and Methods: **We retrospectively analyzed the clinical information of 3 patients with graft-versus-host disease treated at our center from 2016 to 2018. We compared serum samples from the 3 patients with the disease, patients with excellent posttransplant outcomes, and healthy controls using mass spectrometry-based proteomics in discovery study. Probable peptides were further identified by a tandem mass spectrometry system and verified by enzyme-linked immunosorbent assay.**

Results: **Of 343 patients, 3 patients (0.875%) had graft-versus-host disease. Two of these patients died of sepsis and multiorgan failure despite intensive therapy. We observed no correlation between severity of clinical manifestation and prognosis; however, the patients with graft-versus-host disease had early onset and infection and showed worse outcome. Serum peptidome profiling showed 65 differentially expressed peaks among the 3 groups; the 2 peptides with the most significant changes (m/z values of 1950.29 and 2088.16) were further sequenced and identified as ATP citrate lyase and fibrinogen alpha chain. Western blot and enzyme-linked immunosorbent assay showed that both peptides gradually decreased among all groups.**

Conclusions: **Graft-versus-host disease is a complication of organ and tissue transplantation with a high mortality rate. Our identification of potential biomarkers for graft-versus-host disease associated with liver transplant may aid in diagnosis and help to reduce patient mortality in those cases.**

Key words: *Biomarkers, Mass spectrometry, Posttransplant mortality*

Introduction

Graft-versus-host disease (GVHD) is a serious, fatal complication following liver transplantation (LT); acute GVHD usually occurs within the first few weeks after LT.[1,2] The exact mechanisms are still unclear, with mechanisms depending on the balance between the donor and recipient immune systems. Humoral GVHD is mediated by antibodies against the red cell antigen, resulting in self-limiting hemolytic anemia,[3] whereas cellular GVHD occurs as a result of a destructive cellular immune response by immunocompetent donor T lymphocytes against the recipient tissue.[4] Skin, gastrointestinal tract, and bone marrow are the targeted organs of cellular GVHD.[5,6]

The diagnosis of GVHD relies on clinical suspicion and is confirmed by pathology; however, diagnosis may be delayed because of infections and drug reactions with similar presentations.[7] There is growing interest in the detection of chimerism with the use of molecular techniques, such as polymerase

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chain reaction-based techniques or fluorescence in situ hybridization.[8] However, it is still controversial whether persistence of chimerism correlates with GVHD.[9,10] Alexander and colleagues reported that chimerism functions as a nonspecific diagnostic marker, not only for GVHD but also for immune tolerance.[10] Monitoring chimerism may just be a tool in the presence of symptoms. With nonspecific clinical manifestations, invasive biopsies, and limited applications of chimerism, the diagnosis of GVHD remains a challenge. Further investigations on early recognition and diagnosis of GVHD are critical for improvement of patient outcomes.

Ideal biomarkers should be accurate, noninvasive, rapid, and inexpensive. There are numerous strategies in the search for GVHD biomarkers, with technological advancements in chemistry, engineering, and bioinformatics. Over the past decade, proteomic technologies have been successful in elucidating pathogenesis and discovering clinical biomarkers in many diseases such as cancers.[11,12] Antibody microarray profiling and mass spectrometry (MS) have been employed as classical proteomic tools for biomarker study.[13,14] Serum, which may reflect the patient's particular pathophysiological state, is readily used as a classical clinical sample. Today, tumor biomarker discovery in serum is well established and is widely used in the clinic. We hypothesized that identification of serum biomarkers in GVHD after LT could also be a promising application of proteomic research.

In this discovery study, we identified differentially expressed proteins discriminating healthy controls from GVHD patients by MS-based proteomics. The potential biomarkers were then analyzed by bioinformatics and validated by enzyme-linked immunosorbent assay (ELISA) and Western blot. We identified 2 peptides, ATP citrate lyase (ACLY) and fibrinogen alpha chain (FGA), that could function as noninvasive diagnostic tools for GVHD after LT.

Materials and Methods

Patient selection and sample preparation

All patients included in our study had long-term follow-up after standard orthotopic LT. Diagnostic criteria for GVHD in our center include acute onset (within 2 months), typical clinical manifestation (fever, rash, diarrhea, and pancytopenia), and histopathology after exclusion of differential diseases. Skin biopsy revealed skin squamous cell dyskeratosis associated with dermal chronic inflammatory cell infiltration.[15]

In discovery study, we screened 3 manifestations and confirmed GVHD through pathology in 3 patients (patients 1-3); our study also included 10 patients without any complications (excellent posttransplant outcomes) and 10 control patients matched by age and sex; all patients were seen between 2016 and 2018. For this study, GVHD refers specifically to acute cellular-mediated GVHD.

Serum samples for the 3 patients with GVHD were collected immediately after the diagnosis (days 15, 15, and 32); serum for patients with excellent posttransplant outcomes were collected 14 to 30 days after LT. Serum from control patients were matched based on age and sex. Serum samples were centrifuged at 3500 *g* for 20 minutes and stored at -80 °C until use.

Mass spectrometry and analysis systems

We used the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) MS technique. Serum samples were separated with the use of magnetic bead-based weak cation exchange (Bruker). A mixture of same volume eluted peptides and matrix were placed on the MALDI AnchorChip surface (Bruker).

All targets were analyzed by Autoflex analysis software (version 3.0; Bruker), with an optimized protocol of FlexControl software (version 3.0; Bruker). Peptide patterns were identified with the use of ClinProTools software (version 2.2; Bruker).

Peptide identification and bioinformatic analysis

Peptides were measured using liquid chromatography/electrospray ionization tandem MS/MS system, consisting of an EASY-nLC 1000 (Thermo Fisher Scientific) coupled with a nano-electrospray ion source to a Q-Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific), as described previously by Wang and colleagues.[15]

We used gene ontology (GO) enrichment analysis to analyze the identified proteins. We used the STRING database as the source to establish an interaction network of potential biomarkers.

Enzyme-linked immunosorbent assay and Western blot

We determined ACLY and FGA levels using a human ACLY ELISA kit and a human FGA ELISA kit (H-12925 and H-12843; both from Hengyuan Biotech).

Standard curves were generated to determine the concentrations of ACLY and FGA. All serum samples mentioned above were analyzed blindly and run in triplicate.

Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequently transferred to polyvinylidene difluoride membranes. After a blocking procedure, membranes were incubated with primary antibodies overnight and then incubated with secondary antibodies.

Statistical analyses

Statistical analysis was performed with GraphPad Prism version 6.0. Data are expressed as mean ± SD. We used *t* tests to compare means between 2 groups and one-way analysis of variance with subsequent Bonferroni correction to analyze multiple comparisons. Spearman correlation analysis was used to determine the correlation between expression of serum biomarkers and clinical information. $P < .05$ was considered statistically significant.

Ethics statement

All procedures performed were approved by the ethics committee of the First Affiliated Hospital, Xi'an Jiaotong University (No. 20151006). Signed consent was obtained from all participants. Donations after brain death were the source for LT in the study.

Results

Clinical characteristics

Among 343 LT patients seen at our center between 2016 and 2018, there were 3 patients (0.875%) with GVHD. Two of the 3 patients died (66.67%) (Table 1). Table 2 provides background information on all study patients. All 3 patients with GVHD were men versus 7 of 10 patients were men in the group with excellent posttransplant outcomes; patients with GVHD were also older: 52.7 ± 9.8 versus 47.4 ± 8.6 years old. Among patients with GVHD, indications for LT included cirrhosis and hepatocellular carcinoma (HCC); in the patient group with excellent posttransplant outcomes, indications were cirrhosis (8/10), HCC (1/10), and acute hepatic failure (1/10). Two patients with GVHD (66.7%) underwent previous transcatheter arterial chemoembolization (TACE) and 1 patient required retransplant due to tumor recurrence. Patients with excellent posttransplant outcomes did not have any surgical history. Furthermore, patients with GVHD seemed to have relatively higher mean immunosuppression concentration in the first week. In the control group, there were 7 men and 3 women with an average age of 47.3 ± 8.7 years. No differences were found regarding the other factors.

Table 1. Clinical Course and Treatment of 3 Patients With Graft-Versus-Host Disease After Liver Transplant

Signs of GVHD, Treatment, and Outcomes	Patient Number 1	2	3
Fever onset	POD15	POD15	POD32
Maximum temperature, °C	39.6	38.5	38.2
Skin rash onset (area %)	POD16 (90%)	POD17 (60%)	POD35 (40%)
Cytopenia onset	POD16	POD19	POD32
Minimum WBC, $\times 10^9$	0.01	0.75	0.12
Minimum neutrophils, $\times 10^9$	0	0.21	0.02
Minimum platelets, $\times 10^9$	5	26	12
Minimum RBC, $\times 10^9$	2.87	3.14	3.14
Diarrhea onset	POD33		POD37
GVHD onset	POD15	POD15	POD32
Microbial etiology	*E. coli*, MRSA	Candidiasis	
Other	SIADH	AKI	
Treatment			
Stop immunosuppression	Yes	Yes	Yes
Reduce immunosuppression	No	No	No
Antilymphocyte treatment	AGT	Infliimab	ATG
Steroids	Yes	Yes	Yes
G-CSF	Yes	Yes	Yes
IVIG	Yes	Yes	Yes
Outcome	Death	Death	Recovered
Outcome POD	48	23	>300
Granulocyte recovery	No	No	Yes
Cause of death	Sepsis and multiorgan failure	Sepsis and multiorgan failure	

Abbreviations: AKI, acute kidney injury; ATG, antithymocyte globulin; G-CSF, granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; IVIG, intravenous immunoglobulin; MRSA, methicillin-resistant *Staphylococcus aureus*; POD, postoperative day; RBC, red blood cell; SIADH, syndrome of inappropriate antidiuretic hormone secretion; WBC, white blood cell

The clinical characteristics and treatment of patients with GVHD are summarized in Table 1. Average time from surgery until clinical symptom of GVHD was 20.7 days. All patients with GVHD had fever, skin rash, and pancytopenia, which appeared early and shortly after onset of fever; 2 patients (66.67%) had diarrhea and 2 (66.67%) developed infection (1 bacterial, 1 candidiasis); however, liver, renal, and coagulation functions were not remarkable affected (Table 3). Patients also had maculopapular rashes that covered the trunk and spread to other parts of the body (Figure 1A and 1B). There was a rapid drop in white blood cell count, reaching a minimum of 0.01 to 0.75 × 10^9/L, and platelet count, reaching a minimum of 5 to 26 × 10^9/L, with patients

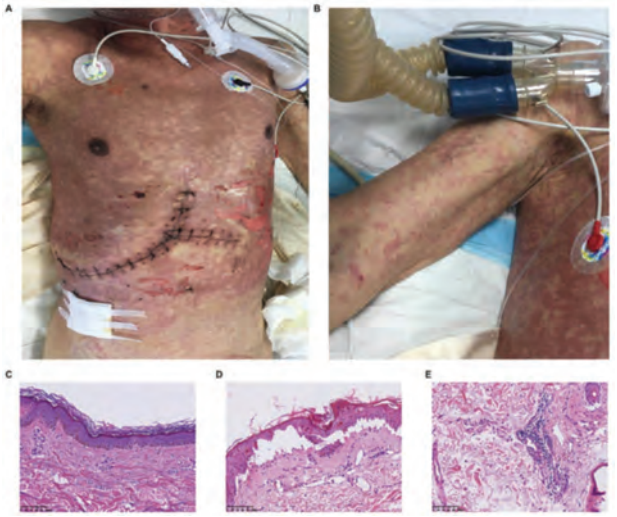
eventually becoming profoundly pancytopenic (Table 1). We found no correlation between grade of fever, range of rash, degree of pancytopenia, and outcome; however, patients with GVHD had early onset of symptoms (postoperative day 15) and complications of infection.

Table 2. Demographics and Characteristics of Patients With Graft-Versus-Host Disease and Patients With Excellent Posttransplant Outcomes

Variable	Patients With GVHD (n = 3)	Patients With Excellent Posttransplant Outcomes (n = 10)
	Donor	
Age, years	40.7 ± 6.9	43.5 ± 15.5
Sex, male/female	3/0	9/1
Body mass index, kg/m^2	24.2 ± 1.3	20.8 ± 3.8
Etiology		
Cerebral hemorrhage	1	3
Craniocerebral injury	2	6
Other	0	1
	Recipient	
Preoperative age, y	52.7 ± 9.8	47.9 ± 8.5
Age >60 y	1	0
Recipient-to-donor age difference, y	16.0 ± 7.3	11.7 ± 9.9
Sex, male/female	3/0	7/3
Preoperative body mass index, kg/m^2	22.6 ± 2.5	20.9 ± 4.2
Etiology		
Liver cirrhosis	1	8
Hepatitis B virus related	1	4
Hepatitis C virus related	0	2
Alcohol related	0	1
Other	0	1
HCC	2	1
Acute hepatic failure	0	1
Child-Pugh score		
B	2	4
C	1	6
MELD score	18.3 ± 2.6	20.9 ± 6.6
ABO compatible		
Yes	3	9
No	0	1
Type 2 diabetes mellitus	1	1
Surgical history		
TACE	2	0
Hepatectomy	1	0
Previous liver transplant	1	0
None	1	10
Intraoperative		
Cold ischemia time, h	8.0 ± 1.6	6.5 ± 2.1
Warm ischemia time, min	6.0 ± 1.4	9.6 ± 0.8
Blood loss, mL/kg	28.1 ± 10.9	28.8 ± 11.7
Operation time, h	8.5 ± 1.8	6.6 ± 1.0
RBC transfusion, U	9.3 ± 3.4	11.3 ± 3.6
Plasma transfusion, mL	1733.3 ± 498.9	1650.0 ± 355.7
Anhepatic phase, min	41.7 ± 2.4	45.6 ± 7.6
Postoperative		
Tacrolimus + MMF + steroids	2	7
Cyclosporine + MMF + steroids	1	3
Week 1 mean immunosuppression concentration		
Low	0	1
Normal	0	4
High	3	5

Abbreviations: GVHD, graft-versus-host disease; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease; MMF, mycophenolate mofetil; RBC, red blood cell; TACE, transcatheter arterial chemoembolization

Figure 1. Appearance of Maculopapular Rashes and Histopathology of Skin Lesion Biopsy of Graft-Versus-Host Disease After Liver Transplant



(A) Maculopapular rashes and desquamation cover the neck, chest, and anterior abdominal wall (patient 2). **(B)** Scattered maculopapular rashes over the right axilla and right arm (patient 3). **(C)** Hyperkeratosis of epidermis and perivascular lymphocytic infiltration in upper dermis (patient 1). **(D)** Many apoptotic keratinocytes, lymphocytic exocytosis, and spongiosis in addition to the subepidermal cleft (patient 2). **(E)** Perivascular lymphocytic infiltration in upper dermis (patient 3). Laboratory images are hematoxylin and eosin stained; original magnification ×20.

Table 3. Laboratory Tests in Study Patients

Variable	Control Group (n = 10)	Patients With Excellent Posttransplant Outcomes (n = 10)	Patients With GVHD (n = 3)
Hematological parameter			
WBC, ×10^9/L	6.52 ± 1.38	6.71 ± 2.63	1.84 ± 1.17
RBC, ×10^{12}/L	4.92 ± 0.67	3.67 ± 0.37	3.72 ± 0.12
Platelets, ×10^9/L	219.40 ± 49.82	225.17 ± 116.16	94.67 ± 63.43
Neutrophils, ×10^9/L	4.51 ± 0.76	5.42 ± 2.00	1.41 ± 0.96
Lymphocytes, ×10^9/L	2.44 ± 0.47	0.72 ± 0.42	0.35 ± 0.07
Monocytes, ×10^9/L	0.41 ± 0.08	0.45 ± 0.19	0.07 ± 0.05
Liver function marker			
AST, U/L	20.09 ± 6.69	18.33 ± 8.19	52.67 ± 47.12
ALT, U/L	22.60 ± 11.27	42.5 ± 20.18	25.33 ± 2.49
GGT, U/L	24.20 ± 10.29	63.67 ± 24.23	55.33 ± 17.44
Cholesterol, mmol/L	3.99 ± 0.94	4.29 ± 0.43	3.07 ± 0.92
Albumin, g/L	46.30 ± 4.35	38.07 ± 3.89	36.50 ± 2.35
TBil, μmol/L	8.94 ± 3.31	24.03 ± 12.46	27.57 ± 6.30
Creatinine, μmol/L	42.67 ± 11.34	46.00 ± 19.54	76.67 ± 25.75
Coagulation function marker			
PT, s	12.40 ± 0.68	13.45 ± 1.01	14.53 ± 0.25
Fibrinogen g/L	3.03 ± 0.56	3.26 ± 0.47	3.95 ± 1.08

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; GVHD, graft-versus-host disease; PT, prothrombin time; RBC, red blood cell; TBil, total bilirubin; WBC, white blood cell

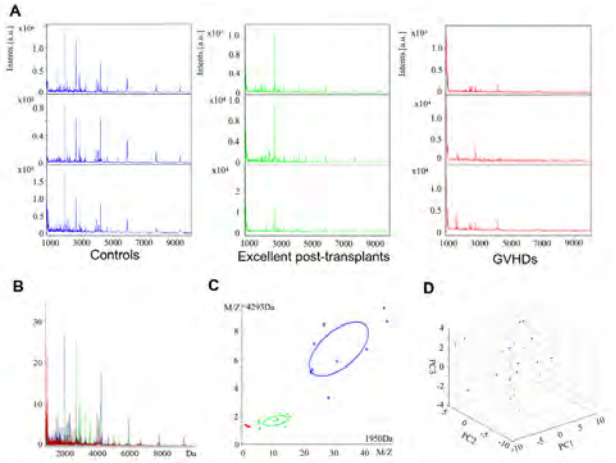
Diagnosis of GVHD was confirmed by clinical manifestation and skin biopsies.[16] Skin biopsy revealed many keratinocytes, perivascular lymphocytic infiltration, and spongiosis (Figure 1C to 1E). Although

there is no clear treatment protocol, our treatment strategies included high-dose methylprednisolone in 3 patients, antithymocyte globulin in 2 patients, and stopping/reducing current immunosuppressive protocol. Empirical antibiotics and antifungal agents play a vital part in bacterial and fungal infections after LT. Hematopoietic cytokine was administered to treat pancytopenia. Patients also received supportive therapy, such as isolation and nutritional support, with the goal of benefiting the entire condition. Despite intensive treatment, 2 of 3 patients (66.67%) with GVHD died due to sepsis and multiorgan failure. The remaining patient was alive after 300 days of follow-up without complications.

Comparison of serum proteomic profiles among the study groups

We tested the system stabilization first, and the results showed that the mean value of the coefficient of variance was <20%, with maximum and minimum values of 20.76% and 8.73%, respectively. We analyzed 3 representative serum samples and found close reproducibility and stability of the mass spectra for the mass range of 1 to 10 kDa (Figure 2A). Figure 2B shows the differentially expressed peaks among the control group, the patients with excellent posttransplant outcomes, and the patients with GVHD. Distribution among the 3 groups showed small overlapping areas, indicating that patients with GVHD could be distinguished from control patients (Figure 2C and 2D).

Figure 2. Proteomic Profiling in the Different Patient Study Groups



Abbreviations: GVHD, graft-versus-host disease

(A) Mass spectra of 3 representative serum samples. Mass ranged from 1 to 10 kDa. **(B)** and **(C)** Comparative analysis of serum proteomic profiles among different groups. **(D)** Three-dimensional plot of the 3 groups in the component analysis.

Selection of differential expressed peptides

When we compared the patient groups, the ClinProTools software identified 65 different peaks, of which 2 were significantly different among the 3 groups (fold-change >1.5; $P < .001$). Peak 1 (m/z: 1950.29) and peak 4 (m/z: 2088.16) were both downregulated in patients with GVHD compared with control patients (Table 4) and showed the same trend when compared with patients with excellent posttransplant outcomes. Peptide mass spectrum comparisons of the 2 peaks in all samples (Figure 3A and 3C) were in line with the results shown in Table 4. The area under the curve values of the 2 peaks were both 1 (peak 1, m/z: 1950.29; peak 4, m/z: 2088.16) (Figure 3B and 3D). Relative expressions of peak 1 and peak 4 are shown in Figure 3E.

Table 4. Mean Levels of Different Peptides Among the Different Patient Study Groups

Peak	m/z	*P* Value	Patient Group		
			Control (n = 10)	Excellent Posttransplant Outcomes (n = 10)	GVHD (n = 3)
1	1950.29	.0000111	34.6 ± 10.5	12.0 ± 4.3	2.0 ± 0.7
2	4293	.0000628	4.2 ± 1.227	1.0 ± 0.2	0.8 ± 0.2
3	4220.51	.0000807	17.8 ± 7.2	12.9 ± 5.7	4.0 ± 1.3
4	2088.16	.0000807	7.4 ± 2.0	4.5 ± 1.4	1.9 ± 0.4
5	3965.64	.00029	10.4 ± 3.4	3.5 ± 2.1	2.0 ± 0.6
6	873.15	.00029	3.3 ± 0.9	4.9 ± 2.0	11.3 ± 0.9
7	2216.34	.00029	8.0 ± 2.9	3.1 ± 1.3	1.6 ± 0.4
8	4101.6	.00029	8.0 ± 2.6	6.5 ± 2.2	2.4 ± 0.7
9	2890.91	.000431	4.4 ± 1.2	7.1 ± 6.4	1.7 ± 0.3
10	5919.02	.000609	8.9 ± 4.3	5.0 ± 2.9	1.0 ± 0.7
11	4277.46	.000653	3.1 ± 1.0	1.1 ± 0.3	0.8 ± 0.2
12	2870.09	.0013	9.3 ± 4.3	14.0 ± 9.6	2.6 ± 0.6
13	2278.3	.001	10.9 ± 6.5	4.0 ± 1.8	1.8 ± 0.1
14	5077.39	.00562	1.4 ± 0.6	2.4 ± 1.7	0.7 ± 0.1
15	2551.51	.00588	3.6 ± 0.9	5.5 ± 0.4	2.4 ± 3.6
16	2177.07	.00857	8.6 ± 7.1	2.9 ± 1.7	1.2 ± 0.2
17	4655.91	.00974	3.2 ± 1.6	0.9 ± 0.3	1.0 ± 1.1

Abbreviations: GVHD, graft-versus-host disease

Peptide identification

Tandem MS/MS and the Uniprot database were used to confirm sequences, which were identified as ACLY and FGA (Table 5). The sequence of identified peptides is shown in Figure 4.

Gene ontology and STRING interaction analysis

Figure 5 shows the results of GO analysis of identified proteins. The identified proteins were scattered among various cellular components, including cell and cell parts, organelle and organelle parts, membrane-enclosed lumen, extracellular region and parts, membrane and membrane parts, protein-containing

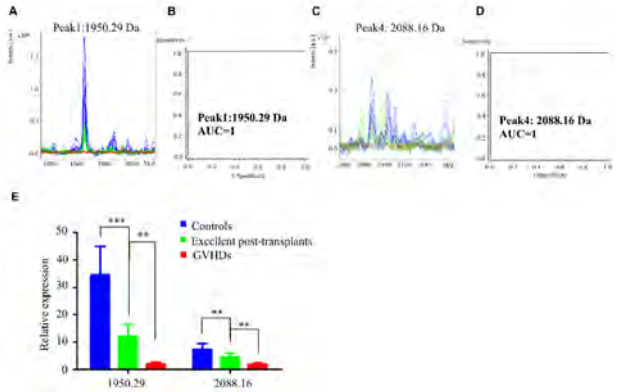
complex, and synapse (Figure 5A). The molecular function identified protein patterns, including binding, structural molecular, and catalytic activity (Figure 5A). In addition, the proteins were involved in a wide range of biological processes, including metabolic process, cellular process, multicellular organismal process, biological regulation, regulation of biological process, positive regulation of biological process, localization, signaling, response to stimulus, negative regulation of biological process, multiorgan process, immune system process, cellular component organization or biogenesis, biological adhesion, and developmental progress (Figure 5A). Moreover, the STRING database was used to explore the network of ACLY and FGA, which showed a close protein-protein interaction network (Figure 5B).

Table 5 Amino Acid Sequences of Identified Proteins That Showed Abundance Among the Patient Study Groups

Mass, Da	Peptide Sequence	Identity
2087.71	Y.KMADEAGSEADHEGTHSTKRGHAKSRPV.R	FGA
1949.9	K.ILIIGGSIANFTNVAATFK.G	ACLY

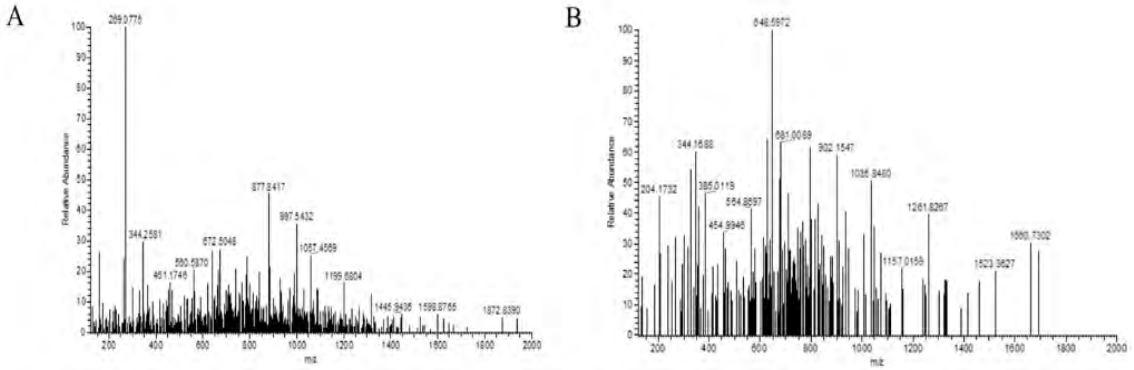
Abbreviations: ACLY, ATP citrate lyase; FGA, fibrinogen alpha chain

Figure 3. Representative Spectra of Peak 1 and Peak 4 in the Different Patient Study Groups



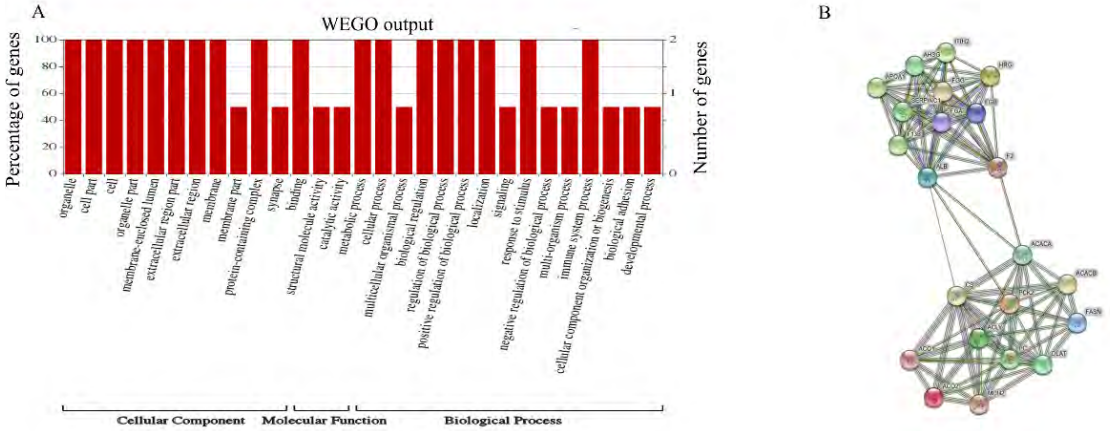
Abbreviations: GVHD, graft-versus-host disease
(A) and **(C)** Comparison of spectra of peak 1 and peak 4. **(B)** and **(D)** Receiver operating characteristics and area under the curve for peak 1 and peak 4. **(E)** Average expression of peak 1 and peak 4

Figure 4. Tandem Mass Spectrometry System Spectrometry Fragment Map



(A) Peak 1; m/z: 1949.9. **(B)** Peak 2; m/z: 2087.71.

Figure 5. Bioinformatics Analysis of Identified Proteins



Abbreviations: ACLY, ATP citrate lyase; FGA, fibrinogen alpha chain
(A) Gene ontology analysis of ACLY and FGA. **(B)** Interaction network between ACLY and FGA.

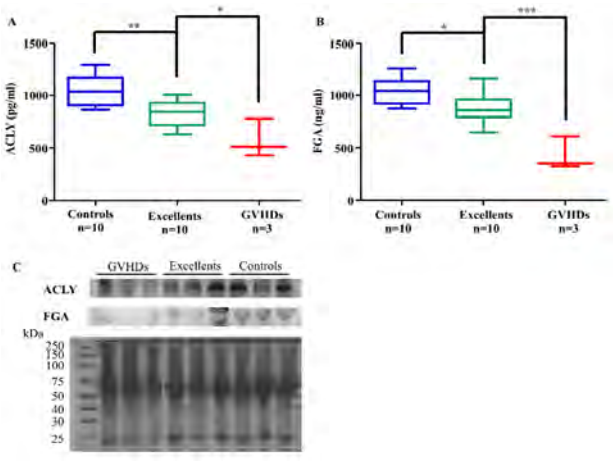
Protein expression of identified peptides

Serum concentrations of ACLY and FGA were examined by ELISA in samples from control patients, patients with excellent posttransplant outcomes, and patients with GVHD. The concentration of ACLY in patients with GVHD (571.6 ± 183.0 pg/mL) was notably lower than in patients with excellent posttransplant outcomes (837.2 ± 124.7 pg/mL) (Figure 6A). There was a lower level of FGA in serum in patients with GVHD (428.1 ± 159.4 ng/mL) than in patients with excellent posttransplant outcomes (880.8 ± 137.8 ng/mL) and control patients (1042.2 ± 237.3 ng/mL) (Figure 6B). Furthermore, Western blot was performed to identify the same expression trend for the ACLY and FGA from 3 serum samples chosen randomly from the control, excellent posttransplant, and GVHD groups (Figure 6C). Results of ELISA and Western blot indicated that ACLY and FGA might be potential diagnostic serum biomarkers for GVHD after LT.

Clinical correlation analysis

Spearman analysis was used to compare the correlations between biomarkers (ACLY and FGA) and clinical information. Our study showed that ACLY was not correlated with any laboratory value; however, FGA was inversely correlated with lymphocyte (P = .018) and monocyte (P = .003) levels (Table 6), which likely was due to the small sample size.

Figure 6. Concentration of ACLY and FGA in Validation Study



Abbreviations: ACLY, ATP citrate lyase; FGA, fibrinogen alpha chain; GVHD, graft-versus-host disease

(A, B) Concentration of ACLY and FGA in control group, patients with excellent posttransplant outcomes and patients with GVHD by enzyme-linked immunosorbent assay. **(C)** Quantification of ACLY and FGA by Western blot. Equal protein loading of serum samples was confirmed using Ponceau S staining.

Discussion

Graft-versus-host disease was first discovered as a common complication after hematopoietic stem cell transplant, with an estimated incidence of 50%.[17] The incidence of GVHD after LT is only 0.1% to 2%; however, the mortality rate is over 75% despite intensive treatment protocols. With the increased number of procedures and the advanced immunosuppressor options, decreased host immune defenses have increased the incidence of GVHD after LT. Treatment strategies remain controversial, and treatments are mostly based on experience. The most common causes of mortality in GVHDs are sepsis-associated complications and bleeding. Risk factors include complete HLA match, recipient age >65 years, age difference >20 years, autoimmune hepatitis, alcoholic liver disease, HCC, retransplant, and glucose intolerance.[18] In our LT center, prevalence of GVHD was 0.875% and mortality rate was 66.67%. Elderly recipients (52.7 ± 9.8 years), large age differences between donors and recipients (16.0 ± 7.3 years), and state of immunosuppression (HCC, TACE history, and higher mean immunosuppression concentration) were factors for more likely development of GVHD (Table 2), which were consistent with previous reports.[19,20] Compatibility testing of HLA between the donors and recipients was not routinely done, so we did not have data on HLA matching in our patients.

In our patients with GVHD, the most common symptoms were fever (100%), followed by skin rash (100%), pancytopenia (100%), and diarrhea (66.67%)

Table 6. Spearman Correlation Analysis Between Identified Serum Biomarkers and Clinical Information

	ACLY		FGA	
	r	*P* Value	r	*P* Value
WBC	-0.931	.239	-0.972	.150
RBC	0.732	.477	0.819	.389
Platelets	-0.613	.580	-0.497	.669
Neutrophils	-0.917	.261	-0.964	.172
Lymphocytes	-0.986	.106	-1.000	.018
Monocytes	-0.990	.092	-1.000	.003
ALT	-0.994	.068	-0.970	.157
AST	-0.383	.750	-0.507	.661
GGT	-0.682	.523	-0.574	.611
Cholesterol	-0.424	.721	-0.295	.810
Albumin	-0.075	.952	0.063	.960
TBil	0.173	.889	0.035	.978
PT	-0.250	.839	-0.382	.750
Fibrinogen	0.502	.665	0.617	.576

Abbreviations: ACLY, ATP citrate lyase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FGA, fibrinogen alpha chain; GGT, gamma-glutamyl transferase; PT, prothrombin time; RBC, red blood cell; TBil, total bilirubin; WBC, white blood cell

(Table 1). We observed that the first symptom of fever occurred on average in 20.67 days, but patients were histopathologically diagnosed with GVHD about 1 to 2 weeks after clinical findings were noted. Late-onset GVHD is associated with a relatively mild and self-limiting process and better prognosis than early-onset GVHD.[16] Because chimerism detection was not yet available in our institute, we employed symptom and histopathological evaluations of skin to diagnose GVHD. Early diagnosis was difficult; the lack of a biomarker and the delay in diagnosis and complexity in treatment of GVHD after LT could influence the prognosis of this disease.

During the past decade, many efforts have been devoted to identifying potential biomarkers to diagnose GVHD without invasive tissue biopsies. For example, several different immune cells, particularly T cells, may serve as promising cellular biomarkers that could diagnose, predict risk, and evaluate responsiveness to treatment in GVHD.[21-23] Furthermore, because of the potential cytokine storm occurring early after LT, interleukin 2 (IL-2) and tumor necrosis factor α have been tested as potential GVHD biomarkers, which was helpful to understanding pathophysiology and target therapy.[24] However, cytokines were not specific for GVHD, often being elevated in inflammation and other complications. In addition to cytokines, microRNA-based diagnostic panel (miR-155 and miR-146a) and lymphocyte surface molecules (CD30 and α4β7 integrin) were found to be upregulated in acute GVHD, showing association with T-cell function, and thus could be biomarkers for GVHD, from mechanism to diagnostic and prognosis.[25-29]

Proteomic studies have provided promising insights into biomarker discovery for GVHD, from mechanism to diagnostic and prognosis. Recent studies have revealed elafin to be a biomarker of skin GVHD[30] and regenerating islet-derived 3-alpha (reg3α) as a relevant biomarker in gastrointestinal acute GVHD[31]; both of these molecules were secreted as a result of end-stage organ damage. These studies mainly focused on patients who underwent hematopoietic stem cell transplant; however, there is still no validated diagnostic blood test that could be used in routine clinical visits. In a study from Meng and colleagues,[32] IL-2, IL-18, and interferon γ were identified as potential biomarkers for early diagnosis and for monitoring the effects of anti-GVHD treatment; however, this was the only other study (other than ours) involved in study of GVHD biomarkers after LT.

Our previous study[15] identified biomarkers after LT by a novel but robust method using untargeted MALDI-TOF proteomics with network analysis and validation by ELISA. Based on this previous method, in this study, we explored the differentially expressed proteins in a small cohort with GVHD of LT. As a result, our identification of ACLY and FGA for GVHD associated with LT might also aid in diagnosis.

The key metabolic enzyme ACLY interconnects glucose and lipid metabolism; ACLY is increased or activated in many different kinds of tumors, whereas inhibition of ACLY could arrest cancer cell proliferation.[33] In addition, ACLY may play an important role in various chronic diseases, including cardiovascular diseases, inflammation, and neuro-degenerative diseases.[34]

Fibrinogen is a plasma glycoprotein, which is involved in many physiopathological processes, such as blood coagulation, inflammation, and angiogenesis.[35] Drew and colleagues found that fibrinogen may regulate tissue repair via supplying the matrix and accelerating cell proliferation and migration.[36] Through evaluation in cancer and other diseases, FGA was identified as an alpha component of human fibrinogen.[37,38]

Our study had several limitations. First, although powerful statistical tests are useful, they are not robust enough to identify biomarkers in such a small population. Sufficient validation is still needed in an independent and diverse cohort. To overcome the challenge of a small sample size and to further confirm the results in patients with GVHD after LT, additional serum and liver tissue samples should be obtained and patients with GVHD after other solid-organ transplant procedures and bone marrow transplant should be investigated. Screening LT recipients who show some symptoms of GVHD is helpful but not for those who have GVHD-like febrile complications or allergic skin rash or cytopenia due to drug toxicity. We acknowledge that ACLY and FGA may be altered in cancers, inflammation, and other conditions, and their use should be combined with other clinical indicators in practice.

Conclusions

In our study, when we reviewed 65 peptide peaks distinguishing patients with GVHD from patients with

excellent posttransplant outcomes and control patients, we identified 2 significantly differently peaks as potential biomarkers. These 2 downregulated peptides were identified as ACLY and FGA and validated by ELISA and Western blot. As far as we are aware, this study is the first to show the downregulation of ACLY and FGA in serum samples from patients with GVHD after LT through untargeted MALDI-TOF proteomics with network analysis, which were thereafter validated by ELISA and conventional statistical analyses, suggesting that ACLY and FGA are potential serum biomarkers for GVHD.

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Correlation of Deceased Donor Factors to Postreperfusion Severe Hyperglycemia in Adult Patients Undergoing Liver Transplant

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Abstract

Objectives: **In this study, our objective was to identify perioperative factors associated with postreperfusion severe hyperglycemia, with a particular focus on deceased donor factors.**

Materials and Methods: **Perioperative data from 100 patients without diabetes who were undergoing liver transplant from deceased donors were reviewed. Mean blood glucose levels were calculated at each liver transplant surgical phase, with a cutoff level of 12.7 mmol/L (230 mg/dL) during the neo-hepatic phase defined as postreperfusion severe hyperglycemia. Patients were divided into those with and without postreperfusion severe hyperglycemia. Selected perioperative variables were compared between the 2 groups.**

Results: **Of 100 patients, 55 developed postreperfusion severe hyperglycemia. Among donor variables, a statistically significant difference between groups was only shown for graft-to-recipient liver weight ratio (*P* < .001). With regard to preoperative recipient variables, the 2 groups showed a significant difference in mean age (*P* = .001). Patients in the postreperfusion severe hyperglycemia group required significantly more packed red blood cell transfusions (*P* = .002), sodium bicarbonate (*P* = .054), and vasopressors (*P* = .002) during the operation. Moreover, in terms of laboratory findings, although the last arterial pH was acceptable in both groups, a last lower arterial pH was observed in patients with postreperfusion severe hyperglycemia (*P* = .011). Higher mean blood glucose levels were detected in the postreperfusion hyperglycemia group during the preanhepatic and anhepatic phases (*P* = .024, *P* = .001, respectively).**

Conclusions: **In patients undergoing liver transplant, incidence of postreperfusion severe hyperglycemia was influenced by graft-to-recipient liver weight ratio. Furthermore, postreperfusion severe hyperglycemia was associated with intraoperative clinical and laboratory disturbances in liver transplant recipients.**

Key words: *Blood glucose, End-stage liver disease, Graft-to-recipient liver weight ratio, Reperfusion*

Introduction

Glucose homeostasis is frequently impaired in patients with end-stage liver disease, which is caused by multiple mechanisms such as peripheral resistance to insulin and hyperinsulinemia.[1] In addition, liver transplantation (LT) is associated with various metabolic disturbances. During LT, blood glucose status often worsens in recipients, and progressive hyperglycemia may occur; indeed, blood glucose levels often peak after liver graft reperfusion.[2] Hyperglycemia during LT can be caused by a variety of exogenous factors, including surgical stress, steroids, blood transfusions, and catecholamine vasopressors.[3] According to results from experimental studies, hyperglycemia increases the inflammatory response, which can exacerbate ischemia-reperfusion injury.[4,5] High blood glucose variability, but not hyperglycemia, during the intraoperative period and the early period after LT may also be associated with the development of certain complications, such as acute kidney injury.[6] Hence, the most logical approach is to manage variabilities in blood glucose during LT.

There are few clinical studies on postreperfusion severe hyperglycemia (PRSH) in LT, and many of these studies have mainly emphasized the effects of

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PRSH on post-LT outcomes, such as infection, renal failure, and graft function.[7]

It is important to identify factors associated with PRSH to ensure appropriate management strategies. However, the relationship between donor factors and PRSH is not yet clear. In one study, Chung and colleagues[8] reported the contribution of donor factors to PRSH in patients undergoing living donor LT. However, to our knowledge, the relationship between perioperative factors of deceased donor LT (DDLT) and PRSH has not been reported. In the present study, our goal was to identify perioperative factors associated with PRSH and with regard to deceased donor factors.

Materials and Methods

Our retrospective study included 100 adult patients without diabetes pretransplant (age ≥18 years) who had undergone DDLT at the Organ Transplant Center of Mashhad University between August 2013 and December 2019. The University Ethics Committee approved this retrospective study (approval number: IR.MUMS.MEDICAL.REC. 1397.174) and waived the need for written informed consent.

Exclusion criteria included recipients who had received dextrose (20%, 50%) during LT for correction of hypoglycemia or hyperkalemia, liver retransplant, and multiorgan transplant.

The classic technique without venovenous bypass or the piggyback technique was used for LT. Donated liver grafts were prepared using University of Wisconsin solution.

All patients received standard induction anesthesia with fentanyl (1-2 μg/kg), propofol (0.5-2 mg/kg), and muscle relaxants with either succinylcholine and/or cisatracurium. Anesthesia was maintained with isoflurane in low to moderate concentrations (0.5-1.0 minimum alveolar concentration) and bolus cisatracurium. A remifentanil infusion (0.05-0.3 μg/kg/min) and bolus fentanyl were administered throughout LT based on the patient's hemodynamic responses. Mechanical ventilation was delivered at a tidal volume of 8 to 10 mL/kg using a mixture of medical air and oxygen at a fresh gas flow rate of 2 L/min, and respiratory rate was adjusted as needed to maintain normocapnia. After induction of anesthesia, a central venous pressure catheter and a radial arterial line were placed to allow continuous hemodynamic monitoring and blood sampling. The intravenous fluid included 1% to 2% albumin in normal saline, and the rate of infusion was regulated according to central venous pressure, urine output, and volume of blood loss. Excessive metabolic acidosis (base excess less than -6.0) was treated with sodium bicarbonate. Body core temperature was maintained using a whole body-sized warm blanket.

The use of inotropes and vasopressors was at the discretion of the anesthesiologist and in response to the patient's hemodynamic status. Transfusion of packed red blood cells (PRBCs) was used to target hematocrit level of 25% to 30%. Coagulation components were replaced under thromboelastography guidance to correct intraoperative coagulopathies.

Intraoperative blood glucose management

Glucose-containing solutions were not routinely used during LT. Blood glucose levels were assessed by arterial blood sampling at least every 1 hour until the end of LT. Mean blood glucose levels ≥12.7 mmol/L (230 mg/dL) during the neo-hepatic phase was defined as PRSH.

During LT, whenever the blood glucose level exceeded 11 mmol/L (200 mg/dL) after a bolus dose of 2 units of regular insulin, continuous infusion of insulin (2 U/h) was started. The target glucose level was 7.7 to 9.9 mmol/L (140-180 mg/dL). Blood glucose was monitored every 0.5 hour after starting the regular insulin infusion; for patients with uncontrolledblood glucose levels, the infusion rate was doubled. Hypoglycemia, blood glucose level <3.9 mmol/L (70 mg/dL), was corrected using 20% or 50% dextrose solution.

Patients were divided into 2 groups (PRSH and non-PRSH) using a mean blood glucose cutoff level of 12.7 mmol/L (230 mg/dL) during the neo-hepatic phase. Donor variables collected for analyses included age, sex, total ischemia time of liver graft, and fatty changes in the graft. In addition, prepared donor livers and recipient livers were weighed after hepatectomy, and the graft-to-recipient liver weight ratio was calculated.

Preoperative recipient variables collected for analyses included age, sex, body mass index, indication for LT, Model for End-Stage Liver Disease (MELD) score, Child-Pugh-Turcotte score, history of ascites, hepatorenal syndrome, hepatic

encephalopathy, gastrointestinal bleeding, concurrent diseases, and echocardiographic and laboratory findings. All preoperative variables were based on the latest data before LT. Intraoperative variables included surgical time, amount of crystalloid or colloid infusion, blood and blood product transfusion, hourly urine output (mL/kg/h), amount of ascites, postreperfusion syndrome (PRS), administered drugs (vasopressors, calcium gluconate, sodium bicarbonate, and regular insulin), and mean blood glucose (mg/dL) at the pre-anhepatic phase, anhepatic phase, and neo-hepatic phase of LT, as well as last arterial pH level.

Statistical analyses

Results are expressed as number (%) for categorical variables and mean ± SD or median (range) for continuous variables. The chi-square test or the Fisher exact test was used for qualitative variables, and the Mann-Whitney test was used for continuous variables. $P < .05$ was considered statistically significant. All statistical analyses were performed with the use of SPSS version 16 software.

Results

This retrospective observational study included 100 patients who underwent DDLT. Of these, 55 patients were classified into the PRSH group.

The mean age of donors was 39 ± 16 years, and most were men (69%). The proportions of graft-to-recipient liver weight ratio <1.09% and >1.1% were 47% and 53%, respectively. The mean total ischemic time of the graft was 204 minutes. On graft biopsy, 46% showed fatty changes <5%, 38% showed fatty changes of 5% to 10%, and 16% had fatty changes of 10% to 30%. The relationship between donor factors and PRSH is shown in Table 1.

Pretransplant recipient variables for both patient groups are shown in Table 2. Mean age of recipients was 45 years, and most were men (75%). Dominant causes for LT in our study group were cryptogenic cirrhosis (28%) and hepatitis B virus cirrhosis (21%). Among recipients, 53% of patients had Child-Pugh-Turcotte class B. The overall MELD score was 19.7 ± 5.12 points. All recipients had endotracheal tube extubation in the operating room.

During DDLT, patients with PRSH required significantly more PRBC transfusions ($P = .002$), more sodium bicarbonate ($P = .054$), and more vasopressors ($P = .002$) than patients without PRSH. With regard to laboratory findings, patients with PRSH had lower last arterial pH ($P = .011$). However, arterial pH was acceptable in both study groups.

Throughout DDLT, blood glucose levels progressively increased (Figure 1). Significantly higher mean blood glucose levels were observed in the PRSH group during the pre-anhepatic and anhepatic phases ($P = .024$ and $P = .001$, respectively). Table 3 shows the comparison of intraoperative variables between the PRSH and non-PRSH groups.

Table 1. Relationships Between Donor Factors and Postreperfusion Severe Hyperglycemia

Variable	Non-PRSH (n = 45)	PRSH (n = 55)	*P* Value
Age, years	40.0 ± 17.2	38.2 ± 15.3	.375
Male sex, No. (%)	31 (68.9%)	38 (69.1%)	.983
History of cardiopulmonary resuscitation, No. (%)	17 (37.8%)	15 (27.3%)	.481
Graft fatty change, No. (%)			.723
<5%	19 (42.2%)	27 (49.1%)	
5%-10%	19 (42.2%)	19 (34.5%)	
>10%-30%	7 (15.6%)	9 (16.4%)	
GRWR, No. (%)			<.001
≤1.09	34 (75.6%)	13 (23.6%)	
≥1.1	11 (24.4%)	42 (76.4%)	

Abbreviations: GRWR, graft–to–recipient weight ratio; PRSH, postreperfusion severe hyperglycemia
Values are expressed as means ± SD or numbers (percent).

Table 2. Relationship Between Pretransplant Recipient Variables and Postreperfusion Severe Hyperglycemia

Preoperative Variable	Non-PRSH (n = 45)	PRSH (n = 55)	*P* Value
Age, years	39.9 ± 15.2	49.9 ± 13.8	.001
Male (%)	33 (73.3%)	42 (76.4%)	.728
Body mass index, kg/m^2	23.5 ± 5.0	24.6 ± 4.8	.276
Cause of LT, cirrhosis/cancer, No.	41/4	51/4	.767
MELD score	19.1 ± 5.0	20.4 ± 5.1	.201
CPT class, No. (%)			.522
A	4 (8.9%)	8 (14.5%)	
B	23(51.1%)	30 (54.5%)	
C	18 (40%)	17 (30.9%)	
Accompanying systemic disease, No. (%)			.313
Hypertension	1 (2.2%)	1 (1.8%)	
Hepatorenal syndrome	0 (0%)	4 (7.3%)	
Severe hepatic encephalopathy (grade III/IV)	5 (11.1%)	5 (9.1%)	
Varix bleeding	12 (26.7%)	9 (16.4%)	
Ascites, L	3.8 ± 2.4	3.2 ± 2.3	.850
Echocardiography findings, No. (%)			
Regional wall motion abnormality	0 (0%)	2 (3.6%)	.139
Diastolic dysfunction	7 (15.6%)	15 (27.3%)	.270
Ejection fraction	55.5 ± 3.3	54.2 ± 5.03	.161

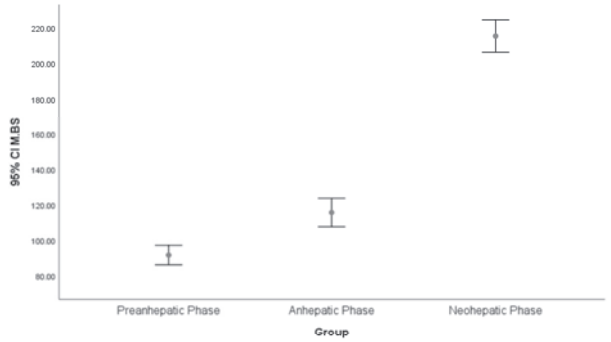
Abbreviations: CPT, Child-Pugh Turcotte; LT, liver transplant; MELD, Model for End-Stage Liver Disease; PRSH, postreperfusion severe hyperglycemia
Values are expressed as means ± SD or numbers (percent).

Table 3. Relationship Between Intraoperative Factors and Postreperfusion Severe Hyperglycemia

Preoperative Variable	Non-PRSH (n = 45)	PRSH (n = 55)	*P* Value
Surgical time, min	327 ± 61	347 ± 67	.123
PRBC transfusion, pints	1.5 ± 1.2	2.4 ± 1.6	.002
Fluid solution, L	3.3 ± 0.67	3.6 ± 0.87	.84
Hourly urine output, mL/kg/h	2.8 ± 1.2	2.4 ± 1.1	.150
Postreperfusion syndrome	3 (6.6%)	5 (9.1%)	.42
Administered drugs			
Calcium gluconate, g	2.6 ± 1.9	3.1 ± 1.9	.146
Sodium bicarbonate, mEq	121 ± 41	139 ± 50	.054
Regular insulin, U	0	10.74 ± 10.30	<.001
Albumin, g	65 ± 20	70 ± 15	.249
Vasopressors, No. (%)	5 (11.1%)	21 (38.2%)	.002
Initial arterial pH	7.40 ± 0.07	7.40 ± 0.06	.461
Last arterial pH	7.37 ± 0.08	7.33 ± 0.07	.011
Mean blood glucose, mmol/L			
Pre-anhepatic phase	4.6 ± 0.9	5.4 ± 1.7	.024
Anhepatic phase	5.8 ± 1.5	6.8 ± 2.6	.001
Neo-hepatic phase	10.1 ± 1.1	13.3 ± 2.2	.118

Abbreviations: PRBC, packed red blood cell; PRSH, postreperfusion severe hyperglycemia
Values are expressed as means ± SD or numbers (percent).

Figure 1. Trends in Blood Glucose Concentrations During Deceased Donor Adult Liver Transplant



Abbreviations: MBS, mean blood sugar

Discussion

Our observational study indicated that, during DDLT, processes leading to PRSH were influenced by donor-related factors, particularly graft-to-recipient liver weight.

To our knowledge, only 1 study evaluated the relationship between PRSH and liver donor factors. This retrospective study, by Chung and colleagues,[8] identified that liver graft size, extent of fatty change, and PRS were independent donor-associated predictors of PRSH during living donor LT.

Graft size plays an important role in determining outcomes after partial graft LT. Indeed, graft–to–recipient weight ratio is an important criterion during selection of donors for living donor LT.[9] In our study, we investigated whole graft LT from deceased donors. Various definitions of estimated standard liver weight have been proposed in the literature.[10] Therefore, we considered the graft-to-recipient liver weight ratio in our study.

Graft size affects glucose influx into the recipient's circulation. The sudden increase of blood glucose in the early neo-hepatic phase can occur as a result of glucose influx from the grafted liver, secondary to ischemic injury.[11,12] Restoration of the transplanted graft function after LT is associated with suppression of PRSH.[13]

Postreperfusion syndrome has been defined as at least a 30% decrease in mean arterial pressure occurring during the first 5 minutes after liver graft reperfusion and lasting longer than 1 minute.[14] The underlying pathophysiological mechanisms of PRS associated with hyperglycemia are not well known. However, production of inflammatory mediators may contribute to PRS, which can eventually lead to activation of hepatic gluconeogenesis and peripheral insulin resistance.[15] Interestingly, hyperglycemia exacerbates ischemia-reperfusion injury by inducing inflammation.[16]

In our study, we found that incidence of PRS was not significantly different between the 2 study groups; thus PRS may have a negative effect on intraoperative glucose homeostasis independent of PRSH. Moreover, patients with PRSH needed more PRBC transfusions and higher doses of vasopressor and sodium bicarbonate during surgery. Hence, PRSH is a complex and unclear issue.

An accepted criteria for liver donation at our center is graft fatty change of ≤30%. In the present study, our 2 study groups showed no significant differences in the extent of graft fatty changes, which is contrary to the results from Chung and colleagues.[8] In light of these contradictions, further studies are recommended to explain the differences in findings between DDLT versus living donor LT.

The main limitation of our study was that glucose-related cytokine levels were not assayed.

Conclusions

Incidence of PRSH in DDLT recipients was strongly influenced by donor-related factors. The graft-to-recipient liver weight ratio was identified as an independent predictor of PRSH. Because PRSH is a complex process and might be an indicator of graft outcome, further studies on this topic are recommended.

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Immediate Tracheal Extubation After Pediatric Liver Transplantation

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Abstract

Objectives: **We examined whether immediate tracheal extubation among pediatric liver transplant recipients was safe and feasible.**

Materials and Methods: **We retrospectively analyzed medical records of pediatric liver transplant recipients at Baskent University Hospital from January 2012 to December 2017. We grouped children who were extubated in the operating room versus those extubated in the intensive care unit.**

Results: **In our study group of 81 pediatric patients, median age was 4 years (range, 4 mo to 16 y) and 44 (54%) were male. Immediate tracheal extubation in the operating room was performed in 39 patients (48%). Children who remained intubated (n = 42) had more frequent massive hemorrhage (14% vs 0%; *P* = .015), received larger amounts of packed red blood cells (19.3 vs 10.2 mL/kg; *P* < .001), and had higher serum lactate levels (9.0 vs 6.9 mmol/L; *P* = .001) intraoperatively. All children with open abdomens postoperatively remained intubated (n = 7). Patients extubated in the operating room received less vasopressors (1 [3%] vs 12 [29%]; *P* = .002) and antibiotics (11 [28%] vs 22 [52%]; *P* = 0.041) and developed infections less frequently postoperatively (3.0 [8%] vs 15.0 [36%]; *P* = .003). Children extubated in the operating room had shorter mean stay in the intensive care unit (2.0 vs 4.5 days; *P* < .001). Hospital mortality was higher in children who remained intubated (12% vs 0%; *P* = .026).**

Conclusions: **Immediate tracheal extubation was well tolerated in almost half of our patients and did not compromise their outcomes. Patients who remained intubated had longer intensive care unit stays and higher hospital mortalities. Therefore, we recommend immediate tracheal extubation in the operating room after pediatric liver transplant among those children without intraoperative requirements for massive blood transfusion, high-dose vasopressors, high serum lactate levels, and open abdomen.**

Key words: *Blood transfusion, Intensive care unit, Intubation*

Introduction

Liver transplant is the most important treatment modality for end-stage liver failure. The first liver transplant was performed in 1963 by Starzl and his team.[1,2] In Turkey, the first liver transplant was performed by Dr. Haberal and his team in 1988. This team also performed the first pediatric liver transplant from a living donor in 1990.[3] Survival after liver transplant has increased due to improvements in surgical and anesthetic methods, postoperative follow-up, and innovations in medical therapies.

Several publications have documented that early tracheal extubation after liver transplant reduces respiratory and infectious complications by shortening the duration of mechanical ventilation in adult patients, shortens the duration of intensive care and hospital stays, and reduces hospital costs.[4-6] Moreover, mechanical ventilation has negative effects on transplant recipients, such as reduced graft blood flow.[7] For this reason, many centers prefer early tracheal extubation to achieve optimal results.[5] However, there are only a limited number of publications on early tracheal extubation in the pediatric patients.[8]

In this study, we aimed to determine the incidence of immediate tracheal extubation in pediatric transplant recipients at our center and compare those who had undergone immediate tracheal extubation versus

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those who did not. We also aimed to determine whether this approach is safe and feasible.

Materials and Methods

This study was approved by the Baskent University Institutional Review Board (project no. KA 17/279).

We retrospectively analyzed the medical records of pediatric patients who underwent liver transplant at Baskent University Hospital from January 2012 to December 2017. Patients were extubated at the end of surgery when they were awake, responsive, and met universally accepted criteria, including metabolic and hemodynamic stability. Children were divided into 2 groups: those who were extubated in the operating room (OR) and those extubated later in the intensive care unit (ICU). Collected data included demographic characteristics of children (age, sex, body weight, body mass index), systemic diseases, drugs, Pediatric End-stage Liver Disease (PELD) score, Model of End-stage Liver Disease (MELD) score, perioperative laboratory values and hemodynamic parameters, intraoperative massive hemorrhage, extubation time, length of ICU or hospital stay, and hospital mortality. We defined massive hemorrhage as blood loss exceeding 150 mL/min.[9]

Patients over the age of 16 years and patients with missing data were excluded from the study. The same anesthetic technique was used in all patients. Anesthesia was induced with a combination of propofol (1.5-2.5 mg/kg) and fentanyl (3-5 μg/kg). Rocuronium was given to facilitate endotracheal intubation (0.5-1.2 mg/kg) and maintain paralysis during surgery (0.01-0.012 mg/kg/min). Anesthesia maintenance was achieved with a sevoflurane-air-oxygen mixture and an infusion of remifentanil (0.1-0.2 μg/kg/min). Routine monitoring included electrocardiography, pulse oximetry, capnography, nasopharyngeal temperature, invasive arterial pressure (radial pressure), and central venous pressure via the subclavian or internal jugular vein. After surgery, all patients were admitted to the intensive care unit. The same surgical, anesthesia, and intensivist teams were assigned during the perioperative period of all liver transplant surgeries.

Statistical analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 20.0, IBM Corporation, Armonk, NY, USA). Results are shown as mean and standard deviation for continuous variables and number (%) for categorical variables. $P \leq 0.05$ was accepted as the cutoff for significance.

Results

During the study period, 81 pediatric liver transplant recipients were admitted to the ICU. The median age of children was 4 years (range, 4 mo to 16 y), and 44 of them were male (54%). The demographic characteristics of patients included in the study are shown in Table 1. The most frequent diagnosis was biliary atresia (n = 18, 22%). Patient diagnosis, PELD score, MELD score, and presence of comorbidities are shown in Table 2. The preoperative laboratory values are shown in Table 3.

Table 1. Demographic Characteristics of the Study Population

Characteristic	Median (Minimum to Maximum) or No. (%)
Age	4 years (4 mo to 16 y)
Body weight, kg	13.9 (6-62)
Height, cm	95 (54-174)
Male	44 (54%)

Table 2. Cause of Liver Failure, Scores, and Presence of Comorbidities

Parameter	Median (Minimum to Maximum) or No. (%)
Cause of liver failure	
Biliary atresia	18 (22%)
Wilson	10 (12%)
Cryptogenic	7 (9%)
Neonatal cholestasis	6 (7%)
Urea cycle defects	5 (6%)
Acute hepatic failure	5 (6%)
PFIC	4 (5%)
Acute hepatitis	4 (5%)
Other	22 (27%)
PELD score	12.0 (-10.0 to 40.0)
MELD score	14.0 (6.0-39.0)
Comorbidity	35 (43%)

Abbreviations: MELD, Model for End-Stage Liver Disease; PELD, Pediatric End-Stage Liver Disease; PFIC, progressive familial intrahepatic cholestasis

Table 3. Preoperative Laboratory Values

Laboratory Parameter	Median (Minimum to Maximum
Hemoglobin, g/dL	10.4 (5.6-14.2)
Sodium, mmol/L	136.0 (128.0-151.0)
Albumin, g/dL	3.2 (1.9-4.7)
Total bilirubin, mg/dL	9.1 (0.1-54.3)
AST, U/L	115.0 (16.0-4200.0)
ALT, U/L	72.0 (8.0-4100.0)
Creatinine, mg/dL	0.4 (0.2-8.7)
INR	1.3 (0.7-4.4)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio

Immediate tracheal extubation was performed in 39 patients (48%) in the OR. Median postoperative duration of mechanical ventilation in those who were

extubated in the ICU was 12 hours (interquartile range [IQR], 5-19). There were no differences in demographic characteristics, PELD score, MELD score, graft type, comorbidities, and preoperative presence of infections and antibiotic use between the 2 groups (Table 4). There were no differences in terms of preoperative laboratory values between the 2 groups (Table 5).

Table 4. Comparison of Patient Groups in Terms of Demographics, Scores, and Preoperative Presence of Infections

Parameter	Median (Interquartile Range) or No. (%)		*P* Value
	With Immediate Extubation (n = 39)	Without Immediate Extubation (n = 42)	
Age, mo	60.0 (12.0-120.0)	39.0 (7.0-84.0)	.155
Male	20 (51%)	24 (57%)	.659
Body weight, kg	16.0 (8.7-37.0)	12.4 (7.7-25.0)	.136
Height, cm	100.0 (71.5-140.5)	83.0 (65.0-126.0)	.241
PELD	10.5 (6.3-22.3)	15.0 (1.0-23.0)	.950
MELD	16.0 (10.0-22.0)	11.0 (8.0-18.0)	.363
Living donor	36 (92%)	38 (91%)	1.000
Comorbidity	17 (44%)	18 (43%)	1.000
Presence of preoperative infection	7 (18%)	8 (19%)	1.000
Use of preoperative antibiotics	13 (33%)	15 (36%)	1.000

Abbreviations: MELD, Model for End-Stage Liver Disease; PELD, Pediatric End-Stage Liver Disease

Table 5. Comparison of Patient Groups in Terms of Preoperative Laboratory Values

Laboratory Parameter	Median (Interquartile Range)		*P* Value
	With Immediate Extubation (n = 39)	Without Immediate Extubation (n = 42)	
Hemoglobin, g/dL	10.0 (9.1-11.3)	10.4 (8.5-11.1)	.932
Sodium, mmol/L	136.5 (133.0-140.0)	135 (133.0-137.3)	.133
Albumin, g/dL	3.2 (2.5-3.6)	3.2 (2.8-3.9)	.377
Total bilirubin, mg/dL	11.4 (2.1-3.6)	4.7 (0.5-18.8)	.06
AST, U/L	148.0 (81.0-233.0)	97.0 (36.3-265.8)	.257
ALT, U/L	79.0 (52.0-189.0)	67.5 (22.0-208.5)	.333
Creatinine, mg/dL	0.4 (0.4-0.5)	0.4 (0.4-0.5)	.872
INR	1.4 (1.1-2.0)	1.3 (1.1-1.7)	.335

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio

Children who remained intubated had intraoperatively more frequent massive hemorrhage (14% vs 0%; $P = .015$), received larger amounts of packed red blood cells (19.3 mL/kg [IQR, 13.1-30.2] vs 10.2 mL/kg [IQR, 0-17.1]; $P < .001$), and had higher serum peak lactate levels (9.0 mmol/L [IQR, 6.9-13.0] vs 6.9 mmol/L [IQR, 4.4-9.0]; $P = .001$) intraoperatively versus those who were extubated in the OR (Table 6). There were no children with open abdomen who were immediately extubated; however, 7 patients (17%) with open abdomen remained intubated postoperatively ($P = .012$). Reintubation rate was less in those extubated in the OR (3% vs 24%; $P = .007$).

Compared with those who remained intubated, patients who were extubated in the OR received less vasopressors (1.0 [3%] vs 12.0 [29%]; $P = .002$) and less antibiotics (11.0 [28%] vs 22.0 [52%]; $P = .041$) and developed infections less frequently postoperatively (3.0 [8%] vs 15.0 (36%); $P = .003$). Compared with patients who remained intubated, the mean length of ICU stay was shorter in children extubated in the OR (2.0 days [IQR, 1.0-4.0] vs 4.5 days [IQR, 3.0-14.0]; $P < .001$). Hospital mortality rate was higher in children who remained intubated after surgery (12% vs 0%; $P = .026$). The overall mortality rate was 6% (n = 5) (Table 7).

Table 6. Comparison of Patient Groups in Terms of Intraoperative Management

Intraoperative Parameter	Median (Interquartile Range) or No. (%)		*P* Value
	With Immediate Extubation (n = 39)	Without Immediate Extubation (n = 42)	
Duration of anesthesia, h	9.5 (9.0-10.0)	9.3 (8.5-11.0)	.721
Cold ischemia time, h	0 (0)	0 (0)	.609
Massive hemorrhage	0 (0)	6.0 (14%)	.026
PRBC per body weight, mL/kg	10.2 (0-17.1)	19.3 (13.1-30.2)	< .001
FFP per body weight, mL/kg	0.9 (0-9.3)	2.9 (0-15.5)	.508
Platelet transfusion per body weight, mL/kg	0 (0)	0 (0)	.972
Crystalloids per body weight, mL/kg	111.5 (79.7-155.7)	113.1 (80.0-144.8)	.880
Use of vasopressors	15 (39%)	23 (55%)	.183
Use of inotropes	8 (21%)	10 (24%)	.793
Acidosis	32 (82%)	39 (93%)	.184
Highest lactate level, mmol/L	6.9 (4.4-9.0)	9.0 (6.9-13.0)	.001

Abbreviations: FFP, fresh frozen plasma; PRBC, packed red blood cells

Table 7. Comparison of Patient Groups in Terms of Postoperative Management and Outcomes

Postoperative Parameter	Median (Interquartile Range) or No. (%)		*P* Value
	With Immediate Extubation (n = 39)	Without Immediate Extubation (n = 42)	
Presence of open abdomen	0 (0)	7 (17%)	.012
Use of vasopressors	1 (3%)	12 (29%)	.002
Use of inotropes	37 (95%)	41 (98%)	.606
Presence of infections	3 (8%)	15 (36%)	.003
Use of antibiotics	11 (28%)	22 (52%)	.041
RRT requirement	0 (0)	5 (12%)	.056
Reintubation	1 (3%)	10 (24%)	.007
Tracheostomy	2 (5%)	5 (12%)	.434
Retransplantation	0 (0)	2 (5%)	.494
Duration of MV, h	0 (0)	12.0 (5.0-19.0)	< .001
Length of ICU stay, d	2.0 (1.0-4.0)	4.5 (2.0-14.0)	< .001
Length of hospital stay, d	30.0 (21.0-37.0)	31.5 (21.0-50.5)	.478
Mortality	0 (0)	5 (12%)	.026

Abbreviations: ICU, intensive care unit; MV, mechanical ventilation; RRT, renal replacement therapy

Discussion

In our retrospective 5-year follow-up of pediatric liver transplant recipients, we noted some differences in perioperative parameters of patients who had undergone immediate tracheal extubation versus those who had not. Immediate tracheal extubation was well tolerated in almost half of our patients and did not compromise their outcomes. Patients who had experienced massive bleeding in the intraoperative period, had higher intraoperative serum lactate levels, and had received more erythrocyte suspension transfusions were observed to be extubated more frequently in the postoperative ICU. The use of vasopressors and antibiotics in the postoperative period, the frequency of infection development, and the presence of open-abdominal activity were also observed to be higher than in patients extubated in the OR. Reintubation frequency, duration of ICU stay, and hospital mortality rates were also reported to be higher in patients who were extubated in the ICU.

In our study, all patients with intraoperative massive bleeding were extubated in the ICU; accordingly, intraoperative transfusion of packed red blood cells was more frequently performed in this patient group. In a retrospective study of 128 pediatric patients conducted in 2017 by Nafiu and associates,[10] incidences of postoperative prolonged mechanical ventilation were higher in patients who had undergone more intraoperative transfusions of packed red blood cells. In a retrospective study of 100 adult patients in which the predictors of early tracheal extubation in the OR after liver transplant were investigated, Zeyneloglu and colleagues[11] found that patients with massive bleeding were mostly extubated in the ICU. The findings in these 2 studies are consistent with the findings of our present study regarding massive bleeding and transfusion findings. However, in a retrospective study by Fullington and associates[8] of 84 pediatric patients, excess blood product transfusions did not prevent early tracheal extubation after liver transplant.

In our clinic, patients with massive blood transfusions are transferred to the ICU while still intubated. However, Fullington and associates concluded that the amount of blood transfusion was not, and should not, be a criterion for extubation; their regular practice was to carry out extubations without considering this. We believe that the difference between our study and the study from Fullington and associates[8] may be a result of differences in clinical approach.

Lactate is an intermediate product that is created by carbohydrate and nonessential amino acid metabolism. It is generated in many places, including skin, erythrocytes, central nervous system, and muscle; it is cleared by 60% in the liver and 30% in the kidney.[12,13] Lactate is seen in cases of imbalance between tissue oxygen delivery and consumption (ie, hypoxia-related cases) and may increase in cases that are not accompanied by hypoxia, drug intoxication, mitochondrial myopathies and hypoglycemia.[14] In our study, there were significantly fewer extubations of patients with high intraoperative serum lactate levels. In a retrospective study with 44 adult patients who were admitted to the ICU after liver transplant, Basile-Filho and associates[15] reported that lactate was effective in predicting 1-month mortality and that serum lactate levels were significantly lower in the surviving group. In a prospective study of 53 patients with postnecrotic cirrhosis, De Gasperi and associates[16] followed lactate levels during different phases of liver transplant (preanhepatic, anhepatic, neohepatic) and during the first 48 hours after revascularization. They reported that a low lactate profile after liver transplant could be used as an indicator of early recovery. Compatible with the literature, our study has shown that high serum lactate levels may be associated with poor patient outcomes, such as prolonged mechanical ventilation. In our study, all patients with an open abdomen were found to be intubated when the surgery was completed and extubated in the ICU during the postoperative period. The literature has many studies in which early complications related to open abdomens have included prolonged intubation, fluid loss, delayed early mobilization, and infection.[17-20] Mechanical ventilation in our patients with open abdomen was also prolonged in accordance with that shown in the literature. Infections and antibiotic use were more frequent among patients who were extubated in the ICU than in those who were extubated in the OR. This may be due to the increased risk of ventilator-associated pneumonia in prolonged mechanical ventilation, as well as peritoneal cavity contamination-related intraabdominal infections caused by an open abdomen. Another reason may be that patients with open abdomens undergo recurrent operations.

In our study, patients who remained intubated because they could not be extubated in the OR required higher doses of vasopressors intraoperatively. In contrast, Fullington and associates[8] reported that the use of vasopressors in pediatric liver transplant recipients did not affect early extubation but reported that they did not immediately extubate patients on high doses of vasopressors due to their protocols. In this regard, the incidence of vasopressor use was higher in patients who could not be extubated, although not to a significant degree.

In our study, the frequency of reintubation in patients who underwent an immediate tracheal extubation in the OR at the end of the surgical procedure was found to be statistically and significantly lower than in those who were extubated in the ICU. In parallel with our study, Hoffmeister and associates[21] reported that reintubation frequencies were lower among patients extubated in the OR after liver transplant. Glanemann and associates[22] reported a significantly higher reintubation frequency in adult patients who underwent prolonged mechanical ventilation after liver transplant. Our finding is consistent with the literature, although there were insufficient data available in the literature related to the pediatric patient population.

In our study, length of ICU stay and hospital mortality rates were lower in children who were extubated immediately compared with those extubated in the ICU. Nafiu and associates[10] reported that prolonged mechanical ventilation extended the length of ICU stay after pediatric liver transplant and increased posttransplant mortality rates. However, Fullington and associates[8] found that ICU and hospital stay durations were longer, and only the length of the hospital stay was statistically significant in the group who had undergone an immediate tracheal extubation.

Our study has several limitations, mainly because of its retrospective design. The first limitation was that some of the perioperative data could not be reached. The second limitation was that, in a pediatric patient group, ages and body weights varied widely. However, to overcome this limitation, we tried to limit the amount of intravenous fluids or blood transfusion products in proportion to body weight. Finally, some parameters did not show statistical significance, which could perhaps be attributed to an insufficient number of patients.

For children with intraoperative massive hemorrhage, massive blood transfusions, high serum lactate levels, open abdomen, and high-dose vasopressor treatment and for those with a higher possibility of developing infection and likely to use antibiotics in the postoperative period, it might be more appropriate to lengthen the intubation period and plan extubation in the ICU after liver transplant. When these conditions are not met and if the patient satisfies universal extubation criteria in the postoperative period, immediate tracheal extubation after pediatric liver transplant is safe and feasible. Nevertheless, studies with more patients should be conducted to verify these results.

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Management of Urological Malignancy in Heart and Lung Transplant Recipients: An Irish National Cohort Study

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Abstract

Objectives: **Following the first heart transplant in Ireland in 1985, there have been almost 700 deceased donor heart and lung transplants carried out in Ireland at a single institution. In this retrospective study, our aim was to assess the incidence and management of urological malignancies arising in this national cohort.**
Materials and Methods: **Our retrospective analysis included all heart and lung transplant recipients identified as having a urological malignancy. Primary outcome variables included incidence, management, and clinical outcomes following cancer diagnosis.**
Results: **A total of 28 patients (4.1%) had radiologically or histologically confirmed urological malignancies. Fourteen patients were diagnosed with prostate cancer, with 13 who underwent radical treatment. Eight renal cell carcinomas were diagnosed in heart transplant recipients, with 5 who underwent nephrectomies. Two bladder cancers and 1 upper tract urothelial carcinoma were diagnosed and managed with endoscopic resection, radiotherapy, and nephroureterectomy, respectively. Two patients were diagnosed with penile squamous cell carcinoma and managed with radical surgery and lymph node dissection/sampling, with 1 patient receiving adjuvant chemoradiotherapy.**
Conclusions: **Urological malignancies are not common in heart and lung transplant recipients; however, standard management options can be safely used, including radical surgery. Prospective monitoring of these patients and potential considerations for screening should be maintained.**

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Key words: *Penile cancer, Prostate cancer, Renal cancer, Urothelial cancer, Screening*

Introduction

Heart transplants have been performed in Ireland since 1985; since the inclusion of lung transplants in 2005, there have been almost 700 heart and lung transplants carried out in total. All transplants have been performed at a single institution in Ireland (the Mater Misericordiae University Hospital [MMUH], Dublin, Ireland).

Organ transplantation is considered lifesaving, is associated with improved quality of life, and confers significant financial savings to health services; however, an increased incidence of cancer has been shown in Irish solid-organ transplant recipients.[1] This study aimed to characterize the urological malignancies within the national heart and lung transplant group thus far and to assess their treatment to date with the hope to inform urologists and transplant physicians regarding optimal management going forward.

Materials and Methods

For this retrospective study, we analyzed all patients who underwent heart transplant since 1985 and lung transplant since 2005 at the MMUH National Heart and Lung Transplant Centre. Medical records of these recipients have been prospectively collected and maintained by the MMUH transplant data managers since the establishment of the respective transplant programs. All patient details are held securely within the hospital-based electronic medical records.

For each heart and lung transplant recipient, we used the hospital's electronic medical records, outpatient letters, histopathology reports, theatre

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reports, and radiology reports to identify those who had been diagnosed with urological malignancies. Transplant details, such as date, indication, immunosuppressive regimen, and overall outcomes, were recorded. Time to diagnosis of malignancy, age at diagnosis, stage of disease (including histopathology and radiology findings), management, and outcomes specifically pertaining to cancer were also recorded. We also gathered information on complications arising from treatment of urological malignancies and outcomes at time of submission of this paper. Departmental approval was granted, although individual patient consent was not obtained due to the retrospective and noninterventional nature of the study.

Results

Prostate cancer

Twelve heart transplant and 2 lung transplant recipients (total 2.8% of all male transplant recipients) were diagnosed with prostate cancer. Average age at diagnosis was 58 years at 9.8 years posttransplant, with average prostate-specific antigen (PSA) level at diagnosis of 16 ng/mL. Staging and treatment decisions had been based on National Cancer Control Programme guidelines for the general population diagnosed with prostate cancer in Ireland.[2] Risk stratification into low, intermediate, or high risk of biochemical recurrence had been based on contemporary European Association of Urology (EAU) guidelines.[3]

Ten patients (71%) received radical radiotherapy, with 9 receiving external beam radiotherapy and 1 patient receiving brachytherapy. Characteristics of this subgroup are shown in Table 1. No patients experienced graft dysfunction or complications of their treatment. There were 3 deaths in this subgroup during the study period; however, no deaths were related to prostate disease. Three patients included in the radiotherapy group also received adjuvant androgen-deprivation therapy. One of these 3 patients also received adjuvant systemic therapy with enzalutamide for biochemical recurrence of high-risk prostate cancer. Characteristics of this subgroup are shown in Table 2.

Three heart transplant recipients with prostate cancer (21%) opted for radical surgery, with 1 having open radical prostatectomy and 2 having robotic-assisted laparoscopic prostatectomies. Characteristics of this subgroup are shown in Table 3. To date, none of these patients have had biochemical recurrence (PSA >0.03 ng/mL) of cancer following treatment. Functionally, all patients were fully continent, although all displayed erectile dysfunction postoperatively. One patient died 8 years after prostatectomy; however, this death was unrelated to prostate cancer (PSA of 0 ng/mL prior to death).

Table 1. Characteristics of Patients Who Underwent Only Radical Radiotherapy for Localized Prostate Cancer

Characteristic	Finding
Age at transplant (range)	57 (48-63) years
Indication for transplant	Pulmonary fibrosis, 1 patient Ischemic cardiomyopathy, 4 patients Dilated cardiomyopathy, 2 patients
Age at prostate cancer diagnosis (range)	68 (58-75) years
PSA at diagnosis (range)	9.5 (5.8-24) ng/mL
Gleason and ISUP grade	
• Gleason 3+3, ISUP 1	2 patients
• Gleason 3+4, ISUP 2	6 patients
• Moderately differentiated	1 patient
Biochemical recurrence	0 patients
Complications from treatment	0 patients
Graft dysfunction	0 patients
Mean follow-up from cancer diagnosis (range)	64 (36-120) months
Deaths within subgroup	3 patients (1 from sepsis, 2 from cardiorespiratory disease)

Abbreviations: ISUP, International Society of Urologic Pathologists; PSA, prostate-specific antigen

Table 2. Characteristics of Patients Who Underwent Androgen Deprivation Therapy for High-Risk, Locally Advanced, or Metastatic Prostate Cancer

Characteristic	Finding
Age at transplant (range)	61 (57-66) years
Indication for transplant	Ischemic cardiomyopathy, 1 patient Dilated cardiomyopathy, 2 patients
Age at prostate cancer diagnosis (range)	70 (69-72) years
PSA at diagnosis (range)	57.6 (16-134) ng/mL
Gleason and ISUP grade	
• Gleason 4+4, ISUP 4	1 patient
• Gleason 5+5, ISUP 6	1 patient
• No histological confirmation	1 patient
Biochemical recurrence	1 (occurred after radiotherapy and ADT in high-risk disease)
Complications from treatment	0 patients
Graft dysfunction	0 patients
Mean follow-up from cancer diagnosis (range)	144 (120-156) months
Deaths within subgroup	0 patients

Abbreviations: ADT, androgen-deprivation therapy; ISUP, International Society of Urologic Pathologists; PSA, prostate-specific antigen

One patient (7%) who developed prostate cancer at age of 69 years, at 4 years after lung transplant, opted for watchful waiting. A Gleason 3+4=7 disease score with a PSA of 4.8 ng/mL was confirmed on

biopsy; however, the patient died within 12 months as a result of severe respiratory disease.

Table 3. Characteristics of Patients Who Underwent Radical Surgery for Localized Prostate Cancer

Characteristic	Finding
Age at transplant (range)	57 (52-61) years
Indication for transplant	Ischemic cardiomyopathy, 2 patients Dilated cardiomyopathy, 1 patient
Age at prostate cancer diagnosis (range)	67 (63-70) years
PSA at diagnosis (range)	19.6 (2.8-30) ng/mL
Gleason and ISUP grade	
• Gleason 3+3, ISUP 1	1 patient
• Gleason 3+4, ISUP 2	2 patients
Surgical pathology	
• Gleason 3+3, bilateral, no EPE, SVI, pT2c	1 patient
• Gleason 3+4, bilateral, no EPE/SVI, pT2c	2 patients
Biochemical recurrence	0 patients
Complications from treatment	0 patients
Graft dysfunction	0 patients
Mean follow-up from cancer diagnosis (range)	80 (24-120) months
Deaths within subgroup	1 patient (transplant related)

Abbreviations: EPE, extraprostatic extension; ISUP, International Society of Urologic Pathologists; PSA, prostate-specific antigen; SVI, seminal vesicle involvement

Two patients (25%) opted for surveillance, with stability seen after an average of 22 months. Significant medical comorbidities have precluded radical surgery in both of these patients. A summary of these findings is shown in Table 4.

Table 4. Characteristic and Management of Patients Diagnosed With Renal Tumors Following Heart and Lung Transplant

Characteristic	Finding
Age at transplant (range)	49 (19-62) years
Interval to renal tumor diagnosis (range)	12 (6-23) years
Radical nephrectomy	
• Papillary RCC, 24-50 mm	3 patients
• Unspecified RCC	2 patients
Radiofrequency ablation	
• Clear cell RCC	1 patient
Surveillance	
• 4.1-cm clear cell RCC	1 patient
• 3.5-cm Bosniak III complex cyst	1 patient
Mean follow-up from cancer diagnosis (range)	74 (18-204) months
Deaths within group	4 patients (1 from complicated diverticular disease, 1 from lower limb acute ischemia, 2 unspecified)

Abbreviations: RCC, renal cell carcinoma

Kidney cancer

Seven heart transplant recipients and 1 lung transplant recipient were diagnosed with renal masses on average 12 years posttransplant (1.17% of all transplant recipients). Average age at transplant was 49 years. All patients (100%) who developed renal tumors following heart or lung transplant had biochemical chronic kidney disease (CKD) at diagnosis. Mean follow-up for this group was 74 months.

Five patients (63%) opted for nephrectomy as the treatment of choice. Histology confirmed papillary renal cell carcinoma (RCC) in 3 patients and unspecified RCC in the remaining 2 patients. All tumors ranged from 24 to 50 mm and were classified as pT1a/b. All patients had preexisting CKD (stage 3-5) at the time of surgery, with 1 patient on dialysis preoperatively. Two patients developed end-stage CKD requiring dialysis in the years after nephrectomy, with 1 of these patients proceeding to renal transplant. Although 3 patients in this subgroup died, no deaths were related to renal malignancy.

One patient (12.5%) opted for radiofrequency ablation of a biopsy that was confirmed to be clear cell RCC on a background of preexisting CKD stage 3 (glomerular filtration rate of 30-59 mL/min/1.73 m^2). This patient ultimately died from complicated diverticular disease 4 years later; however, the renal lesion had remained stable following ablation.

Urothelial cancer

Two transplant recipients (0.3% of all heart and lung transplant recipients) were diagnosed during the study period with bladder cancer during workup for hematuria as shown in Table 5. Average age at time of transplant was 56 years, and interval from transplant to bladder cancer diagnosis was 17 years. Both patients underwent transurethral resection of bladder tumor. One patient required multiple subsequent fulgurations of recurrent low-grade tumors on surveillance cystoscopies; however, this patient was shown to be disease free at a recent follow-up of 36 months. The remaining patient was diagnosed with aggressive T4 transitional cell carcinoma. The patient succumbed to progressive bladder cancer following palliative radiotherapy after 11 months of follow-up.

A further transplant recipient (0.15% of all heart and lung transplant recipients) was diagnosed with upper tract urothelial carcinoma of the ureter following multiple episodes of visible hematuria and recurrent urinary tract infections at 4 years posttransplant. The patient proceeded to have an uncomplicated robot-assisted laparoscopic nephroureterectomy without any interruption to his immunosuppression. Histopathological analysis confirmed high-grade ureteric transitional cell carcinoma, pT3, with carcinoma in situ. The patient has not had any adverse outcomes or complications at a 3-month follow-up.

Table 5. Characteristic and Management of Patients Diagnosed With Bladder and Upper Tract Urothelial Malignancy Following Heart and Lung Transplant

Characteristic	Finding
Age at transplant (range)	58 (55-63) years
Indication for transplant	Pulmonary fibrosis, 2 patients Ischemic cardiomyopathy, 1 patient
Age at urothelial cancer diagnosis (range)	71 (67-79) years
Bladder urothelial carcinoma	
• pTa high-grade G2	1 patient
• >pT2, T4 on staging	1 patient
UTUC	
• pT3 high-grade UTUC with CIS	1 patient
Mean follow-up from cancer diagnosis (range)	16 (3-36) months
Deaths within subgroup	1 (metastatic bladder cancer)

Abbreviations: CIS, carcinoma in situ; UTUC, upper tract urothelial cancer

Table 6. Characteristic and Management of Patients Diagnosed With Penile Malignancy Following Heart and Lung Transplant

Characteristic	Finding
Age at transplant (range)	44 (25-63) years
Indication for transplant	Pulmonary fibrosis, 2 patients Dilated cardiomyopathy, 1 patient
Age at penile cancer diagnosis (range)	52 (29-65) years
Invasive penile carcinoma	
• pT2 N3 M0 poorly differentiated SCC	1 patient
• pT2N3M1 poorly differentiated usual SCC	1 patient
PeIN	
• Undifferentiated PeIN	1 patient
Mean follow-up from cancer diagnosis (range)	23 (6-48) months
Deaths within subgroup	1 (metastatic penile SCC)

Abbreviations: PeIN, penile intraepithelial neoplasia; SCC, squamous cell carcinoma

Penile cancer

Three male patients were diagnosed with penile cancer posttransplant (0.6% of male heart and lung transplant recipients). Two were diagnosed with invasive penile cancer an average of 10 years posttransplant. Both patients developed clinically apparent tumors of the glans penis with palpable inguinal lymphadenopathy and proceeded to have partial penectomy with bilateral inguinal lymph node dissection. One patient developed pulmonary metastases and subsequently died within 6 months of diagnosis. The other patient had positive inguinal lymph node disease and proceeded to pelvic lymph node dissection as well as adjuvant chemotherapy (carboplatin and paclitaxel) with radiotherapy to the lymph node beds. A local stump recurrence required revision distal penectomy. At 15-month follow-up, this patient showed no clinical recurrence of disease. The final patient was diagnosed with penile intraepithelial neoplasia at 3 years after lung transplant at circumcision and was managed with topical 5-fluorouracil. At 48-month follow-up, the patient showed no further clinically appreciable disease. A summary of these findings is shown in Table 6.

Discussion

Since the first heart transplant in 1985 conducted at MMUH, over 400 heart transplants and almost 300 lung transplants have been performed.[4] With improving perioperative treatment and optimized immunosuppressive regimens and aftercare, life expectancy of transplant recipients continues to improve, with recent data showing that 60% and 40% of Irish patients are alive 10 years after their heart and lung transplants, respectively.[5] Because these patients are immunosuppressed, they may be more likely to develop malignancies and at a younger age.[1,6,7] Tacrolimus or cyclosporine is used in all patients, as well as prednisolone and/or mycophenolate mofetil for immunosuppression; however, no patients in this series required immunosuppression interruption during cancer treatment. In transplant recipients, bladder, renal, and prostate cancers have been specifically implicated as having a worse disease-specific survival compared with outcomes in the general population.[8]

Data on urological cancers in heart and lung transplant recipients are sparse. Nevertheless, surgical management of any organ-confined malignancy should be offered to transplant recipients where suitable. An expected survival of >50% at 5 years to justify surgery and thorough multidisciplinary team meetings involving the treating oncologist (surgical/medical/radiation) with the primary transplant physician are general guidelines to assist in decision making.[9]

Prostate cancer

On average, heart and lung transplant recipients are diagnosed with prostate cancer at age 68 years, which is comparable to the national average of 67 years; stage of disease has also been favorable in heart and lung transplant recipients, with 86% diagnosed with T2 tumors.[10] Irish demographics from 2000 to 2014 showed a decrease in all patients opting for surgery, with a corresponding increase in radiotherapy. This trend has also been seen in Irish renal transplant recipients, with radiotherapy being the preferred method of treatment for 53%, and

also in patients presented in our study, with 71% receiving radiotherapy.[10,11] However, in a systematic review of kidney transplant recipients with prostate cancer, 82% of patients underwent surgery for organ-confined prostate cancer.[12] Surgery clearly allows maximum control in avoiding the nearby renal graft in the iliac fossa, as opposed to radiotherapy, which may inadvertently subject the graft to radiation, potentially increasing the risk of graft loss, although this is clearly not of significance in heart and lung transplant recipients. No treatment-specific complications or prostate cancer deaths were observed in the patients actively treated in our series; thus, all options appear safe for heart and lung transplant recipients.

Kidney cancer

Data from the United Kingdom have suggested that heart and lung transplant recipients are 2.5 and 4.4 times more likely to be diagnosed with kidney cancer compared with the general population, respectively, with highest incidence among male patients and those over 60 years of age.[13] In kidney transplant recipients, increasing age and acquired renal cystic disease specifically are thought to be behind the increase in RCC.[13] Furthermore, it must be considered that a certain proportion of transplant recipients will develop chronic renal impairment, a well-recognized complication of immunosuppression after organ transplant and an independent risk factor for development of renal malignancies.[14] In our series, all patients with renal tumors had some degree of chronic renal impairment.

Partial nephrectomy is the standard of care in T1 (<7 cm) tumors and has been shown to have a more favorable overall survival over radical nephrectomy in patients under 65 years of age, although there are limited reports in transplant recipients.[15] In a study from Tollefson and colleagues,[16] partial nephrectomy was successfully performed in 3 transplant recipients with T1 tumors as well as 8 radical nephrectomies. In a study of 5 patients with RCC after heart transplant, Peled and colleagues[17] also showed safety in performing radical nephrectomies in 3 patients, with the remaining 2 managed with surveillance. Although interventional procedures such as radiofrequency ablation appear feasible and safe without compromising oncological outcomes in renal transplant recipients, they should be reserved for frail patients or those with significant comorbidities that preclude surgery.[3,15] In our series, no patients experienced perioperative complications and there were no cancer-specific deaths, although 2 patients progressed to end-stage renal disease. We have also demonstrated that surveillance is safe in appropriately selected patients.

Bladder cancer

Bladder cancer, although uncommon, has been noted to be particularly aggressive following heart transplant.[18] Diagnostic transurethral resection of bladder tumor is the mainstay of obtaining a tissue diagnosis and can be therapeutic in nonmuscle-invasive bladder tumors.[19,20] There is a risk of BCG (Bacillus Calmette-Guérin) infection in immunosuppressed patients with its use; therefore, this treatment is used mainly for high-grade tumors, multifocal tumors, recurrences, or those with carcinoma in situ disease.[9,19,20] Treatment of muscle-invasive bladder cancer, when the patient is deemed fit for surgery and in the absence of metastatic disease, relies on radical cystectomy and an appropriate diversion method for curative intent. Although no patients in our series were appropriate, radical cystectomy has been reported to be successful in heart and lung transplant recipients.[19,21] Currently, EAU recommendations include neoadjuvant cisplatin-containing chemotherapy, if suitable, because of the demonstrated improved overall survival of 5% to 8% at 5 years.[8] In unsuitable patients, immunotherapy in programmed death-ligand 1-positive patients in a trial setting can be considered.[8] Outcomes are of course variable and based on disease stage but appear comparable with outcomes in the nontransplanted population when early, aggressive therapy is initiated.[20]

Testicular cancer

Testicular malignancies are rare in organ transplant recipients and in most papers account for 1 to 2 cases per series.[6,7,22] Because of the inherent risk of developing testicular cancer with cryptorchidism, some authors have advocated orchidectomy in an atrophic, undescended testicle in immunosuppressed patients due to the potential for accelerated malignant growth.[23] Because there were no patients with testicular tumors in our series and surgical management is generally straightforward, patients with a suspicious lump should obtain a definitive histological diagnosis via inguinal radical orchidectomy, as recommended by the EAU.[24] Because of the risk of secondary non-germ cell malignancies

in patients treated with radiotherapy who are already at risk because of immunosuppression, chemotherapy, if suitable, should likely be considered as adjuvant treatment where required.[24]

Penile cancer

Although rare, most reported cases of penile cancer in organ transplant recipients have been managed with conventional methods of wide local excision of the tumor for treatment and diagnosis with good success.[25] Human papillomavirus (HPV) is known to be associated with the development of approximately one-third of invasive penile cancers and up to 100% of intraepithelial neoplasia with HPV-related malignancies known to occur in excess in solid-organ transplant recipients.[26,27] In our series, we had 2 patients with invasive penile carcinoma, resulting in an incidence of 0.4%, which is notably higher than quoted figures in other series of 0.004%.[25] In terms of management, there are several effective modalities under local, regional, or general anesthesia, making surgical treatment suitable for virtually all transplant recipients regardless of fitness for anesthesia. Radical lymphadenectomy in clinically apparent nodes can be lifesaving and therefore should performed in all patients, including pelvic lymphadenectomy, if inguinal nodes are positive during initial sentinel lymph node biopsy.[28] In our series, we demonstrated the success and safety of multimodality therapy with surgery, chemo-radiotherapy, and topical treatment in penile intraepithelial neoplasia in immunosuppressed transplant recipients.

Screening recommendations

Skin malignancies are the only formal screening programs in transplant recipients that have been advocated; therefore, prudent monitoring and guidelines for detection of urological cancers should be considered on a case-by-case basis by the treating physician when there is clinical suspicion.[1] In line with recommendations, PSA results are checked yearly in all male transplant recipients >40 years old at our center, with patients referred directly to a urologist if results are persistently elevated or if patients have an abnormal digital rectal examination.[29] For bladder cancer, no specific screening recommendations exist, with the exception of yearly cytology and imaging for patients who have undergone renal transplant and who have a history of analgesia abuse.[29] Renal transplant recipients are generally recommended to have yearly native kidney ultrasonographic evaluations to monitor for development of RCC, and this could be considered for any heart or lung transplant recipients with coexisting renal impairment.[30] All male transplant recipients should be encouraged to self-examine their testicles for abnormalities, particularly those with a history of cryptorchidism.[23]

Limitations

Our study had limitations because of limited availability of certain data that predated electronic medical records, as the national heart transplant program commenced in 1985. Data are retrospective and were collected primarily by transplant physicians; therefore, data pertaining to the patient's urological issues may be scant in some records. Selection bias of a small number of transplant recipients with a urological malignancy in over 700 transplant recipients in total may also apply. These small numbers practically only suffice for a narrative review rather than possibly more meaningful statistical analyses. Furthermore, although all patients underwent follow-up at a single national center from a transplant perspective, their urological care was often performed in other hospitals; therefore, some details may be unavailable. With the consideration that this study spanned many years, there have clearly been significant developments in management of cancer and outcomes over this period (eg, neoadjuvant chemotherapy for muscle invasive bladder cancer, use of robot technology for minimally invasive surgery). Thus, there may be some heterogeneity of histopathological reporting, treatment modalities, and outcomes.

Conclusions

Urological cancer in the transplant population is uncommon in terms of isolated figures; however, this population is clearly at higher risk of developing malignancy, which must be considered in follow-up. The striking finding in this study is that 100% of heart and lung transplant recipients who developed malignant renal masses had underlying chronic renal impairment, although this may be a sequelae of immunosuppressive therapy. This specific subgroup may benefit from routine surveillance with ultrasonography in follow-up posttransplant. Otherwise, no findings in this national cohort study were shown

that might advocate for additional screening programs within the heart and lung transplant recipient group, although each patient must be assessed on individual risks. Surgery, radiotherapy, and systemic therapy options were all performed in all patient subgroups, in line with treatment options in the standard population, with equitable success, safety, and outcomes. We do advocate that these patients undergo treatment in specialist transplant centers shared by a uro-oncologist with multidisciplinary facilities available to ensure contemporary and individualized specialist management.

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Nontuberculous Mycobacterial Pulmonary Infection Among Lung Transplant Recipients

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Abstract

Objectives: **Data are limited regarding the clinical significance of nontuberculous mycobacteria pulmonary infections among lung transplant recipients. We investigated the incidence and characteristics of pulmonary nontuberculous mycobacteria infection in our lung transplant patient population.**

Materials and Methods: **We obtained data of the patients who underwent lung transplant in our center from January 1997 to March 2019.**

Results: **Of 690 patients, nontuberculous mycobacteria were identified in 58 patients (8.4%) over a median follow-up of 3 years. Types of species were as follows: *Mycobacterium simiae* (n = 24), *avium complex* (n = 12), *abscessus* (n = 9), *fortuitum* (n = 6), *chelonae* (n = 2), *szulgai* (n = 1), *kansasii* (n = 1), *lentiflavum* (n = 1), and undefined mycobacteria (n = 2). When we compared infections in the early versus late period posttransplant (before and after 6 months), infections with *Mycobacterium simiae* (16 vs 8 incidents) and *Mycobacterium fortuitum* (5 vs 1 incident) were more often observed within the early period, whereas most *Mycobacterium abscessus* (7 vs 1 incident) and *Mycobacterium avium* complex (9 vs 3 incidents) were observed in the later period. The median forced expiratory volume in 1 second over time did not differ significantly between patients with and without nontuberculous mycobacteria infection (*P* = .29). Nontuberculous mycobacteria acquisition was significantly associated with decreased survival (relative risk of 2.41, 95% CI, 1.70-3.43; *P* < .001).**

Conclusions: **The nontuberculous mycobacteria species isolated varied according to the time elapsed since transplant. Among lung transplant recipients, nontuberculous mycobacteria infection was associated with increased mortality but not with lung dysfunction.**

Key words: *Nontuberculous mycobacteria, Prognosis, Pulmonary function*

Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment; more than 190 species have been identified.[1] Over the past few years, trends of increasing prevalence of NTM pulmonary infection have been reported in the general population.[2-4] Solid-organ transplant recipients have an increased risk of infection, including NTM pulmonary infection, due to suppressed cell-mediated immunity.[5-7] Although these infections are less common than other infections in the transplant recipient population, NTM is a source of significant morbidity and mortality. This is partly because of delayed diagnosis, which frequently results from difficulties in disease recognition.[5-7]

Among solid-organ transplant recipients, lung transplant is associated with the highest risk of NTM pulmonary infections.[6,7] Nonetheless, published data regarding clinical and prognostic implications of NTM pulmonary infection occurring after lung transplant are scarce.[8-13] We therefore investigated the occurrence and species of NTM pulmonary infections following lung transplant and assessed the associations between NTM infection and pulmonary function and long-term survival.

Materials and Methods

Study population and design

This retrospective study was conducted at the Rabin Medical Center, the sole center for lung

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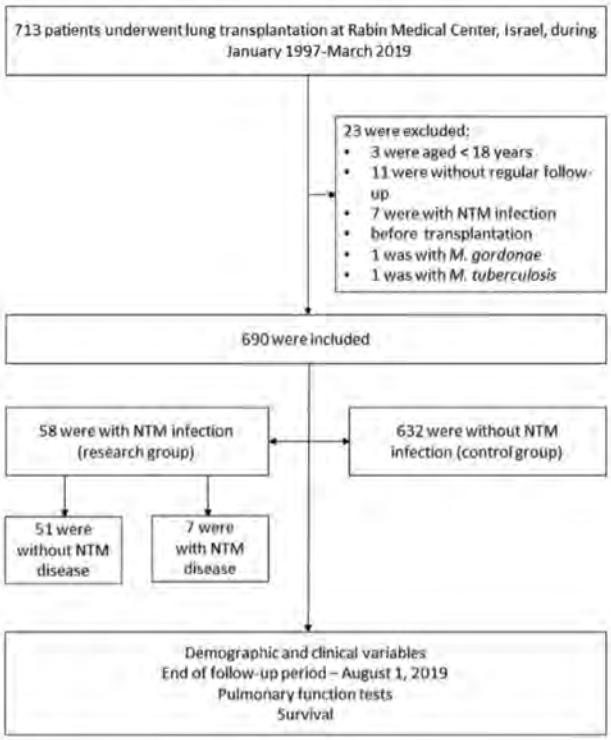
transplantation in Israel. Figure 1 presents the study design. From January 1997 to March 2019, 713 lung transplantations were performed. We identified 699 patients according to the following eligibility criteria: age ≥18 years and regular follow-up. Of eligible patients, NTM pulmonary infections were diagnosed in 67 patients. We excluded 9 patients due to the development of NTM before transplant (n = 7) or infection with *Mycobacterium gordonae* (n = 1) or *Mycobacterium tuberculosis* (n = 1). Of the 690 patients included in the cohort, 58 (8.4%) developed NTM pulmonary infections after lung transplant.

The study was carried out in accordance with the Declaration of Helsinki and was approved by our Institutional Ethics Committee.

Figure 1. Flowchart Presenting the Study Design



Abbreviations: *M., Mycobacterium*; NTM, nontuberculous mycobacteria

Follow-up protocol

Lung transplant recipients are routinely evaluated in the ambulatory clinic of our institution at 2 and 4 weeks posttransplant and then every 2 months, on average, thereafter. More frequent visits are scheduled when a clinical need arises. Follow-up includes a detailed medical interview, physical examination, chest radiograph, and pulmonary function test. At our center, all pulmonary function test measurements are performed on the ZAN 530 system (Zan Messgeräte GmbH). The measurement technique and reference values are calculated according to European clinical practice guidelines.[14,15] Chest computed tomography (CT) scans are routinely performed at 6 and 12 months posttransplant and then annually.

Bronchoscopy

All bronchoscopic examinations included in this study were performed at our pulmonary institute. Three scheduled bronchoscopies with bronchoalveolar lavage (BAL) are performed routinely at 1 and 2 weeks and at 1 month posttransplant. Additional bronchoscopic examinations are performed according to clinical need and in patients with clinical deterioration (ie, worsening dyspnea and pulmonary function tests), abnormal findings on chest CT, unresolved pneumonia, lobar atelectasis, suspected lung rejection, or bronchial stenosis.

Bronchoalveolar lavage procedure

To prevent contamination by upper airway flora, the BAL trap used to collect the specimen is connected to the suction channel of the bronchoscope only after the bronchoscope traverses the vocal cords. The bronchoscope is wedged into a subsegmental bronchus (usually toward the place of infiltrate, when present), and 3 aliquots of sterile saline (50 mL each) are instilled and aspirated.

Microbiologic samples

Bronchoalveolar lavage samples are plated on blood, Chocolate, and MacConkey agars. Specimens are analyzed for mycobacteria using Ziehl-Neelsen stain and cultured on Lowenstein medium and in *Mycobacterium* growth indicator tubes (Bactec MGIT 960, Becton Dickinson). *Mycobacterium* growth indicator tubes and Lowenstein-Jensen cultures are discarded after 6 to 8 weeks. We use the GenoType *Mycobacterium* DNA strip assay (Hain Lifescience GmbH) to detect and identify *Mycobacterium* species obtained from positive liquid and solid mycobacterial cultures. Quantitative NTM cultures are not performed in our center.

Definition of nontuberculous mycobacteria pulmonary infection and disease

Nontuberculous mycobacteria pulmonary infection is diagnosed based on 1 positive culture from a BAL sample or 2 positive sputum cultures.[3,14] During the

study period, NTM pulmonary disease was defined using the following criteria: (1) presence of clinical pulmonary symptoms, (2) evidence of nodular or cavitary opacities on chest radiograph or multifocal bronchiectasis with multiple small nodules on CT, and (3) microbiologic confirmation of NTM pulmonary infection.[3,16]

Nontuberculous mycobacteria treatment

In general, we do not treat NTM pulmonary infections; however, patients are closely observed for development of NTM pulmonary disease. Treatment of NTM pulmonary disease is performed according to published guidelines.[3,16] Treatment consists of multiple antibiotic medications, selected according to NTM species, sensitivity, expected toxicities, and interactions with immunosuppressive agents and other medications. The duration of treatment depends on the clinical course of the NTM pulmonary disease and usually lasts at least 1 year following repeat negative NTM cultures.

Data collection

Data were obtained from electronic medical record database systems that integrate medical information from all hospitals in Israel. These data included the following variables: age, sex, single or double lung transplant, diagnosis that led to transplant, the type of *Mycobacterium* species, and the forced expiratory volume in 1 second (FEV1) over the duration of the study period. At the end of the follow-up period, vital status or date of death were ascertained from hospital records and the registry of the Israeli Ministry of Internal Affairs.

Statistical analyses

Descriptive data are expressed as means and SD or numbers (percentages) of presented cases. We used the chi-square test for categorical variables and the *t* test for continuous variables. $P < .05$ was considered statistically significant. We compared FEV1 values between patients with and without NTM pulmonary infection using a linear mixed model with random effects. A Cox proportional hazards model with time-dependent covariates was used for analysis of the association between NTM pulmonary infection and survival. Statistical analysis was performed using SAS software, version 9.2 (SAS Institute Inc).

Results

Baseline characteristics

Table 1 presents the baseline characteristics of the study population (n = 690). Mean age of the study population was 53.9 ± 13.2 years; 63.1% were males. Pulmonary fibrosis and emphysema were the most common etiologies for lung transplant. Nontuberculous mycobacteria isolates were identified on BAL cultures in 58 patients (8.4%). Demographic characteristics, transplant types, and the etiologies for the lung transplantation were comparable between the patients with and without NTM pulmonary infections.

Table 1. Baseline Characteristics of Lung Transplant Recipients With and Without Nontuberculous Mycobacteria

Variable	Patients With NTM (n = 58)	Patients Without NTM (n = 632)	*P* Value
Age (mean ± SD), years	56.3 ± 10.0	53.6 ± 13.4	.06
Male patient, No. (%)	39 (67.2%)	397 (62.8%)	.57
Unilateral/bilateral transplant, No. (%)	34 (58.6%)/24 (41.3%)	354 (56.0%)/278 (43.9%)	.78
Etiology for lung transplant, No. (%)			.20
Pulmonary fibrosis	22 (37.9%)	264 (41.8%)	
Emphysema	22 (37.9%)	177 (28.0%)	
Cystic fibrosis	3 (5.2%)	64 (10.1%)	
Bronchiectasis	4 (6.9%)	31 (4.9%)	
Silicosis	5 (8.6%)	25 (4.0%)	
Scleroderma	2 (3.4%)	22 (3.5%)	
Primary pulmonary HTN	0 (0.0%)	18 (2.8%)	
Re-transplantation	0 (0.0%)	9 (1.4%)	
Other disorder	0 (0.0%)	22 (3.5%)	

Abbreviations: HTN, hypertension; NTM, nontuberculous mycobacteria

Spectrum of nontuberculous mycobacteria pulmonary infections

During a median follow-up period of 3 years, the following NTM species were identified among 58 patients: *M. simiae* (n = 24), *M. avium complex* (n = 12), *M. abscessus* (n = 9), *M. fortuitum* (n = 6), unidentified mycobacteria (n = 2), *M. chelonae* (n = 2), *M. szulgai* (n = 1), *M. kansasii* (n = 1), and *M. lentiflavum* (n = 1). Figure 2 illustrates the distributions of NTM species in the early period versus the late period after transplant (before and after a 6-month median point). In the early period posttransplant, cultures were more often positive for *M. simiae* (16 vs 8 patients) and *M. fortuitum* (5 vs 1 patient). In the late period posttransplant (later than 6 months), *M. abscessus* (8 vs 1 patient) and *M. avium complex* (9 vs 3) were more commonly identified.

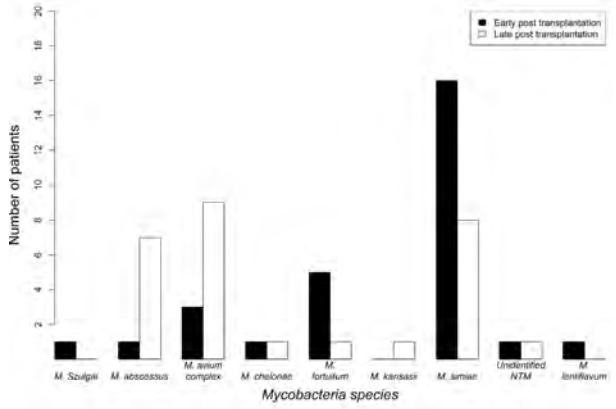
Of the 58 patients with positive NTM cultures, 51 had positive BAL cultures for NTM species that did not meet diagnostic criteria for NTM pulmonary disease. The remaining 7 patients met the diagnostic

criteria for NTM pulmonary disease. Causes of NTM disease were by *M. abscessus* in 5 patients and *M. avium* complex in 2 patients.

Pulmonary lung function

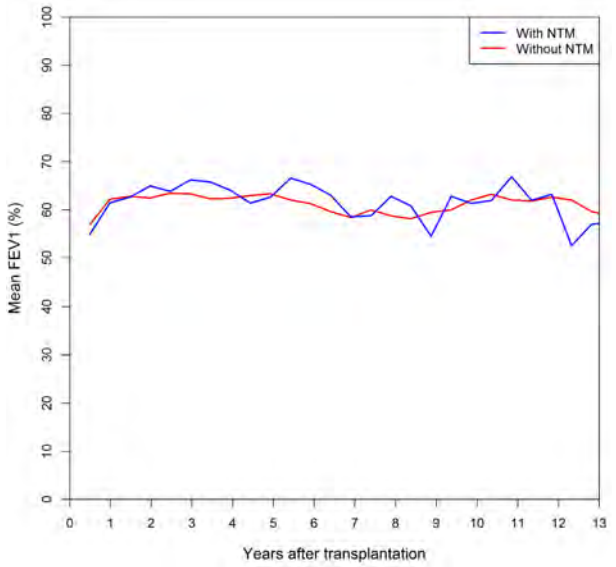
During the follow-up period, mean FEV1 did not differ significantly between patients with and without NTM isolates ($P = .29$; Figure 3).

Figure 2. Spectrum of Nontuberculous Mycobacteria Species in the Early and Late Posttransplant Periods According to 6-Month Median Cut-off



Abbreviations: *M., Mycobacterium;* NTM, nontuberculous mycobacteria

Figure 3. Mean Values of Forced Expiratory Volume in 1 Second in Lung Transplant Recipients With and Without Nontuberculous Mycobacteria Pulmonary Infections ($P = .29$)



Abbreviations: FEV1, forced expiratory volume in 1 second; NTM, nontuberculous mycobacteria

Survival

During the study period, 393 of the lung transplant patients died (56.9%). The mortality rates were higher among those with NTM pulmonary infection, that is, in 34/51 (67%) with NTM versus in 357/632 (57%) without NTM. Of the 7 patients with NTM pulmonary disease, 5 survived and 2 died. On multivariate analysis, NTM acquisition (infection or disease) after lung transplant, as a time-dependent variable, was significantly associated with increased mortality (relative risk of 2.41, 95% CI, 1.70-3.43; $P < .001$).

Discussion

This study reported the isolation of culture-positive NTM in 8.4% of lung transplant recipients. This is within the range of incidence of NTM isolates after lung transplant reported in other studies of 3.3% to 22.4%.[8-12] Discrepancies between studies in the prevalence of NTM isolates may be explained by differences in the median follow-up periods, which ranged from 97 to 1205 days.[8-12] The variance in incidence of NTM isolates among lung transplant recipients may also be attributed to the varied prevalence of pulmonary NTM infection across geographic locations.[3,13] Even within Israel, a broad spectrum of prevalence of NTM infections has been reported, with higher rates in humid coastal cities and lower rates in the dry mountainous area.[17]

Among our study patients, half the NTM pulmonary infections were identified during the first 6 months posttransplant. This is within the range of 3 to 9 months as the median time for the development of NTM pulmonary infection posttransplant reported by previous studies.[8-10] Several reasons may explain the early occurrence of NTM pulmonary infection posttransplant. First, chronic pulmonary NTM colonization before lung transplant could be underrecognized. Second, NTM pulmonary infection might be vertically transferred from the lung donor. Third, a nosocomial source of NTM infection is possible.[6,18] Finally, lung transplant recipients are more heavily immunosuppressed in the first than in the subsequent months following transplant, and thus patients can be presumably more vulnerable to NTM pulmonary infections.[6,7] Of note, Huang and colleagues[8] reported that single lung transplants were associated with increased incidence of posttransplant NTM infection. They proposed that the native abnormal lung may harbor NTM at the time of transplant and facilitate NTM infection posttransplant.[8] However, in our cohort, we did not find a significant difference between patients with

unilateral and bilateral lung transplant procedures with regard to occurrence of NTM infections within the first 6 months posttransplant.

The most common NTM species isolated in our study were *M. simiae* (41.4%), which is generally considered nonpathogenic, and *M. avium* complex (20.7%), which is generally considered pathogenic. Diversity in the spectrum of NTM species among lung transplant recipients has been previously reported.[8-11] Our results concur with other studies that identified *M. avium* complex as one of the common NTM species among lung transplant recipients.[8-11] In contrast, *M. abscessus* was identified as the most prevalent isolate in 2 studies[9,11] and *M. simiae*[8] and *M. fortuitum*[10] were the most prevalent in 1 study each. Our finding of *M. simiae* prominence corroborates other studies conducted in Israel that identified this as the most common NTM species among patients with cystic fibrosis[17] and bronchiectasis.[19]

We reported different distributions of NTM species isolated within 6 months and at more than 6 months posttransplant. Specifically, isolates of *M. simiae* and *M. fortuitum* were more often identified early, within 6 months of transplant, whereas *M. abscessus* and *M. avium* complex were more often isolated at a later date. Moreover, *M. simiae* and *M. fortuitum* are considered less virulent and generally colonizers,[3,16] whereas *M. abscessus* and *M. avium* complex are generally associated with NTM pulmonary disease. With a longer time elapsed since transplant, the cumulative effect of the immunosuppressive drug regimen more likely increased the risk of pulmonary infection with more virulent NTM species. The clinical significance of the prevalence of various NTM species at different lapses of time following lung transplant should be investigated in larger patient populations.

Interestingly, among lung transplant recipients with NTM pulmonary acquisition, the decline in FEV1 over the study period was not significantly greater than among patients without NTM infection. We did not find studies that investigated an association of NTM infection with lung function in lung transplant recipients. In their study of non-lung transplant patients, Park and colleagues[20] reported a significant decline in lung function over time among those with NTM pulmonary disease. The decline in lung function was greater among patients who did not respond to treatment for NTM pulmonary infection than among those who were successfully treated. Notably, most of the patients in that study had NTM pulmonary disease, whereas most of our patients with NTM colonization did not develop NTM pulmonary disease. Thus, it appears likely that the structural damage from NTM colonization, if any, does not substantially impair lung function.

Another important finding of the current study is the association of NTM pulmonary acquisition with poor long-term survival. Three studies did not find a significant association of NTM pulmonary infection with mortality in similar patient populations.[9-11] However, as in the present study, Huang and colleagues[8] and Friedman and colleagues[13] reported an association of posttransplant NTM pulmonary infection with a higher risk of mortality. Several explanations are possible for the discrepancy between our findings and others. First, in the previous reports, the number of patients with NTM pulmonary infections was relatively small, ranging from 33 to 53,[9-11] which may have impacted the statistical comparison. Second, the possibility of bias arises due to the time-dependent development of NTM pulmonary infection among lung transplant recipients. Thus, those who survived in the first months posttransplant were more likely to be infected with NTM over time. To mitigate this possible bias, we analyzed the association of NTM infection with long-term survival using a Cox proportional hazards model with time-dependent covariates. The underlying mechanisms for the association between posttransplant NTM pulmonary infection and decreased survival are not clear. For some lung transplant recipients, the development of NTM pulmonary infection might be related to severe immunosuppression, which may increase the mortality risk from non-NTM infections.[8] In addition, NTM pulmonary infection may be a marker of more severe lung damage, which could lead to poor outcome. Associations have been reported of NTM pulmonary infections with pulmonary damage,[3,16] chronic lung rejection,[11] and bronchial posttransplant complications.[8] Nonetheless, we did not find a significant association between NTM pulmonary acquisition and impaired lung function.

Limitations of the present study include its retrospective single-center design, which represents 1 geographical region. Additionally, the number of patients with NTM pulmonary infections was relatively small and may have affected statistical

power for comparisons of some relevant data. Notwithstanding, our study represents one of the largest investigations that have been conducted to date on lung transplant recipients with NTM pulmonary infection.

Conclusions

Nontuberculous mycobacteria pulmonary infections are not uncommon following lung transplant. Although most species identified do not cause NTM pulmonary disease, especially in the first 6 months posttransplant, the risk for NTM disease increases with longer time elapsed since transplant. Although NTM pulmonary acquisition was not found to be significantly associated with decreased pulmonary function, it was associated with increased mortality.

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HLA Matching Status Among Patients Requiring Hematopoietic Stem Cell Transplant: The Need for a National and Regional Stem Cell Bank

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Abstract

Objectives: **Hematopoietic stem cell transplant is a strategic treatment for many malignant and nonmalignant blood diseases. Finding an HLA-matched donor is a requirement for a successful transplantation. The aim of the current study was to explore indications, demographics, and HLA patient-donor matching status among Bahraini patients requiring this transplant.**

Materials and Methods: **Records of 100 patients who required hematopoietic stem cell transplant at the Salmaniya Medical Complex, Ministry of Health tertiary hospital in Bahrain were retrospectively studied. Data were analyzed and compared with data from similar studies.**

Results: **For the 100 patients, 294 potential donors were HLA typed. Indications for transplant included malignant diseases (50%) and hereditary blood diseases (50%). For those in the 0- to 5-year age group, the main indication was acute lymphoblastic leukemia, whereas acute myeloid leukemia was the main indication for those who were >5 years old. Sex distribution showed that 55% of patients were males and 45% were females. With regard to age distribution, 22% of patients were less than 5 years old, 30% were 5 to 17 years old, and 48% were 18 years and older. Patient-donor HLA matching status was 50% HLA identical, 32% haploidentical, 15% more than haploidentical, and 3% less than haploidentical. The number of potential donors per patient ranged from 1 to 11 typed for each patient (average of 2.94 ± 1.86).**

Conclusions: **The rate of finding a family member as HLA-matched donor for hematopoietic stem cell transplant in our study on Bahraini patients was higher than reports in western countries yet close to other reports from countries with almost similar family sizes. We recommend forming a national Bahrain registry in addition to a regional Eastern Mediterranean stem cell bank to increase the success rate of finding an HLA-matched donor.**

Key words: *Bahrain, Hereditary blood disease, HLA-matched donor*

Introduction

Hematopoietic stem cell transplantation (HSCT) plays a major role in the treatment of many diseases. The results of the HLA-matching process can decide the success of HSCT procedures. However, finding an HLA-matched donor can be a major challenge. The ideal donor is a fully HLA-matched sibling. The probability of finding an HLA identical donor among siblings depends on the number of siblings, with success rates of 25% if there is 1 sibling and up to 90% for patients who have 8 siblings.[1] This fact predicts that a higher number of patients with matched donors can be found in regional Eastern Mediterranean countries compared with western societies because of the higher number of consanguineous marriages and the larger family size.

Malignancy is the major indication for HSCT.[2] However, given differences in incidence of hereditary blood diseases in our region compared with western societies, we expect that a higher number of HSCT procedures are performed because of blood disease-related causes.

Research on HSCT and HLA has recently focussed on forming HLA registries and on cord blood stem cell transplant; however, HLA is highly variable among ethnicities and different cultures. Therefore, it is important to study the HLA status patterns in each country. In this study, our aim was to

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explore the different indications for HSCT, the demographics, and the HLA patient-donor matching status among Bahraini patients requiring this treatment, thus aiming to determine the rate of finding an HLA identical donor.

Materials and Methods

This study was conducted at Salmaniya Medical Complex, Ministry of Health, a tertiary hospital in Bahrain. Records of 100 patients who required HSCT and their 294 family members (potential donors) were retrospectively studied in the 3-year period from 2017 to 2019. HLA class I and II analyses were performed by polymerase chain reaction and/or microcytotoxicity methods for patients and donors. An HLA-matched donor was defined as matching in class IA, B, and C and class II DR and DQ.

Official ethical approval for this study was given by the Bahrain Ministry of Health, Secondary Healthcare Research Committee.

Statistical analyses

We used Microsoft Excel 2016 and the SPSS (Statistical Package for Social Sciences) program version 20 for statistical analyses. Quantitative variables are presented as mean ± standard deviation. Categorical data are shown as counts and percentages.

Results

One hundred patients were enrolled in our study. Of 294 potential donors, 197 were siblings, 76 were parents, 4 were daughters, 1 was a grandmother, 15 were sons, and 1 was an uncle. Sex distribution showed that was 55% were male patients and 45% were female patients. Among the patients, 22% were less than 5 years old, 30% were 5 to 17 years old, and 48% were 18 years and older. The minimum number of donors tested for each patient was 1 and the maximum number was 11. The overall average number of donors typed for each patient was 2.94 ± 1.86. Patient-donor HLA matching status was 50% HLA identical, 32% haploidentical, 15% more than haploidentical (more than 50% matching and less than 100%), and 3% less than haploidentical (less than 50% matching), as illustrated in Figure 1.

Figure 2 shows that patients were more likely to find an HLA-matched donor if they were older; 68.70% of those who were above 18 years old had an HLA-matched identical donor compared with 43.3% of those who were >5 to 18 years old and 18.20% of those who were 0 to 5 years old. Table 1 shows the average number of donors tested per patient in each age group and the average number of matching donors for each patient.

Indications for HSCT were 50% because of malignant diseases (leukemia/lymphoma) and 50% because of benign hematological disorders (mainly sickle cell disease, thalassemia major, aplastic anemia, Fanconi anemia, hemophagocytic lymphohistiocytosis, and myelodysplastic syndrome). In the 0- to 5-year age group, the main indication was acute lymphoblastic leukemia, whereas acute myeloid leukemia was the predominant indication in those older than 5 years old. The most common indication with regard to benign hematological disorders was sickle cell disease followed by thalassemia major and aplastic anemia.

Figure 1. Patient/Donor HLA Matching Status

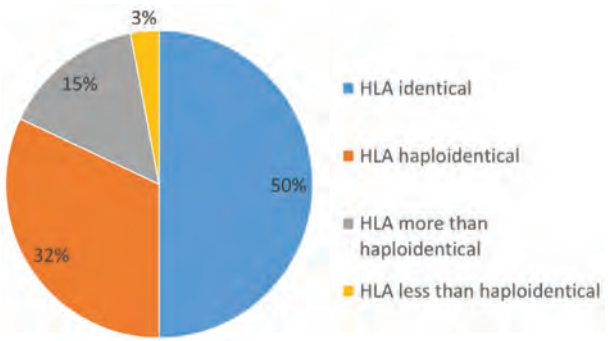


Figure 2. Age Distribution of Studied Patients and Donors

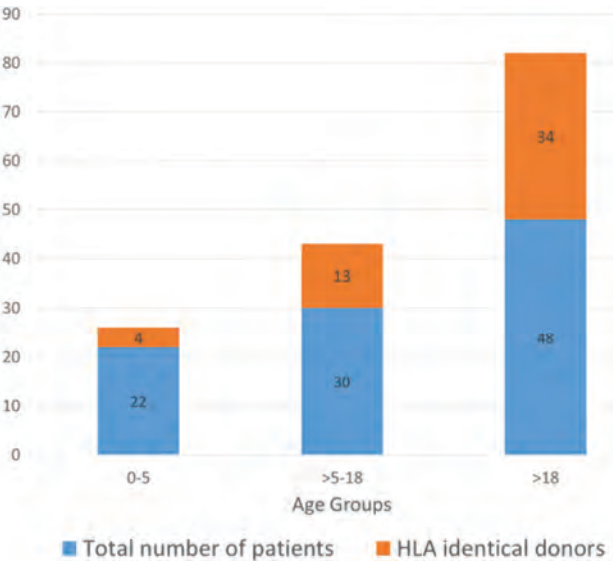


Table 1. Average Number of Tested Donors and Matching Donors Per Patient in Each Age Group

Age Group, years	Average Number Donors Tested Per Patient	Average Number Matching Donors Per Patient	Total Number of Patients
0 to 5	2.81 ± 1.56	0.54 ± 0.35	22
>5 to 18	2.4 ± 1.35	0.69 ± 0.32	30
>18	3.33 ± 2.18	0.50 ± 0.30	48

Discussion

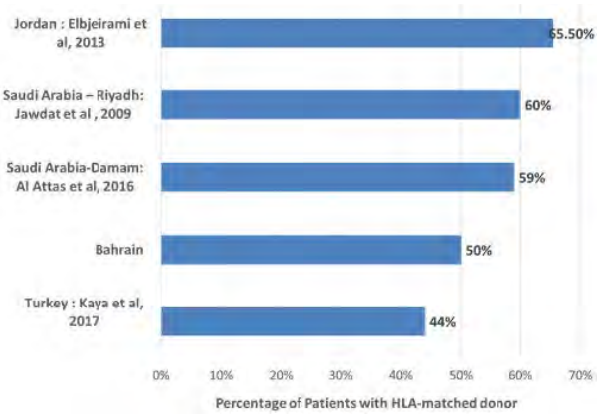
The overall rate of finding a fully matched HLA identical donor (10 of 10) in our study was 50%, which is higher than the percentage reported in Turkey (44%)[3] and lower than that reported in Saudi Arabia (60% in Riyadh4 and 59% in Damam[5]) as well as in Jordan (65.5%),[6] as illustrated in Figure 3. However, our rate was significantly greater than in western countries. The total number of HCSTs from 2013 to 2017 performed in the United States with an HLA-matched sibling or other related donor was 18%,[7] and the percentage of HLA-matched related donors among HSCTs was 36% in Europe as reported in data survey study from 2014.[2] The greater rate reported in our study can be explained by the larger family size and the higher rates of consanguineous marriage found in Middle Eastern societies. The total consanguineous marriages reported in Bahrain in 2008 was 10.9% and 11.4% in 2009.[8] A study on Saudi Arabia families reported 57.7% of included families were consanguineous marriages.[9] Among Jordanians, the rate of consanguineous marriage is 49%.[10] In western countries, one-third of patients for HSCT have HLA identical siblings.[11]

The likelihood of finding an HLA identical donor increases significantly with age, with a rate of 68.70% in those >18 years old and only 18.20% in those between 0 and 5 years old. This may be attributed to older patients having larger families and greater numbers of siblings to screen and therefore having a higher chance of finding a matched donor, which agrees with findings from previous studies in Saudi Arabia[4] and Jordan.[6]

Nonmalignant disease-related causes as an indication for transplant were found in 50% of our cases, with most related to hereditary blood diseases. This finding is in contrast to the European data survey study on HSCT from 2014,[2] in which transplant because of nonmalignant indications was only 6%. This can be explained by the high prevalence of hereditary blood diseases in Bahrain. A study published in 1995 reported that 2% of newborns had sickle cell disease and 18% had sickle cell trait, whereas 24% were carriers of the thalassemia gene and 10.4% of non-neonates had sickle cell disease.[12]

Although the chance of finding an HLA-matched donor reported in our study and other studies in the region is high, there is still a large percentage of patients who are not able to find an HLA-matched donor. This places a need for constructing a national Bahrain donor registry, which is important for HSCT. A good example is Turkey, where a national registry has been in place since 2015 ("TURKOK"); this registry has had a key role in finding unrelated donors. National donor banks are important for both finding an unrelated donor within a shorter time than international donor searches and for their economic impact. In addition, there is a need for a regional stem cell bank for HSCT, which could provide great opportunities to study HLA haplotypes prevalent in our region in addition to providing higher chances of finding a suitable matched donor for patients. The common ethnic origin, higher rates of consanguinity, and larger family size present in our region are strong factors in favor of a successful bank. Cooperation between the Kingdom of Bahrain and Turkey in the field of oncology has already started.

Figure 3. Percentage of Finding an HLA Identical Donor in Current Study and Other Studies



Conclusions

The rate of finding HLA-matched family members as donors for HSCT in our study on Bahraini patients was higher than the rate reported in western countries yet close to other reports from countries with almost similar family sizes. We recommend

forming a national Bahrain registry in addition to a regional stem cell bank to increase the success rate of finding an HLA-matched donor.

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Comparison of the Success Rate of Two Different Marking Techniques (F-Mark and Asymmetric Triangular Mark) to Orient the Donor Graft During Descemet Membrane Endothelial Keratoplasty

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Abstract

Objectives: **We evaluated the effects of various graft-marking techniques on surgical results in patients undergoing Descemet membrane endothelial keratoplasty.**

Materials and Methods: **In this single-center retrospective study, 65 eyes from 55 patients that had received various types of marking or no marking and that had been used for Descemet membrane endothelial keratoplasty endothelial graft preparation were included. Patients were divided into 3 groups according to the marking technique used: group I (F-marked graft; 17 eyes), group II (asymmetric triangle-marked graft; 12 eyes), and group III (unmarked graft; 36 eyes). The main outcome measurements were best-corrected visual acuity, endothelial cell density, central corneal thickness, postoperative complications, rebubbling, and secondary keratoplasty rates.**

Results: In groups I, II, and III, rates of patients with 6-month best-corrected visual acuity ≥ 20/32 were 35.7%, 77.8%, and 71.9%, respectively (*P* = .04). The mean 6-month endothelial cell density decrease for each group was 43.3%, 48.8%, and 46.4%, respectively (*P* = .589), whereas the mean 6-month central corneal thickness decrease for each group was 7.7%, 15.8%, and 34.0%, respectively (*P* = .001). Rates of primary graft failure for groups I, II, and III were 35.3%, 8.3%, and 13.9%, respectively. Rebubbling was performed in 21.5% of eyes, and secondary keratoplasty was required in 29.2% of eyes.

Conclusions: **Although graft-marking techniques for Descemet membrane endothelial keratoplasty greatly facilitate graft positioning during surgery, both the potential toxic effects of alcohol on the endothelium when marking with gentian violet dye and the risk of graft detachment with asymmetric marking must be considered.**

Key words: *Corneal graft detachment, Corneal transplantation, Gentian violet dye, Graft orientation*

Introduction

Descemet membrane endothelial keratoplasty (DMEK) was introduced by Melles and colleagues in 2006[1] and has become popular because of the better visual outcomes with more rapid visual rehabilitation. In addition, DMEK has lower endothelial rejection and general complication rates than Descemet stripping automated endothelial keratoplasty and penetrating keratoplasty (PKP).[1-5] However, the learning curve for performing DMEK can be steep for surgeons new to the technique because of the more difficult donor tissue preparation, implantation, orientation, and unscrolling.[6]

One of the most difficult stages of DMEK is positioning the graft correctly in the anterior chamber and then unscrolling it. However, positioning is the most important factor determining surgical success; accidental upside-down placement of the graft results in iatrogenic primary graft failure.[7] Various techniques have been defined to ensure placement of the graft in the correct position, and instruments and techniques that help orientation include the handheld slit beam,[8] endoilluminator-assisted transcorneal illumination,[9] and intraoperative optical coherence tomography.[6,10] Marking the stromal endothelial graft aspect with a surgical skin marker (S-stamp, F-mark) and marking the graft periphery with a semicircular punch or

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with the single triangular technique have also been reported as methods to facilitate graft positioning.[11-15]

The aim of this study was to determine the effects of 2 endothelial graft-marking techniques (F-mark and asymmetric triangular mark) on best-corrected visual acuity (BCVA), endothelial cell density (ECD) decrease, central corneal thickness (CCT), postoperative complications, and need for rebubbling and secondary keratoplasty. We are not aware of any other published study that has reported effects of multiple graft-marking techniques on DMEK success.

Materials and Methods

The Institutional Review Board and Ethics Committee of the Dr. Lutfi Kirdar Kartal Education and Research Hospital approved the initiation of the study (IRB No: 2018/514/143/1). The study was conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all individual study participants.

Patients

This study included 65 eyes of 55 patients who met all inclusion criteria and had undergone the DMEK procedure between May 2014 and February 2018 as a result of diagnosis of Fuchs endothelial dystrophy, pseudophakic bullous keratopathy, or failed DMEK. All procedures were performed at a single center. Patients were divided into 3 groups according to the marking technique used during the endothelial graft preparation stage: group I (F-marked endothelial graft; n = 17 eyes), group II (asymmetric triangle-marked endothelial graft; n = 12 eyes), and group III (endothelial graft prepared without any marking technique; n = 36 eyes). Patients who were aphakic or had a dislocated intraocular lens, a history of glaucoma surgery, iris or pupil disorders, uveitis, or microcornea and those on whom an F-mark or asymmetric triangle could not be observed on the intraoperative graft were excluded from the study. There were 20 male (36.4%) and 35 female (63.6%) patients with a mean age of 69.78 ± 9.82 years (range, 49-84 y). The 65 surgeries were performed by a single experienced surgeon (BK) and consisted of 48 DMEK cases (73.8%), 13 triple DMEK cases (20%), and 4 repeat DMEK (re-DMEK) cases (6.2%). The mean follow-up was 19.2 ± 13.8 months (range, 6-48 mo). Demographic data, DMEK indications, and surgeries performed are presented in Table 1.

Donor tissue preparation and marking technique

The corneoscleral button was affixed onto a suction holder (Barron; Katena Products, Inc., Parsippany-Troy Hills, NJ, USA) while the donor Descemet-endothelium complex was being prepared. The peripheral endothelium was marked with a Y-shaped hook or a 9.0- to 9.5-mm trephine. The mark was then made visible using the 60-second 0.06% trypan blue staining procedure (Vision Blue; DORC, Zuidland, the Netherlands). The Descemet-endothelium complex was partially detached by pulling with 2 forceps in a parallel and centripetal manner. The graft was then placed in the same position and cut with 8.0-, 8.25-, or 8.50-mm trephine.

After the graft cut with the punch trephine was stripped halfway, viscoelastic substance (sodium hyaluronate 1.4%; Bio-hyalur EV; Biotech, Gujarat,

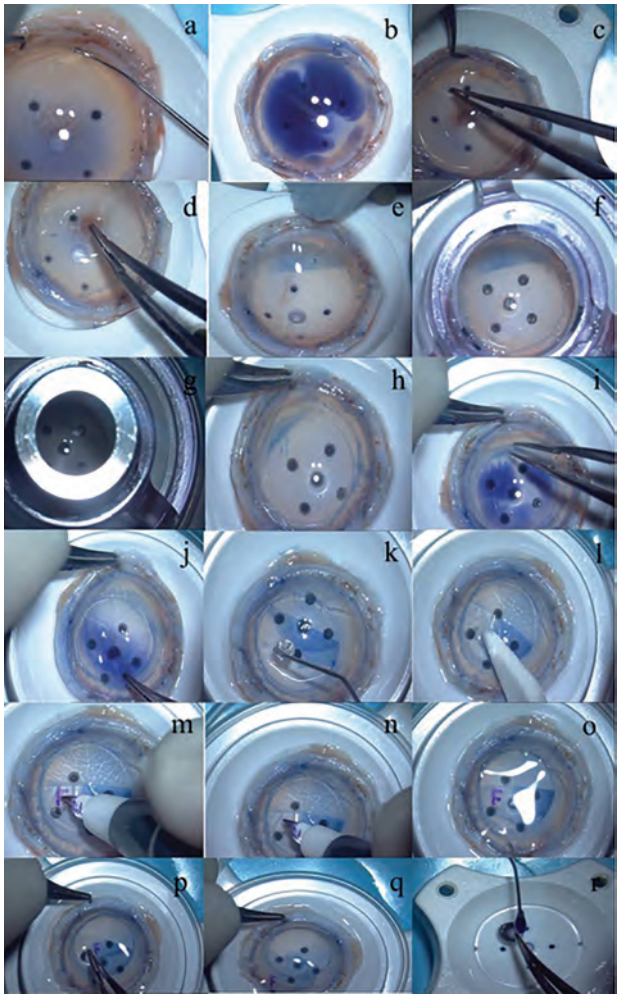
Table 1. Demographic Data, Indication for Descemet Membrane Endothelial Keratoplasty and the Surgeries Performed

	Group I: F-Mark	Group II: Asymmetric Triangle	Group III: No Mark	All Groups
Number of eyes	17	12	36	65
Female/male patients, No. (%)	13/4 (76.5%/23.5)	5/4 (55.6%/44.4%)	17/12 (58.6%/41.4%)	35/20 (63.6%/36.4%)
Mean age (SD), y	70.00 ± 11.01	67.33 ± 15.54	69.93 ± 7.34	69.78 ± 9.82
Indications for DMEK, No. (%)				
FECD	6 (35.3%)	4 (33.3%)	9 (25%)	19 (29.2%)
BK	10 (58.8%)	7 (58.3%)	25 (69.4%)	42 (64.6%)
Failed DMEK	1 (5.9%)	1 (8.3%)	2 (5.6%)	4 (6.2%)
Preoperative lens status, No. (%)				
Pseudophakic	11 (64.7%)	10 (83.3%)	28 (77.8%)	49 (75.4%)
Phakic	6 (35.3%)	2 (16.7%)	8 (22.2%)	16 (24.6%)
Surgery				
DMEK	10 (58.8%)	11 (91.7%)	27 (75%)	48 (73.8%)
Repeat DMEK	1 (5.9%)	1 (8.3%)	2 (5.6%)	4 (6.2%)
Triple DMEK	6 (35.3%)	0 (0%)	7 (19.4%)	13 (20%)

Abbreviations: BK, bullous keratopathy; DMEK, Descemet membrane endothelial keratoplasty; FECD, Fuchs endothelial corneal dystrophy; SD, standard deviation

India) was inserted between the endothelial surfaces in the region to be marked to prevent contact in the F-mark group. An F-mark was placed directly onto the stromal aspect of the Descemet-endothelium complex with a surgical skin marker, and the graft was brought into its original position using balanced salt solution. The marked graft edge was then grasped using non-toothed forceps to enable stripping from the stroma (Figure 1).

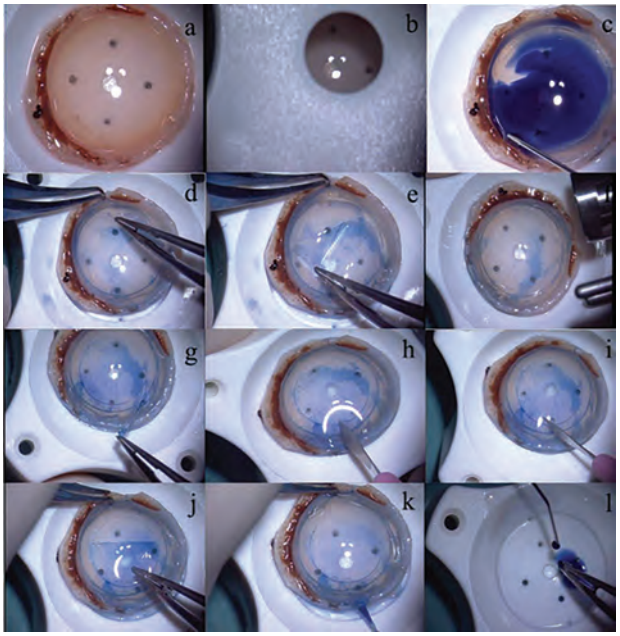
Figure 1. Graft Preparation With the F-Mark Technique



(a) The corneoscleral button is placed on the suction holder while the donor Descemet-endothelium complex is prepared. The peripheral endothelium is detached 360 degrees using a Y-shaped hook. **(b)** The detached area is made visible by using trypan blue dye. **(c, d)** The Descemet-endothelium complex is partially detached using 2 forceps to pull with a parallel centripedal motion. The partially detached graft is placed in the proper position with balanced salt solution (BSS) **(e),** which is then cut with a punch trephine **(f, g).** The cut graft Descemet-endothelium complex is denuded half-way, and viscoelastic material is then placed on the endothelial interface at the region to be marked **(h-k)**. Once the area to be marked is dried with a sponge triangle **(l),** the F-mark is placed with a surgical skin marker **(m, n)** and the graft put back to the original position with BSS **(o). (p, q)** The graft edge is grasped gently with a nontoothed forceps and fully detached from the stroma. **(r)** Trypan blue dye is used to dye the detached graft.

The asymmetric triangular marking group went through the same initial procedures, after which the graft was cut with 8.0-, 8.25-, or 8.50-mm trephine. An asymmetric triangle was created on the Descemet-endothelium complex at a single region and from the endothelial surface, using an ophthalmic stab knife at 45° in a counterclockwise manner (Figure 2).[15] The prepared Descemet-endothelium complex was stained with 0.06% trypan blue and then placed into a standard lens cartridge (Zaraccom cartridge and injection system; Sivas, Turkey).

Figure 2. Graft Preparation With the Asymmetric Triangle Mark Technique



(a) The corneoscleral buttons are placed on the suction holder when the donor Descemet-endothelium complex is being prepared. **(b)** The peripheral endothelium is cut with a 9.0- or 9.50-mm punch trephine. **(c)** The detached area is made visible by using trypan blue dye. **(d, e)** The Descemet-endothelium complex is partially detached using 2 forceps in a parallel centripedal pulling motion. **(f)** The partially detached graft is placed in its location with balanced salt solution and then cut with a smaller (8.00-, 8.25-, or 8.50-mm) punch trephine. **(g)** The intermediate endothelial remnant is detached with forceps. **(h, i)** An asymmetric triangle shape is created on the Descemet-endothelium complex using an ophthalmic stab knife at 45° from the endothelial side in a counterclockwise manner. **(j, k)** The graft edge is held gently with nontoothed forceps, and the graft is completely separated from the stroma. **(l)** Trypan blue is used to dye the detached graft.

Surgical technique

Retrobulbar anesthesia was administered to all patients with the modified Van Lint facial block technique using a 4-mL mixture of 2% lidocaine hydrochloride and 0.5% bupivacaine hydrochloride. Intraocular pressure was then decreased with 5 minutes of ocular massage and 15 minutes of Honan balloon use. After routine surgical preparations were

completed, the corneal epithelium was denuded to obtain a clear image when needed. The 2 side ports and the corneal surface were marked at an approximately 9.0-mm diameter using a surgical skin marker. The anterior chamber was filled with air after the side ports were opened at the 10:30 and 1:30 clock positions. A reverse Sinskey hook was used to perform a Descemetorhexis (removal of the Descemet membrane) with a diameter of 9 mm by scoring a circular Descemet membrane area and then stripping it from the posterior stroma.

Graft insertion was then performed through a 3.0-mm tunnel incision created at the limbus 12-o'clock position. Miosis was created by administering carbachol (Miostat Single 0.01%, Novartis Pharma GmbH, Nürnberg, Germany) into the anterior chamber. A peripheral iridectomy was performed at the 6-o'clock position using a 23-gauge vitrectomy probe. A repeat iridectomy was not performed as one was already present in cases with repeat DMEK. The cartridge was then used to insert the graft into the anterior chamber. A 30-gauge cannula was used for corneal anterior surface tapping while unfolding the graft. After confirmation that the graft had opened in the correct position and the marks were in the appropriate places, adhesion of the graft to the receiver posterior stroma was ensured by filling the anterior chamber with air.

Triple Descemet membrane endothelial keratoplasty

The triple procedure consisted of endothelial keratoplasty after standard phacoemulsification surgery and intraocular lens implantation.[16] For these surgical cases, we performed a Descemetorhexis of approximately 9.0 mm in diameter under viscoelastic material (sodium hyaluronate, Bio-hyalur EV, Biotech, India) after cataract surgery and intraocular lens implantation. The viscoelastic material and Descemet membrane remnants in the anterior chamber and behind the intraocular lens were then removed with irrigation-aspiration. Miosis was ensured by administering carbachol (Miostat Single, Novartis Pharma GmbH) to the anterior chamber. Peripheral iridectomy was performed at the 6-o'clock position using a 23-gauge vitrectomy probe. The graft was implanted with a standard lens cartridge and then fully opened, with air then administered between the graft and the iris. We confirmed that the DMEK graft was transplanted on the receiver graft stroma after it was completely unfolded.

The corneal entry sites were sutured, and gentamycin (Gentamicin, DEVA Holding, Istanbul, Turkey) and dexamethasone (Dekort, DEVA Holding) were injected subconjunctivally at the end of the surgery. The patients were then instructed to remain supine for 48 hours to ensure adhesion of the graft to the stroma.

Postoperative follow-up

All eyes received 0.5% moxifloxacin hydrochloride (Vigamox, Alcon, Fort Worth, TX, USA) and 0.1% dexamethasone (Maxidex, Alcon) 5 times per day postoperatively. The corneal sutures were removed within 15 days. Topical antibiotic treatment was stopped on postoperative day 10 in all patients. Topical steroid treatment was decreased 1 drop per day every month and changed to 0.5% loteprednol etabonate (Lotemax, Bausch & Lomb, Bridgewater, NJ, USA) 3 times per day at the end of month 3. The local steroid treatment was then gradually decreased to a once daily maintenance dose.

Patients with corneal graft detachment on microscopy were evaluated together with anterior segment optical coherence tomography results to determine whether the rebubbling procedure was necessary. Rebubbling was performed in the first month for peripheral detachments that were large or showed scroll formation or interfered with the visual axis. This procedure consisted of unrolling the graft and keeping the patient in a supine position afterward as necessary. Peripheral detachments that were straight or did not interfere with the visual axis were followed up without intervention.

Patients whose cornea did not clear postoperatively were considered to have primary or secondary graft failure. Primary graft failure was defined as grafts that actually attached but did not clear at any time, whereas secondary graft failure was defined as attached grafts that had shown signs of becoming clear but that had then decompensated.

Statistical analyses

We used PASW Statistics software (SPSS: An IBM Company, version 17.0, IBM Corporation, Armonk, NY, USA) for statistical analyses. Results are shown as means ± standard deviation. Before comparisons, the Shapiro-Wilks test was used to determine whether the group data had a normal distribution. The chi-square test, Wilcoxon test, paired sample *t* test, Fisher exact test, and independent sample *t* test

were used for evaluation. Statistical significance level was accepted as $P < .05$.

Results

Visual outcomes

After exclusion of patients with low visual acuity potential (diabetic macular edema, age-related macular degeneration) and those who had undergone unsuccessful DMEK surgery (repeat DMEK or secondary PKP) before 6 months, the overall preoperative and postoperative 6-month mean BCVA values were 2.02 ± 0.87 and 0.50 ± 0.69 logMAR, respectively ($P < .001$). Preoperative mean BCVA values for groups I, II, and III were 2.11 ± 0.99, 2.04 ± 0.98, and 1.98 ± 0.81 logMAR, respectively, whereas postoperative mean BCVA values were 0.86 ± 0.90, 0.33 ± 0.59, and 0.39 ± 0.58 logMAR, respectively (group I vs II: $P = .871$; group I vs III: $P = .648$; group II vs III: $P = .855$). Overall, 63.6% of eyes attained a BCVA ≥ 20/32 (≥ 0.6 Snellen fraction [decimal]) and 27.3% attained ≥ 20/20 (≥ 1.0).

Distributions of ocular comorbidities within the groups were as follows. In group I, 2 eyes had age-related macular degeneration and 1 eye had diabetic macular edema. In groups II and III, 1 eye in each group had age-related macular degeneration. Patients who underwent unsuccessful DMEK surgery before 6 months were distributed to the groups as follows: secondary PKP before 6 months in 2 eyes in group II and secondary PKP before 6 months in 1 eye and re-DMEK before 6 months in 2 eyes in group III.

When the 6-month BCVA results were evaluated in the 3 groups after ocular comorbidities and graft failure instances were omitted, the percentage of patients with a BCVA of ≥ 20/32 (≥ 0.6) in groups I, II, and III was 35.7%, 77.8%, and 71.9%, respectively ($P = .04$). A 6-month BCVA result of ≥ 20/32 (≥ 0.6) was statistically significantly lower in group I than in groups II and III (group I vs II: $P = .04$; group I vs III: $P = .02$). There was no significant difference in BCVA between group II and group III ($P = .72$). In groups I, II, and III, a 6-month BCVA value of ≥ 20/20 (≥ 1.0) was seen in 7.1%, 33.3%, and 34.4%, respectively. The percentage of patients with a BCVA of ≥ 20/20 (≥ 1.0) was higher in groups II and III than in group I, but there was no statistically significant difference between the groups (group I vs II: $P = .26$; group I vs III: $P = .073$; group II vs III: $P = 1.0$). Table 2 presents the visual acuity percentages for each group.

Table 2. Postoperative Visual Acuity Levels at 6 Months After Descemet Membrane Endothelial Keratoplasty

	Group I: F-Mark (n = 14)	Group II: Asymmetric Triangle (n = 9)	Group III: No Mark (n = 32)	Overall Group (n = 55)
≥ 20/40	5 (35.7%)	7 (77.8%)	25 (78.1%)	37 (67.3%)
≥ 20/32	5 (35.7%)	7 (77.8%)	23 (71.9%)	35 (63.6%)
≥ 20/20	1 (7.1%)	3 (33.3%)	11 (34.4%)	15 (27.3%)

Patients who had low visual acuity potential and who underwent unsuccessful Descemet membrane endothelial keratoplasty (DMEK) surgery (repeat DMEK or secondary penetrating keratoplasty) before 6 months were excluded.

Endothelial cell density

We excluded 14 of the 65 eyes (21.5%) from the ECD analysis (6 had a secondary reoperation before the 6-month follow-up and 8 had corneal edema that prevented proper evaluation). In the remaining 51 eyes, the mean donor ECD was 2817.07 ± 310.13 cells/mm^2 before and 1496.45 ± 336.82 cells/mm^2 6 months after surgery, indicating a decrease of 46.01 ± 11.96%. In groups I, II, and III, the percent ECD decrease at 6 months was 43.3%, 48.8% and 46.4%, respectively, with no significant difference between groups (group I vs II: $P = .176$; group I vs III: $P = .468$; group II vs III: $P = .647$).

Central corneal thickness

In all groups, the mean CCT decreased 23.29% ± 17.06%, from 761.87 ± 148.40 μm preoperatively to 569.52 ± 70.88 μm at postoperative month 6 ($P < .001$). The decrease in CCT at month 6 was 7.7%, 15.8%, and 34.0% in groups I, II, and III, respectively. The percent CCT decrease was significantly higher in group III patients than in the other groups (group I vs III: $P < .001$; group II vs III: $P = .002$). The percent CCT decrease was also significantly higher in group II patients than in group I patients (group I vs II: $P = .006$).

Complications

Postoperatively, 14 eyes (21.5%) required rebubbling (air reinjection), 5 eyes (7.7%) required secondary PKP, and 14 eyes (21.5%) required repeat DMEK. We encountered primary graft failure in 12 eyes (18.4%), secondary graft failure in 6 eyes (9.2%), and graft separation in 16 eyes (24.6%).

Rates of primary graft failure in groups I, II, and III were 35.3%, 8.3%, and 13.9%, respectively.

Primary graft failure rate was higher in group I than in the other groups, although not significantly (group I vs II: $P = .187$; group I vs III: $P = .143$; group II vs IIIL $P = 1.000$). Rates of secondary graft failure rate were 0% in group I, 41.7% in group II, and 2.8% in group III. The secondary graft failure rate was significantly higher in group II than in the other groups (group I vs II: $P = .007$; group I vs III: $P = 1.0$; group II vs III: $P = .002$).

Rates of graft detachment were 17.6%, 50.0%, and 19.4%, respectively, in groups I, II, and III, that is, higher in group II than in the other groups (group I vs II: $P = .106$; group I vs III: $P = 1.00$; group II vs III: $P = .039$). The rebubbling rate was 17.6% in group I, 50% in group II, and 13.9% in group III (group I vs II: $P = .106$; group I vs III: $P = .701$; group II vs III: $P = .01$). Although rebubbling rate was not significantly different between groups I and II and between groups I and III, the rate in group II was significantly higher than the rate in group III.

A secondary corneal transplant was required in 29.2% of the eyes overall. The secondary corneal transplant rate was 35.3%, 50%, and 19.4%, respectively, in groups I, II, and III (group I vs II: $P = .428$; group I vs III: $P = .211$; group II vs III: $P = .039$). Although secondary corneal transplant rate was not significantly different between groups I and II, group II rate was significantly higher than the rate in group III.

Glaucoma was present in 4.6% of eyes (n = 3, with 1 in group I and 2 in group II) in the postoperative period, and treatment was started with 0.1% topical combined timolol and dorzolamide (Cosopt, Merk & Co, Inc., Whitehouse Station, NJ, USA) twice daily. Allograft rejection developed in 1 eye (1.5%) from group III, which was treated with topical steroid eye drops containing 0.1% dexamethasone sodium phosphate (Maxidex, Alcon). Table 3 presents resurgery data and postoperative complications for the groups.

Discussion

We compared the 6-month clinical outcomes of patients who had DMEK performed after graft marking with the F-mark or an asymmetric triangle and after those performed without a graft mark. A total of 65 eyes that had undergone DMEK surgery by a single surgeon were included in our study. We evaluated the BCVA, ECD, CCT, secondary keratoplasty, and complication rates in DMEK cases with and without preliminary graft marking, considering that the marking techniques used on grafts could influence surgical success because of the possible toxic or traumatic effects on the endothelium.

There are limited numbers of studies on graft-marking techniques in the literature.[7,11-15] Marking techniques used for graft orientation include the F-mark,[11] S-stamp,[12] 3 circular marks,[13] 4 asymmetric marks,[14] and a single peripheral triangular mark.[15] The reason that such marking techniques are needed is because correct anatomic orientation is the most important factor for DMEK success. The DMEK tissues tend to create a scroll, leading to a rotation potential during the injection or unfolding phases. A device to ensure continuous correct orientation during insertion and manipulation so far unfortunately does not exist. Other causes of difficulty in orienting the graft include suboptimum intraoperative anterior chamber view, for example, due to edematous and scarred corneas, and poor graft scrolling tendency as with older donor grafts.[15]

Several in vitro and in vivo studies have evaluated the effects of the F-mark and S-stamp techniques using gentian violet dye on the graft and the relevant surgical results.[7,12,17] In an in vitro study on graft marking with the S-stamp technique using pixel calculation with special software after the graft was stained with calcein, associated endothelial cell loss was shown to be 0.6%. This loss is reported to make up 4.2% of all the endothelial loss at the

Table 3. Repeat Surgeries and Postoperative Complications

	Group I: F-Mark (n = 17)	Group II: Asymmetric Triangle (n = 12)	Group III: No Mark (n = 36)	*P* Value
Repeat surgeries, No. (%)	9 (52.9%)	7 (58.3%)	10 (27.8%)	.078
Rebubbling	3 (17.6%)	6 (50%)	5 (13.9%)	
Secondary keratoplasty	6 (35.3%)	6 (50%)	7 (19.4%)	
Repeat DMEK	6 (35.3%)	3 (25%)	5 (13.9%)	
Secondary PKP	0 (0.0%)	3 (25%)	2 (5.5%)	
Postoperative complications	10 (58.8%)	8 (33.7%)	13 (36.1%)	.105
Primary graft failure*	6 (35.3%)	1 (8.3%)	5 (13.9%)	
Secondary graft failure**	0 (0.0%)	5 (41.7%)	1 (2.8%)	
Allograft rejection	0 (0.0%)	0 (0.0%)	1 (2.8%)	
Glaucoma	1 (5.9%)	2 (16.7%)	0 (0.0%)	
Cataract surgery after DMEK	0 (0.0%)	0 (0.0%)	2 (5.5%)	
Detachment***	3 (17.6%)	6 (50%)	7 (19.4%)	

Abbreviations: DMEK, Descemet membrane endothelial keratoplasty; PKP, penetrating keratoplasty.

*Primary graft failure refers to an attached graft where the cornea fails to clear at any time. **Secondary graft failure refers to an attached graft where the cornea starts to clear but is followed by corneal decompensation. ***Includes all graft detachments as observed at the 6-mo follow-up.

endothelial preparation stage.[17] In a study that compared the 6-month clinical results of 133 DMEK cases that had the S-stamp graft-marking technique and 31 DMEK cases without marking, there were no significant differences between the 2 groups with regard to BCVA, ECD decrease, and rebubbling rates.[12] The upside-down graft implantation rate was 0% in the DMEK S-stamp group and 9.4% in the DMEK group without marking. In our study, we found that the primary graft failure rate was higher (35.3%), although not significantly, in patients who had undergone DMEK after the F-mark was placed directly with the surgical skin marker compared with the other groups. The percentage of patients with a 6-month BCVA of ≥ 20/32 (≥ 0.6) was 35.7%, 77.8%, and 71.9%, respectively, in the F-mark, asymmetric triangular mark, and the unmarked groups, with levels significantly lower in the F-mark group than the other groups.

The 6-month ECD decrease was 43.3%, 48.8%, and 46.4%, respectively, in groups I, II, and III in our study. Because it was not possible to obtain postoperative corneal measurements, we could not include 29.4%, 33.3%, and 13.8% of these eyes, respectively, in calculations for groups I, II, and III. We believe the reason for the lack of a significant difference regarding ECD measurements between the groups could be because only data of measurable eyes were evaluated and because of the low number of patients in the groups. The 6-month CCT decrease was 7.7%, 15.8%, and 34% in groups I, II and III, respectively, in our study, with this decrease significantly lower in group I patients versus the other groups. The BCVA levels were lower in the F-mark group as the CCT measurement could be performed in all patients in the groups and because the CCT decrease is directly related to the BCVA level.

Stoeger and associates stated that gentian violet dye ink can be safely used in the S-stamp technique for Descemet stripping automated endothelial keratoplasty grafts.[18] However, the authors emphasized that one needs to wait for 10 seconds before stromal surface marking is performed so that the alcohol can dry after ink is applied to the stamp.[12,18] The ink was applied to the stamp, followed by waiting 10 seconds for the alcohol carrier to dry, and then the dried dye was applied to the stromal surface. In our study, we believe that the alcohol in the ''wet'' gentian violet dye used directly on the graft stromal surface without a stamp in our study created a toxic effect on the endothelium and decreased the surgical success rates in the F-mark group.

Making asymmetric cuts in the endothelium is another option for correct orientation of the graft. Three techniques have been defined in the literature: 3 circular marks (Bachmann technique)[13], 4 asymmetric marks (Matsuzawa technique),[14] and a single peripheral triangular mark[15] (Bhogal technique). The marking is created in the graft periphery using dermatologic biopsy punches with the Bachmann[13] and Matsuzawa[14] techniques and a 30-degree incision knife with the Bhogal technique. The theoretical endothelial cell loss due to marking has been reported as 2.5% with the Bachmann technique, 5.8% with the Matsuzawa technique, and 0.7% with the Bhogal technique. The authors stated that these loss numbers are acceptable compared with the results that occur with incorrect graft positioning. The Bachmann technique was reported to cause a 1-month ECD loss of 37% and primary graft failure rate of 12%; the Matsuzawa technique resulted in a 6-month ECD loss of 44% with no primary graft failure. In our study, the 6-month ECD decrease was 48.8% and the primary graft failure rate 8.3% in the DMEK cases performed with an asymmetric triangle. The ECD loss rates were similar in the cases that underwent the procedure with the Bachmann or Matsuzawa technique and our asymmetric triangle group. However, we found the secondary keratoplasty rates in the asymmetric triangle group to be higher than with the Bachmann or Matsuzawa technique. We believe this could be due to the high graft separation and rebubbling rates in the asymmetric triangle group.

The rebubbling, primary graft failure, and secondary keratoplasty rates with the F-mark and asymmetric triangle marking techniques in our study group were markedly higher than the rates in other DMEK series in the literature. However, the results for our group without marking were similar to those reported in other studies.[19,20]

Conclusions

Our study is the first to report the clinical results of multiple graft-marking techniques to the best of our knowledge. These techniques greatly facilitate graft orientation in cases where the intraoperative anterior segment view is suboptimum and the graft has poor

scrolling tendency. However, we encountered primary graft failure due to alcohol toxicity in our cases where gentian violet dye was used for marking as we had directly used a surgical marker for the marking procedure. We also observed graft separation starting from the marking area with asymmetric triangle marking that resulted in similar increases in rebubbling, secondary keratoplasty, and secondary graft failure rates. Although the graft-marking technique greatly facilitates intraoperative graft position orientation during DMEK surgery, one must consider the toxic effects of alcohol on the endothelium when marking with the gentian violet dye and the increased graft detachment risk with the asymmetric graft-marking technique.

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ARTICLE

Development, Implementation, Evaluation, and Long-Term Outcome of a Program to Increase Student Interest in Anesthesia and Intensive Care Training

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Abstract

Objectives: **The increasing need for anesthetists has been coupled with a rising number of open training positions. Thus, there is an increased need to attract future anesthetists among students and graduates from medical universities. Using results from a questionnaire, we designed an information and training program to increase interest in anesthesia and intensive care.**

Materials and Methods: **With the use of semistructured interviews, medical students were questioned about factors influencing their decision for a speciality. We used the results to design an information and practice program for students and young doctors. This program was held 12 times at different anesthesia departments in different hospitals. Evaluation was obtained through a feedback questionnaire at the end of each sessions and with another questionnaire 2 to 4 years after the program.**

Results: **Feedback showed positive responses concerning utility for practical work, actuality, and relevance for daily practical work. There was a 22.7% response from participants for the follow-up questionnaire. Of these, 87% stated that interest in anesthesia was increased by the program, and 74% underwent practical training in an anesthesia department. Seventeen participants started a speciality training for anesthesia and intensive care medicine.**

Conclusions: **The design of this practice-oriented program was effective in eliciting, spreading, and increasing interest and attracting students to a medical specialty.**

Key words: *Anesthesiology, Human resources, Lack of personnel, Speciality training, Training program*

Introduction

The scope of speciality anesthesia and intensive care medicine has recently considerably changed. Today, the specialty includes not only core competence of treating the patient in the operating room but also, in most countries, postoperative intensive care treatment, emergency medicine and resuscitation, and pain management. In Austria, this development together with the implementation of the European Working Time Directive for Medical Doctors has led to a doubling of the number of anesthetists in the past 20 years.[1] However, as prognostic evaluations point out, even this increase is not sufficient to cover the future need for doctors in this speciality. In Europe, this will be further aggravated by the fact that many anesthetists are nearing retirement.

In the Kingdom of Saudi Arabia, the problem is urgent. Anesthesia is regarded as a speciality that requires hard work but provides only small rewards; this specialty has not been able to compete with specialities that allow future doctors better income and the satisfaction of a clinic for managing treatment and following patients throughout their disease course. With the rising importance of anesthesia and intensive care medicine, income levels have increased considerably; however, interest among medical students and graduates remains low. Thus, attracting new students to this specialty will require high efforts in the recruitment of graduates from medical universities so that the supply of

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specialists for anesthesia and intensive care meet the number required to allow this needed function to continue in the medical system.

In an effort to study and improve strategies to increase the interest in this speciality, we investigated the following aspects: (1) factors influencing the decision of medical students and graduates for the selection of a speciality; and (2) design and evaluation of a program to increase interest in the speciality of anesthesia and intensive care medicine.

Materials and Methods

This study was conducted in Vienna, Austria, where the biggest medical university of Austria is located. The Vienna Hospital Service allowed and supported the study to take place in one of the hospitals. In Austria, the speciality of anesthesia automatically includes the speciality of intensive care medicine.

In a first step, we evaluated the recognition of the speciality in students and the wishes of students with regard to future working conditions by means of semistructured interviews. We used these results to design a program for the recruitment of young graduates and students. Results from this program were evaluated and compared with results from recent publications to extract common aspects and conditions in the picture of the speciality and to allow an objective communication of the pros and cons of the speciality as well as the development of practical means for the recruitment of anesthetists.

Interviews regarding the perception of the speciality

To determine factors influencing the decision for the selection of a speciality among medical students, we used a questionnaire to conduct semistructured interviews among randomly selected students (n = 20; median age of 23 y, 8 male and 12 female participants) We searched for factors influencing interest and attractiveness in the process of decision of a medical speciality.

Focus:Practice Program

We used the Focus:Practice© Program (a product of Health Care Communication) in the development and implementation of a program to improve interest among medical students and graduates for speciality anesthesia and intensive care medicine; the program was based on results from the interview stage. All participants gave written consent to use of their data in anonymized form for documentation and publication. The goal of the program was to give participants a broad view of the speciality in order to increase the attractiveness of the program and to motivate students to opt for this speciality.

Four topics were selected: (1) practical pain management, (2) perioperative anaesthesia, (3) perioperative intensive care medicine, and (4) emergency management. The duration of each Focus:Practice lecture was 4 hours and was offered at several anesthesia departments in Vienna, Austria. The number of participants was limited to 30. Each event consisted of 3 parts. In part 1, there were 3 or 4 presentations with a duration of 20 to 25 minutes and an emphasis on practical working. In part 2, there was hands-on training at 3 to 4 different locations where participants had the possibility of experiencing and training different techniques relevant to the topic (eg, airway management, intraosseous access for application of drugs, and different applications of ultrasonography). For this part, participants were divided into small groups and rotated between locations to allow optimal participation in the training. Part 3 consisted of a debriefing to allow a discussion of the contents and experiences with the referents. Referents were anesthetists from different departments of the Vienna hospitals.

Extra presentations were offered on the topics "cardiovascular anesthesia" and "mother and child, anesthesia in obstetrics and neonatal resuscitation."

All events were offered on the homepage of the student's association of the Medical University of Vienna. All participants were offered a practice turn in one of the participating anesthesia departments to further increase their knowledge of the speciality.

Evaluation of the program

All participants received an evaluation questionnaire at the end of each event. Here, they could rate different aspects of the event on a scale from 1 (worst value) to 5 (best value). They could also add free comments on the evaluation sheet. The questionnaires were completed at the location and were immediately handed over to the organization team.

To investigate whether an evaluation from a participant was not a momentary impression and whether the program elicited a sustained value for their decision of a further career, we performed a second evaluation from March 2020 to May 2020 by E-mail, where we questioned whether any of the

participants completed a practice turn in an anesthesia department or whether they had decided on that speciality. The online questionnaire consisted of 4 questions and the possibility of free comments.

All data from participants were strictly anonymous and with their consent; no data concerning the health of the participants were noted, and no inclusion of a participant was due to a disease. In addition, none of the participants was from a vulnerable group as defined in the Helsinki Declaration. The Institutional Review Board did not classify this investigation as a clinical study and made no objections to the performance of our work.

Results

The number of applications exceeded the possible number of participants by 4-fold. A total of 186 participants were accepted, who could apply for participation in several events, with the total number of participants in the 12 events performed between 2016 and 2018 of 331.

Interviews with students

Practical working, the wide spectrum of the speciality, and the care of patients in emergencies were named by the students in the interviews as arguments for choosing anesthesia. Further positive aspects were the "high-tech" working field, the high number of available positions, and the interdisciplinary work, especially in the operation room. A high homogeneity was also found in answers on factors that hampered the choice of anesthesia: a lack of knowledge about possibilities of work and career in the speciality and a general lack of contact with anesthesia and intensive care during the university training. Furthermore, a lack of training positions, the dependance of work and career in a hospital, and the lack of communication and follow-up with patients were also named.

Results of evaluation of events

Feedback data collected at the end of the events showed very positive responses. Average satisfaction throughout all events concerning the items relevant to practical work and actuality was 4.83. Hands-on training feedback received a score of 4.80 (Table 1).

The positive results were also supported by the free comments added by the participants in the feedback protocols. A total of 118 participants added remarks, and 103 participants (87.3%) emphasized a high degree of positivity toward clinical work in the presentations and the hands-on training. The possibility for practical experience and the interdisciplinary approach were also highlighted.

Results of the 2020 follow-up evaluation

The results of the follow-up evaluations are shown in Table 2. Of the 186 participants, 172 (92.5%) had a valid E-mail address. Of these, 39 participants (22.7%) responded to the request for follow-up at 2 to 4 years after the original events, with 97.5% responding a very positive (82%) or positive recollection of the event. Of responding participants, 87% stated that the event had influenced their interests for this speciality very positively (49%) or positively (38%). Of note, 74% of

Table 1. Evaluation of Events by Participants at the End of Each Event

Characteristic		Score
Referents	Competence	4.86
	Presentation	4.71
	Practical training	4.8
Relevance for practical work		4.86
Actuality		4.87
Relevance for daily work		4.78

118 feedback questionnaires were completed by the 186 participants (62% female participants, 83% younger than 30 years old, 91% students). Rating followed a 5-step scale with 5 as the best value.

Table 2. Feedback at Follow-Up From Former Participants With Valid E-mail Accounts

Question	Number (%)				
	Very Positive (yes)	Quite Positive	Neutral	Quite Negative	Very Negative (no)
What is your impression when you recall the Focus:Practice events?	32 (82.05%)	6 (15.38%)	1 (2.56%)	0	0
How did the events influence your view on a career in anesthesia and intensive care medicine?	19 (48.72%)	15 (36.46%)	5 (12.82%)	0	0
Have you applied for a practice course in anesthesia and intensive care medicine?	28		1*		10
Did you decide for a training position in a department of anesthesia and intensive care medicine?	17		8*/2§		12

There were 186 former participants, with 172 having valid E-mail addresses at follow-up (92.5%) in which accounts were still valid and 39 (22.7%) returning responses in 2020.
*Not yet decided. §Changed field of work.

these respondents completed a practical training in a department of anesthesia or intensive care. The final question ("Did you decide for the specialties of anaesthesia and intensive care?") was answered with "yes" by 17 participants (43.6%), amounting to 10% of total participants (n = 172) with a still valid E-mail account. Another 20.5% had not yet taken the decision. In the area dedicated to free comments, multiple remarks were made on the value and the sustained effect of hands-on training.

Discussion

Results of our evaluation demonstrated that interest among students and young graduates can be greatly improved by the concept of the Focus:Practice Program. Information relevant to the practical work and content of the speciality was greatly welcomed by the participants. The events that were developed with suggestions from the initial questionnaire, which included the suggestion of hands-on training, had a very positive effect on interest for the specialty among participants, which led to a number of students deciding to train in anesthesia and intensive care medicine.

To ensure a sufficient number of anesthetists for delivery of medical care, improvements in the image of anesthesia are needed, especially among graduates and medical students. Contact with this speciality is sparse in many countries, and the impression often delivered is that of dull but very hard work with few rewards. However, studies have shown that the information available to medical students is extremely important for their future decision on which speciality to choose.[2] Thus, it is essential to supply medical students a comprehensive view of modern anesthesia and intensive care medicine as a complex and broad field of work.

For Austria, the president of the Austrian Society for Anaesthesiology, Intensive Care Medicine, and Reanimatology (ÖGARI) addressed the importance of this problem in a presentation at the 2019 Austrian International Congress (Graz, Austria). Measures are urgently needed against the impending shortage of anesthetists and are receiving the highest priority to avoid a serious threat to medical care.[3] An investigation by BDO Health Care Consultancy, which was mandated by ÖGARI and presented to the directors of Austrian anesthesia departments, concluded that immediate measures are needed to ensure continued demand for anesthetists. This was also emphasized in a position paper of the ÖGARI.[4]

In Saudi Arabia, the situation is almost the same. Active measures are needed to encourage new graduates to choose training in anesthesia and intensive care medicine. The shortages for these speciality are more than for any other country. More than 90% of intensivists and anesthesiologists in Saudi Arabia are not Saudi citizens.

A survey of the Austrian Board of Medical Doctors showed that young doctors who had decided upon anesthesia as a specialty were rating their training on average much higher than young doctors in other specialities.[5] Thus, if the training itself has this positive image, the problem must be faced earlier in the process, that is, when students are deciding upon their speciality. Students and young doctors should be given information in a timely manner on the broad possibilities and the attractiveness of anesthesia, which definitely are in contrast to the widespread image of the speciality in the public and among medical students.

This is also in accordance with many investigations from other countries and specialities.[1,2,6,7] With this knowledge, deficits in information can be corrected, possibilities can be highlighted, and old and wrong images of the speciality can be eliminated. The first part of our investigation in 20 students showed a deficit in knowledge on practical possibilities owned by the speciality and especially a lack of contact with anesthesia and intensive care during training at the university. This result is in accordance with a study from the University of Bochum, which investigated the image of modern anesthesia in medical students as well as their request for working conditions and their perception of the speciality.[2] Piontek concluded that, despite prior experiences and high satisfaction with lectures in anesthesia and intensive care medicine, the speciality is still predominantly identified with the work in the operating room and only to a lesser degree with intensive care medicine, emergency medicine, and pain management. Piontek suggested intensifying communications on the image of modern anesthesia for the further development of the speciality.[2]

In their investigation of emergency medicine, Chew and colleagues evaluated 800 responses to questionnaires and concluded that early contact with a speciality is important for the decision to choose a speciality.[8] Thus, efforts are needed toward providing

students opportunities to become acquainted with different specialities and to support their decision by practical knowledge.

During application of results obtained during the first part of our study, we found it was possible to develop a program that focused on practice and insight on broad aspects of a speciality. In our study, all presentations and teachings were performed by volunteers from participating departments. Publicity was organized by the Students Association of the Medical University of Vienna and was so successful that 4 times as many applications were received as participants could be accepted. We take this as evidence that students are deeply interested and that there is need for such events. During feedback at the end of the event and during follow-up 2 to 4 years after initial participation, participants also stated that they valued content and practice-related exercises.

Bias has to be expected with this follow-up as the call was only answered by 22.4% of participants, with answers naturally more likely given by those who kept the event in a more positive memory. However, 17 of our participants had chosen at that point (2 to 4 years following the event) to accept a training position in a department of anesthesia and intensive care medicine, accounting for almost 10% of participants. Although some may have already had interest in anesthesia before joining our program, the effect of the program was positive by either amplifying their wish or by correcting wrong expectations, which some participants may have had and which could have caused them to leave the training prematurely.

Conclusions

We found that our program was successful in eliciting and spreading interest for anesthesia and intensive care medicine and that a considerable number of students and young doctors were supported in their decision for anesthesia and intensive care medicine.

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Giant Idiopathic Lymphocele 18 Years After Kidney Transplantation, Treated Using Lymphatic Embolization With Lipiodol: Report of a Rare Case

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Abstract

Kidney transplant is the best therapeutic option for patients with end-stage kidney disease. However, kidney transplant is not exempt from postoperative complications. One of the most frequent urological complications is lymphocele, which can appear in up to 20% of patients. Lymphocele most often appears during the first month after surgery. However, its appearance after the first year is completely infrequent. Here, we report a case of a giant idiopathic lymphocele 18 years after kidney transplant and its resolution with lymphatic embolization. The patient, a 34-year-old man who received a deceased-donor kidney transplant in 2002, had presented with no complications until the lymphocele was diagnosed. The lymphocele presented as a voluminous organ-compressing mass. A percutaneous drainage was placed, and 3600 cm³ of lymphatic fluid were drained. After that, 800 cm³ continued to leak every day. An intranodal lymphography and lymphatic embolization with Lipiodol Ultra-Fluide (Guerbet Australia) were performed, owing to the high amount of leakage. At 50 days after embolization, an ultrasonograph showed no fluid collections, so the percutaneous catheter was removed. In most patients, the treatment of the lymphocele after kidney transplant is frequently conservative. However, for patients whose situation cannot be resolved spontaneously, there are few therapeutic choices. As described here, intranodal lymphatic embolization is a mini-invasive option, with a success rate of up to 80%, and should be offered as the first approach.

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Key words: *Lymphatic vessel injury, Renal transplantation, Urological complication*

Introduction

Kidney transplant (KT) is the best therapeutic option for patients with end-stage chronic kidney disease. However, urological complications can occur, with a reported incidence after KT of 2.5% to 30%.[1,2] Lymphocele, a fluid collection between the kidney allograft and the bladder, is the most common urological complication, with a rate of incidence of up to 40%.[1] This complication is caused primarily by extravasation of the lymph from the lymphatic vessels injured during preparation of the iliac vessels of the recipient and the unligated lymphatic system from the renal hilum of the donor. Factors associated with the etiology of lymphoceles include acute rejection, length of surgery, extent of dissection, urinary obstruction, graft decapsulation, and use of immunosuppressants, such as mechanistic target of rapamycin (mTOR) inhibitors.[1,3,4] Small lymphoceles are usually asymptomatic, and spontaneous resolution occurs after a few months. In contrast, large lymphoceles can cause compression of the pelvicalyceal system, resulting in hydronephrosis and worsening renal function. Several choices have been described to treat this complication, from open fenestration to ultrasonogram-guided intranodal lymphatic embolization.[5,6]

Lymphography and lymphatic embolization, which were originally described to visualize lymphatic leakage, have been reported to be effective as not only a diagnostic but also as a therapeutic tool.[7] Furthermore, open or laparoscopic fenestration could be used for refractory lymphoceles as a last option if lymphatic embolization fails. Here, we present a rare case of idiopathic giant lymphocele that appeared 18 years after KT, which was treated

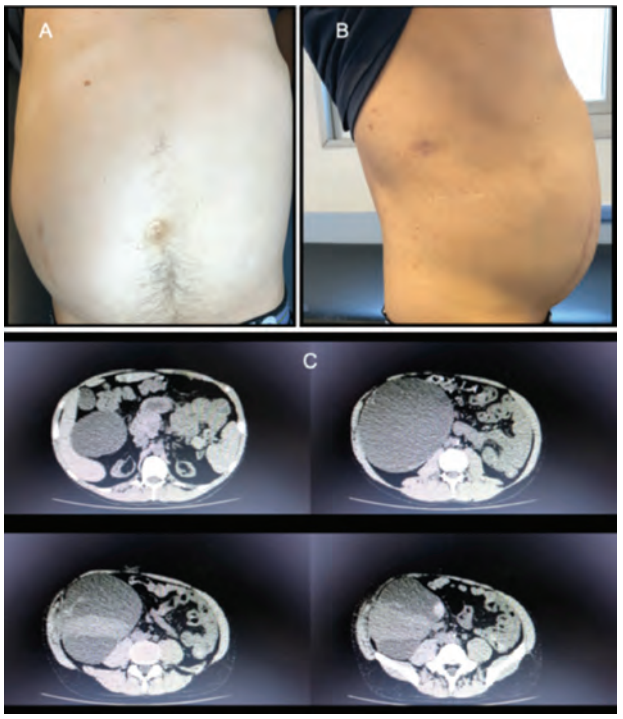
with lymphatic embolization and Lipiodol Ultra-Fluide (Guerbet Australia).

Case Report

Our patient was a 34-year-old man who received a transplant in 2002 due to an unknown end-stage kidney disease. The transplant procedure, which occurred at a separate center before the patient presented to our center, was performed using a graft from a deceased donor, and no complications were recorded during the follow-up.

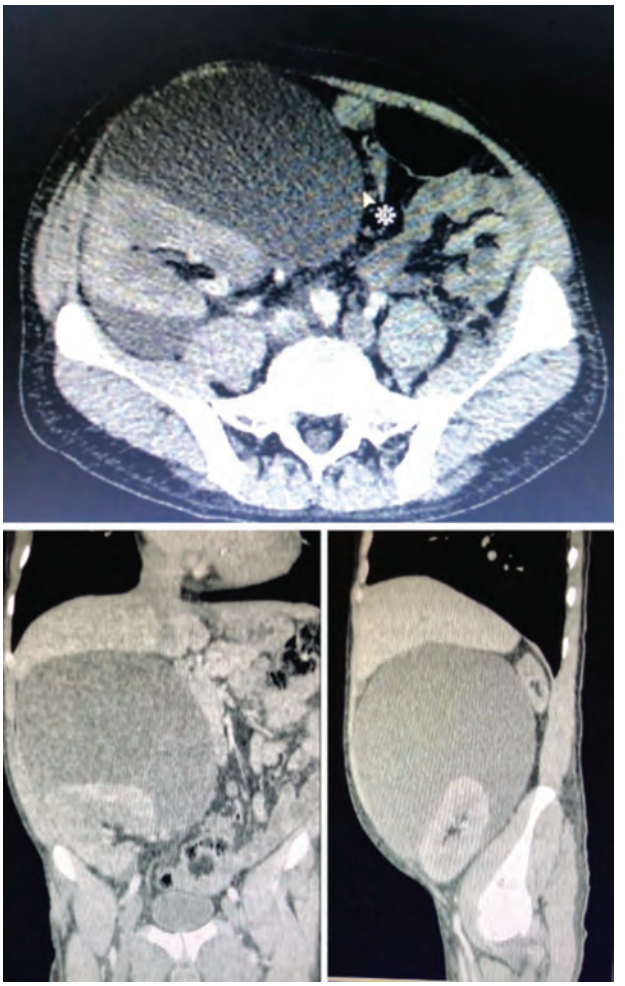
Because of the SARS-CoV-2 pandemic, the patient was referred to our hospital. He presented with a voluminous mass on the right flank that compressed the other abdominal organs. Along with both abdominal pain and early satiety (Figure 1), the patient also had increased levels of creatinine and urea. At presentation, the patient's immunosuppressive treatment included 1500 mg/day of mycophenolate mofetil and 8 mg/day of methylprednisone; neither calcineurin inhibitors nor mTOR inhibitors were being used at that time. An enhanced computed tomography scan showed an enormous amount of fluid collection, with no pathological findings in the kidney allograft (Figure 2). Hydatid disease was dismissed by serological tests.

Figure 1. Images of Patient at Presentation Due to Right Flank Mass



(A) Aspect of the abdomen at frontal view. **(B)** Aspect of the abdomen at side view. **(C)** Sequence shows fluid collection, which occupies the right flank surrounding the graft.

Figure 2. Enhanced Computed Tomography Scan Showing Fluid Collection



As a first approach, an ultrasonograph-guided percutaneous drainage was placed, and 3600 cm^3 of a clear fluid was drained. The biochemical and bacterial findings are shown in Table 1. Despite the drainage, 800 to 1000 cm^3 of fluid continued to leak every day. There was suspicion of occurrence of a urinary fistula; for that reason, an isotope renogram was performed to identify the urinary leakage, which presented normal findings (Figure 3).

Intranodal lymphography and lymphatic embolization were then performed using Lipiodol Ultra-Fluide. First, we performed an ultrasonogram-guided lymph node puncture of both the right and left sides. However, the lymph nodes were too small, and it was difficult to perform the puncture and the Lipiodol was staining the field. After a 40-minute unsuccessful

attempt, we decided to make an incision, desiccate the lymph node, and perform the puncture into the lymph node.

Figure 3. Isotope Renogram, Performed to Identify the Urinary Leakage, Showing Normal Findings

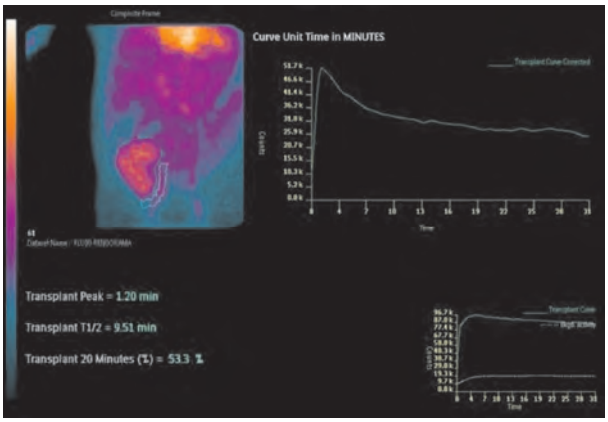


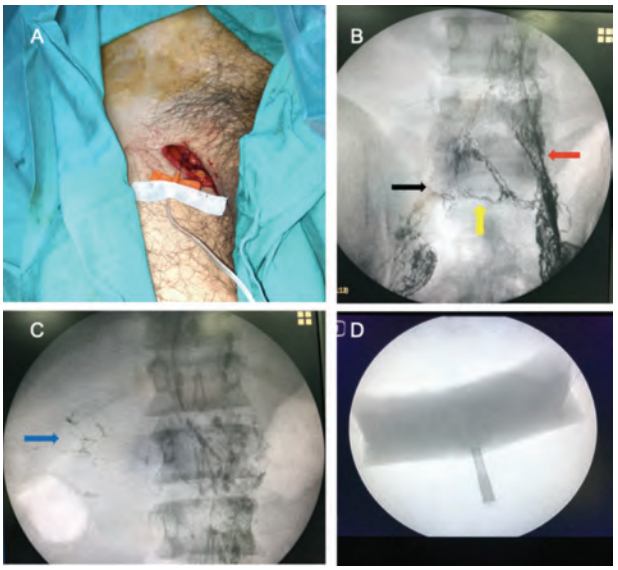
Table 1. Biochemical Determinations in Lymphocele Compared With in Blood

	Lymphocele	Blood
Aspect	Murky	
Color	Slightly xanthochromic	
Glucose, mg/dL	88	115
Proteins, g/dL	<0.800	
Lactate dehydrogenase, IU	31	
Albumin, g/dL	<0.400	
Urea, mg/dL	180	197
Cell account, mm^3	8	
Creatinine, mg/dL	3.82	3.93
Sodium, mmol/L	136	142
Potassium, mmol/L	4.90	5.27
Chloride, mmol/L	118	114.9
Cytology	Mesothelial cells; no malignancies	
Culture	Negative	

During the procedure, we observed some interesting findings: (1) the right lymphatic channels had been amputated in accordance with lymphatic ligation during transplant surgery, (2) collateral circulation was on the left side, and (3) there was lymphatic hypertension on the left side. We were finally able to prove and confirm lymphatic leakage by radioscopy (Figure 4).

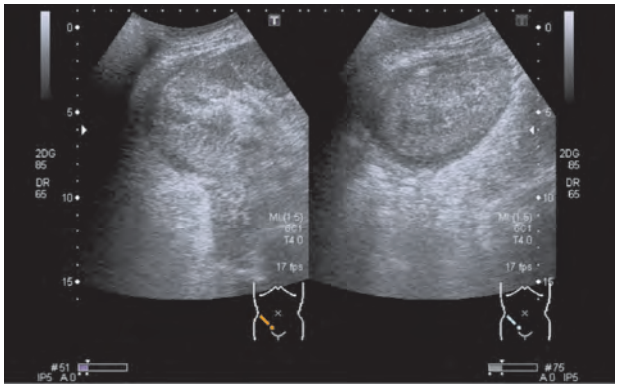
At 1 week after lymphatic embolization, leakage volume decreased from 800 to 200 cm^3 and continued to progressively decrease to 100 cm^3 per day. On day 50 and before the catheter was removed, bleomycin was used as an irritating agent. A subsequent ultrasonogram showed a peripheral sheet of fluid around the kidney allograft (Figure 5), which has not since increased. No other invasive treatment was needed to resolve the giant lymphocele.

Figure 4. Incision and Lymphatic Leakage Confirmed by Radioscopy



(A) Needle into the inguinal lymph node. **(B)** Right lymphatic channel amputated (black arrow), collateral circulation to left side (yellow arrow), and lymphatic hypertension at left side (red arrow). **(C)** Contrast leakage. **(D)** Contrast in the percutaneous bag, 24 hours after lymphography.

Figure 5. Ultrasonogram Showing Peripheral Sheet of Fluid Around the Kidney Allograft



Discussion

As presented here, intranodal lymphatic embolization may not only be a diagnostic tool but may also be an effective, minimally invasive, safe method to treat lymphatic leakage after KT. This was also previously demonstrated by Iwai and colleagues.[6] The most interesting fact of this case was that the leakage appeared 18 years after KT. It should be pointed out that the presence of lymphocele is very uncommon at this late follow-up.

Lymphoceles are diagnosed primarily by ultrasonographic imaging and usually occur from 2 weeks up to 6 months after transplant, with a peak incidence at 6 weeks.[2] The reason for occurrence is

not yet understood, but some theories suggest that it may be the result of damage to the lymphatic vessels of the graft or recipient. Furthermore, immunosuppressants, such as mTOR inhibitors or mycophenolic acid derivatives, may influence the occurrence of lymphoceles.[8,9] Because lymphoceles typically appear within the first few months after transplant, mTOR inhibitors should be avoided from the time of transplant to about 3 months posttransplant to prevent such problems. In addition, Giessing and colleagues, in a comparison of KT recipients who received sirolimus versus patients who did not receive mTOR inhibitors, found that patients treated with sirolimus after KT developed lymphoceles significantly more frequently.[10,11] However, our patient never received mTOR inhibitors, so this cause was dismissed.

A symptomatic lymphocele is a clear indication for decompression procedures. To our knowledge, several treatment methods exist, such as lymphocele aspiration, sclerotherapy, drainage placement, and laparoscopic or open surgery.[4] The advantage of intranodal lymphography is the mini-invasive approach. Selective puncture can be easily performed under fluoroscopic and/or C-arm computed tomography guidance because the leakage point and its closest upstream lymph node are easily visible as a result of the accumulated Lipiodol following initial lymphography. The reported efficacy of lymphography and lymphatic embolization with Lipiodol ranges from 46% to 86%.[7] The period for this technique to be effective is from a few days to weeks; about 50% of patients may require another intervention. Therefore, we suggest that lymphography and the lymphatic embolization with Lipiodol Ultra-Fluide should be offered as a first treatment option to those patients who present recurring lymphoceles after aspiration or do not stop leaking after the placement of drains. This procedure has both a high success rate and a low complication rate[6,7]; therefore, other more invasive procedures, which may be unsuccessful, can be avoided.

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Maximizing Deceased-Donor Allograft Utilization: Management of a Celiac Artery Aneurysm in a Deceased-Donor Liver

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Abstract

As the scarcity of transplantable organs continues to rise, compounded with an aging donor population, transplant surgeons are increasingly confronted with organ offers from less than ideal donors. The presence of a celiomesenteric aneurysm involving the vascular supply of a donor allograft may predispose to vascular complications in the transplanted liver. We present a 61-year-old brain-dead donor who was discovered to have a celiac artery aneurysm during organ recovery. After gross atherosclerotic or mycotic involvement was ruled out and after careful consideration of the vascular reconstructive options, the donor common hepatic artery was divided distal to the aneurysmal dilatation and anastomosed to the recipient bifurcation of the left and right hepatic artery in an end-to-end beveled anastomosis. The postoperative course was unremarkable, with normal blood flow through the anastomosis and no significant complications. The recipient is doing well 6 months after transplant. The presence of a celiomesenteric aneurysm should not discourage the use of an otherwise adequate liver graft. Careful vascular reconstruction is encouraged to increase the rate of marginal graft utilization and minimize vascular complications. Liberal postoperative imaging can enable early detection of vascular complication and prompt intervention. Through this case, we demonstrate the remarkable potential of less-than-ideal grafts with acceptable posttransplant outcomes.

Key words: *Celiomesenteric aneurysm, Extended criteria donor, Liver transplant*

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Introduction

Splanchnic artery aneurysms (SAA) are rare vascular pathologic entities involving the celiac artery, superior mesenteric artery, inferior mesenteric artery, and their branches.[1-4] Splanchnic artery aneurysms have a reported incidence ranging from 0.1% to 2% and were previously only diagnosed following rupture or during autopsies.[1,5] However, with the increased utility of diagnostic and therapeutic abdominal procedures, more vascular anomalies are being incidentally diagnosed, although up to 22% of SAAs still present as clinical emergencies.[4-7] Splanchnic artery aneurysms are more common in men; however, when found in women, they are most likely to involve the splenic artery, which is the most common site (60% to 80%) of SAAs in general.[2,4,8] Hepatic arteries are the second most common site (20%), followed by the celiac artery and superior mesenteric artery, which make up 4% to 6% of SAAs.[1,2,8] Arteriosclerosis is a frequently cited cause of SAAs, especially in older men, although its histopathologic cause has been suggested to be more of a secondary rather than a primary process.[2,3,9] The presence of an aneurysm may complicate the arterial anastomosis of the liver transplant, leading to posttransplant vascular complications like hepatic artery thrombosis and hepatic artery stenosis. Careful arterial reconstruction of this vascular anomaly may reduce this risk of vascular complications and improve utilization of a graft that would have been discarded. Herein, we present a case of celiac artery aneurysm (CAA) incidentally found during brain-dead donor liver recovery; the liver was subsequently successfully transplanted after adequate management of the CAA.

Case Report

A 61-year-old African American man with medical history significant for hypertension, hypercho-

lesterolemia, chronic renal disease, chronic substance abuse since age 21, and smoking 1 pack per day since 17 years old was found unresponsive in a hypertensive crisis with fixed, dilated pupils. A head computed tomography showed an intracranial hemorrhage, cerebral edema, and subfalcine herniation. He was pronounced brain dead 24 hours after admission, and his family decided to pursue organ donation. A noncontrast abdominal computed tomography scan showed a prominent celiac trunk (Figure 1).

During liver recovery, we identified a CAA 18 mm in diameter, with the common hepatic, splenic, and left gastric arteries originating from the aneurysm (Figure 2). There was no gross evidence of atherosclerotic or mycotic degeneration, and the liver graft was deemed appropriate for transplant.

The recipient was a 55-year-old man with end-stage liver disease secondary to Laennec cirrhosis. His Modified End-Stage Liver Disease score at the time of transplant was 32 based on a creatinine level of 1.26 mg/dL, a bilirubin level of 17.3 mg/dL, and international normalized ratio of 2.86. The recipient hepatectomy was unremarkable. Venovenous bypass was not used during the hepatectomy. We performed a side-to-side vena caval anastomosis. The liver portal vein was flushed with 750 mL of cold 5% albumin. We performed an end-to-end anastomosis of the portal vein. Once the venous reconstruction was performed, the recipient's proper hepatic artery was dissected down to the level of the hepatic artery bifurcation to create the anastomosis. The donor's hepatic artery was divided away from the aneurysmal dilatation to the level of the common hepatic artery. The hepatic artery reconstruction was performed in an end-to-end fashion using a Carrel patch from the recipient's proper hepatic artery bifurcation to donor's common hepatic artery.

The recipient had good postoperative recovery. His transplant liver ultrasonography scan showed good flow throughout the liver, and the arterial anastomosis flow was 64 cm/s on postoperative day 1. He was extubated on postoperative day 2. His postoperative course was remarkable for acute blood loss anemia and steroid-induced hyperglycemia, which were adequately managed. The patient was started on oral feeding on postoperative day 3 and began ambulating on the same day. With a progressive improvement in his condition and liver function, he was discharged home on postoperative day 10. Since discharge, the recipient has had 2 episodes of cholestasis-induced pruritus at 7 weeks and 2 months posttransplant, which were managed by endoscopic retrograde cholangiopancreatography with sphincterectomy, dilation, and stent placement. At his last (3 months posttransplant) follow-up visit, his ascites and encephalopathy had completely resolved, and abdominal imaging (Doppler ultrasonography and computed tomography) showed normal flow through the hepatic artery, hepatic veins, and portal vein. The flow through the arterial anastomosis has demonstrated optimal postoperative flow patterns since transplant.

Figure 1. Noncontrast Abdominal Computed Tomography Scan Showing Prominent Celiac Trunk

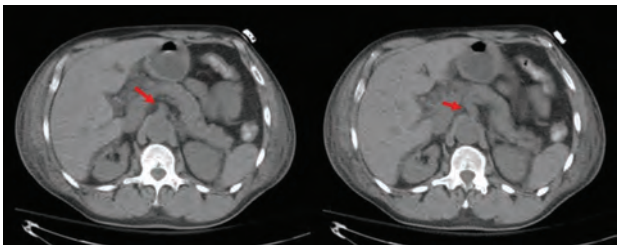
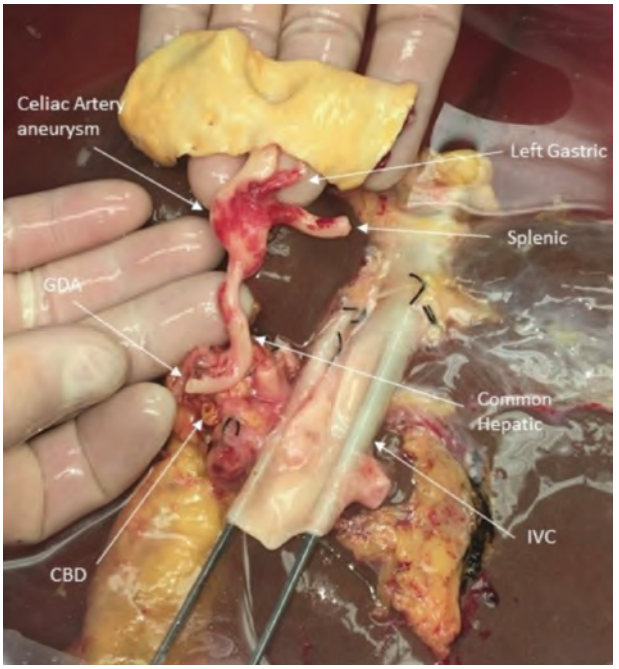


Figure 2. Celiac Artery Aneurysm Shown With Common Hepatic, Splenic, and Left Gastric Arteries Originating From the Aneurysm



Abbreviations: CBD, common bile duct; GDA, gastroduodenal artery; IVC, inferior vena cava

Discussion

The demand for donor organs far outnumbers available grafts. In the past 10 years, an annual

average of 2700 patients in the United States either died waiting for a donor liver or had to be removed from the transplant wait list because they became too sick to undergo liver transplant, accounting for 20% to 26% of all removals from the wait list.[10] Thus, it is incumbent on the transplant community to utilize every accessible graft as long as recipient outcomes are not compromised. Surgeons are now increasingly presented with organ offers from traditionally deemed high-risk or extended criteria donors; these include grafts from elderly donors, donors with hepatis B virus or hepatitis C virus infection, and donors with high body mass index.[11,12]

Despite success shown with liver transplant, vascular complications remain the Achilles heel of the operation. They often occur at the site of anastomosis, with hepatic artery thrombosis (shown in up to 20% of recipients[13]), hepatic artery stenosis (shown in 11% of recipients[14]), and other vascular complications associated with high morbidity and mortality. These complications may lead to endothelial necrosis, biliary tree necrosis, or even graft loss, necessitating retransplant.[14,15] Vascular variations or anomalies that require reconstruction predispose to technical errors and risk for vascular complications.[13,16]

The presence of an aneurysm in a donor graft may complicate the vascular reconstruction, distorting the arterial supply of the graft. However, a thorough work-up for causes of the aneurysm, with specific attention to infectious sources, should render otherwise discarded grafts adequate for transplant. Our review of literature for CAAs in liver donors yielded only 2 case reports, illustrating the apprehension by transplant surgeons to utilize these grafts, which have the potential to achieve good results.[17,18]

Conclusions

Herein, we illustrate that a CAA should not dissuade the use of a liver graft if there is no compromise to the integrity of the vessel or vascular reconstruction. It is critical that abnormalities in vessel wall integrity be evaluated in the operating room and infectious sources excluded. We believe that concerns about the risk of vascular complications may be addressed by liberal use of ultrasonographic imaging in the immediate postoperative period to enable early diagnosis of vascular complications and prompt intervention. Flint and associates had a 92% sensitivity in the use of Doppler studies to diagnose hepatic artery thrombosis,[19] whereas Langnas and associates had positively diagnosed 91% of patients with hepatic artery thrombosis, 100% of patients with portal vein thrombosis, and 100% of patients with combined hepatic artery thrombosis and portal vein thrombosis using Doppler imaging.[15] Thus, our case demonstrates the potential that less-than-ideal liver grafts can possess, which can only be achieved by thorough evaluation of all accessible donor sources.

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Liver Transplantation for Rapidly Progressive Giant Hepatic Hemangioma With Diffuse Hemangiomatosis

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Abstract

Cavernous hemangiomas are the most common benign tumors of the liver and are usually asymptomatic. On the other hand, giant hemangioma and diffuse hepatic hemangiomatosis may become symptomatic by causing compression on adjacent structures, rupture, or consumptive coagulopathy. The coexistence of these 2 entities in an adult is extremely rare, and the literature, especially on their management, is sparse. We report the case of a young woman who developed a rapidly growing recurrent giant hemangioma and diffuse hepatic hemangiomatosis with significant pressure effects, raising the suspicion of a malignant tumor. She had previously undergone a liver resection and an aborted attempt at liver transplant elsewhere. As a preoperative measure, with an aim to shrink the tumor, she underwent arterial embolization and chemotherapy. After this procedure, she underwent deceased donor liver transplant. Her postoperative period was uneventful, and she was well at her 6-month follow-up. We highlight the challenges involved and the need for a multidisciplinary approach in managing these lesions. Liver transplant is an excellent option for patients who develop life-threatening complications or poor quality of life due to these benign liver tumors.

Key words: *Adult, Diffuse hepatic hemangiomatosis, Giant hemangioma*

Introduction

Diffuse hepatic hemangiomatosis (DHH) is a condition characterized by multiple hemangiomas involving one or both lobes of the liver. It is usually found in the pediatric age group and is associated with other anomalies. The coexistence of a giant hemangioma (defined as hemangiomas of more than 10 cm in size) with DHH in an adult, in the absence of extrahepatic manifestations, is extremely rare.[1,2] In the absence of an identifiable trigger, rapid growth is uncommon, and these lesions are usually asymptomatic.[1-3] Here, we present an adult patient with a history of ruptured solitary hemangioma during pregnancy treated by left lateral sectionectomy that was followed by rapid development of a giant hemangioma in the remnant liver with quick progression to DHH. The patient was successfully treated by orthotopic liver transplant (LT), thus expanding the list of unconventional conditions that can be successfully managed by LT. As described below, this case also supports the role of a multidisciplinary preoptimization prior to LT in such patients.

Case Report

A 40-year-old woman was referred to our unit in December 2019 for LT. Her symptoms started in February 2018 when she developed acute-onset abdominal pain and distension during the third trimester of her second pregnancy. On evaluation, she was diagnosed with a ruptured hemangioma in segments 2 and 3 of the liver. She underwent emergency caesarean section, and a simultaneous perihepatic packing of the liver was done. She was transferred to a specialist center, where she underwent a relaparotomy and left lateral sectionectomy.

Intraoperative findings included a large actively bleeding hemangioma in the left lateral segment, with the rest of the liver appearing grossly normal apart from a few subcentimeter hemangiomas on the surface of segment 4. Her postoperative recovery was uneventful, and pathological examination of the

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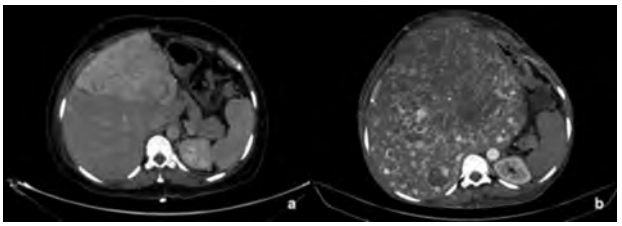
excised liver segment revealed a cavernous hemangioma. One year later, in March 2019, she underwent a laparoscopic hernia repair for incisional hernia. A computed tomography (CT) scan done at that time showed a large hemangioma measuring 17.6 × 19.6 × 17 cm in segment 4, with a few smaller hemangiomas in the right lobe of the liver. Four months later, in July 2019, she developed recurrent abdominal distension, and paracentesis revealed hemorrhagic fluid. A CT scan showed a rapid increase in size of the segment 4 hemangioma, for which a transarterial embolization (TAE) of the segment 4 hepatic artery was performed.

Over the next 4 months, her condition deteriorated, and she developed recurrent ascites requiring frequent large volume paracenteses, pedal edema, significant weight loss, and liver dysfunction with recurrent bloodstream infections. She was evaluated and listed for deceased donor LT (DDLT) at another center. In November 2019, her DDLT was aborted intraoperatively due to massive bleeding and dense adhesions. She gradually recovered from the attempted surgery and was referred to our unit for a second opinion.

On evaluation, she was severely malnourished (transverse psoas muscle thickness sarcopenia index of 16.2 mm/m) with Eastern Cooperative Oncology Group performance status 2. Blood investigations were unremarkable, with normal liver function tests and tumor marker levels. When 2 CT scans done 8 months apart (March vs November 2019) were compared, a 150% increase in the size of the segment 4 lesion was shown, to 32 × 23 × 20 cm, along with multiple small hemangiomas involving the entire liver, with multiple aberrant feeding arteries (Figure 1). The massively enlarged liver was now compressing the inferior vena cava, causing secondary Budd-Chiari syndrome. A positron emission tomography CT, performed to exclude malignancy and extrahepatic disease, was unremarkable. A specimen slide review from the previous surgery was performed, which showed no evidence of malignancy.

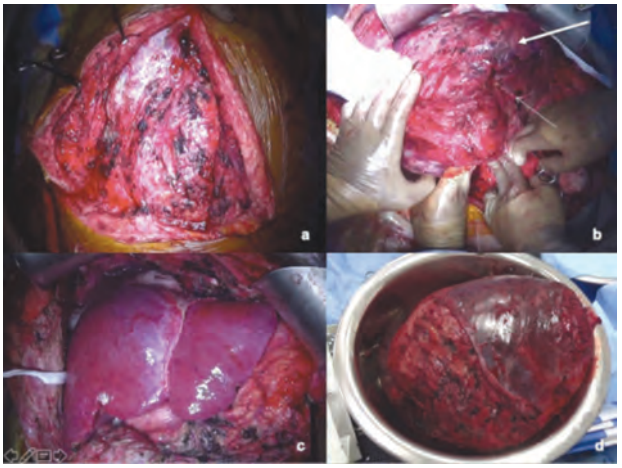
After the case was discussed at a multidisciplinary team meeting, the patient was offered DDLT with a preprocedure plan to control the growth of the lesions using chemotherapy with vincristine and prednisolone. She also underwent TAE of the feeding arteries on the day of the transplant procedure to reduce the risk of intraoperative bleeding and to aid explant hepatectomy. Access to the peritoneal cavity and the hepatic hilum was challenging due to dense vascularized adhesions between the abdominal wall, the previously placed inlay mesh, and the abdominal viscera. After an arduous and meticulous dissection, the explant hepatectomy was completed along with resection of the retrohepatic inferior vena cava and the right dome of the diaphragm. The explanted liver weighed 4580 grams (Figure 2). The deceased donor liver graft was implanted with caval replacement. Total intraoperative blood loss was 3 liters with an operative time of 660 minutes.

Figure 1. Contrast-Enhanced Computed Tomography of the Liver



(a) March 2019 scan, showing a hyperintense lesion in segment 4 following a left lateral segmentectomy with relative sparing of right lobe. **(b)** November 2019 scan, showing rapid increase in size of the segment 4 tumor along with multiple smaller lesions distributed throughout the liver. The lesions show typical centripetal filling in portal venous phase.

Figure 2. Intraoperative Findings

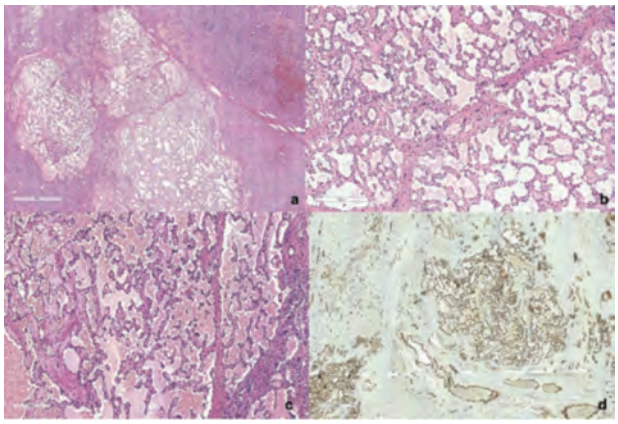


(a) and **(b)** Large tumor with dense adhesions, with solid arrow **(b)** showing giant hemangioma in segment 4 and thin arrow **(b)** showing hypertrophied right hepatic artery feeding the tumor. **(c)** Implanted whole liver graft. **(d)** Explanted liver with tumor.

The patient was electively ventilated overnight and extubated the next day. Doppler scans of hepatic vasculature showed a triphasic flow in the veins and normal portal venous and arterial wave forms. An oral diet was commenced on day 4 posttransplant. She was transferred to the hospital care unit on day 6 and discharged home on day 16 after transplant. Her explant histopathology confirmed the diagnosis

of DHH with a giant hemangioma. There was no evidence of mitosis, and immunohistochemical markers suggestive of malignant vascular tumors (STAT6, WT1, DESMIN, p53, D2-40) were all negative (Figure 3). Six months after transplant, she has remained well on follow-up, with a dramatic improvement in her physical condition.

Figure 3. Immunohistological Examination of the Tumor



(a), (b), and **(c)** Brightfield microscopy showing a multifocal tumor composed of blood-filled, variably sized vascular channels lined by a single layer of endothelial cells supported by fibrous tissue without mitosis or necrosis. **(d)** CD34 immunostaining showed strong positivity.

Discussion

Diffuse hepatic hemangiomatosis usually presents in infancy and is associated with Rendu-Osler-Weber syndrome or skeletal hemangiomatosis, resulting in high-output cardiac failure.[3] Isolated DHH without extrahepatic lesions is extremely rare in adults. Even rarer is its co-occurrence with a giant hepatic hemangioma. Together, these benign lesions are typically slow growing, and their growth is often linked to triggers like pregnancy or estrogenic therapy.[3,4]

Our patient initially presented with an isolated ruptured cavernous hemangioma during her pregnancy, with no other discernible major lesions in the liver. Intriguingly, despite the absence of any known stimulating events, within 1 year, there was the development of a rapidly growing giant hemangioma along with DHH. This precipitous growth in the lesions raised suspicion of malignancy. However, imaging and pathological slide reviews, including immunohistochemistry, were unremarkable, allaying our fears. Because of its rarity, the management of these vascular lesions remains undefined. Various modalities, including TAE, radiofrequency ablation, and radiotherapy, have been attempted with varying degrees of success.[1,3,5-7] Surgery in the form of resection of enucleation is reserved for the management of complications or when malignancy cannot be ruled. Liver transplant is an unconventional therapy for DHH, performed in the presence of life-threatening complications or liver failure.[1,7-9]

Our patient was offered LT as the rapidly growing lesion was not amenable to resection. She also was clinically deteriorating due to the secondary Budd-Chiari syndrome and had poor quality of life with severe malnutrition and sarcopenia. To reduce the risks involved in an already once aborted LT, medications (vincristine and corticosteroids) were started to stall the growth of the lesions. These antiangiogenic drugs have proven to be effective in hemangiomatosis and were used in our patient during the DDLT listing period.[4,7,10] Because a difficult and bloody hepatectomy was anticipated, TAE was performed immediately before the patient was transferred to the operating room for the DDLT. This procedure may have helped temporarily reduce the inflow to the lesions, facilitating the transplant procedure.

This case represents an uncommon indication for LT; in this case, the rate of growth demonstrated by this "benign" tumor was exceptional. Our case is unique because all previously reported cases had a longer duration of progression before ultimately requiring LT. The case also demonstrates the challenges involved in managing such patients and the need for a multidisciplinary approach for successful treatment. Awareness of the coexistence of such benign tumors and their natural course of progression is important while making clinical decisions. Orthotopic LT is a feasible and excellent option in cases of development of life-threatening complications or poor quality of life in these patients with benign liver tumors.

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CASE REPORT

Real-Time Intraoperative Assessment of Microcirculation in Living-Donor Small Bowel Transplant: A Case Report

Arshad Khan,[1,2] Gulnaz Choudhary,[1] Tzvetanov Ivo,[1] Hoonbae Jeon,[3] Enrico Benedetti[1]

Abstract

Living-donor small bowel transplant has emerged as a modality to transplant patients with short bowel syndrome without prolonged wait time, albeit at the cost of technical challenges associated with vascular anastomosis due to the small size of vessels. Suboptimal perfusion in a transplanted bowel can lead to a devastating outcome, and clinical judgment alone is not completely reliable for assessment of bowel microcirculation. Here, we report a 55-year-old female patient who underwent flow cytometric cross-match-positive living-donor bowel transplant from her daughter. Initial suboptimal perfusion prompted a revision of the arterial anastomoses. Despite normal Doppler signals over the mesenteric vessels, the bowel had a variegated appearance. The microcirculation of the bowel wall was subsequently assessed in a real-time fashion by indocyanine green fluorescence angiography, which showed improved perfusion indices with time. Hence, this simple test helped us to avoid another unnecessary exploration and revision of the anastomoses. At present, the patient is thriving on an enteral diet. This case underpins the importance of real-time intraoperative assessment of bowel perfusion and microcirculation in difficult cases. These assessments are needed to help surgeons identify tissues at risk for ischemia and necrosis, thereby allowing for maneuvers to improve intestinal viability.

Key words: *Bowel perfusion, Fluorescence, Indocyanine green, Near-infrared fluorescence*

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Introduction

Central to the success of bowel transplant is adequate revascularization and perfusion during the process of implantation. Compared with deceased-donor small bowel transplant, vascular anastomosis is more challenging in living-donor small bowel transplant procedures due to the small size of donor vessels. Despite improvements in vascular implantation techniques, vascular complications in organ transplant still pose a significant problem and may jeopardize outcomes.[1] Most early vascular problems are due to technical inaccuracies. Clinical assessment of intestinal perfusion and viability based on the color of the serosal surface, the presence of bowel peristalsis, pulsation, and bleeding from the marginal arteries may be subjective and deceptive.[2] Moreover, it does not provide objective information about microcirculation. Numerous techniques for assessing various parameters of intestinal viability have been described in gastrointestinal surgery.[3] However, surgeons have been unable to incorporate these technologies into clinical practice as they are highly user dependent and involve a steep learning curve. Therefore, simple, quantitative, real-time, intraoperative methods of assessment of bowel microcirculation are of paramount importance. The ability to recognize suboptimal bowel perfusion intraoperatively could help avoid disastrous posttransplant consequences.

Recently, near-infrared fluorescence (NIRF) imaging using indocyanine green (ICG) has emerged as a valuable method, offering real-time intraoperative imaging, perfusion, and microcirculation assessment capabilities in a number of clinical scenarios.[4] Indocyanine green is an inert, safe, water-soluble, nonradioactive, and nontoxic contrast agent, which was approved by the US Food and Drug Administration in 1959. After intravenous administration, it is rapidly bound to plasma proteins and

exclusively excreted through the biliary system, with a half-life in blood of 2.5 to 3 minutes. With the use of a laser light source at 40 mW/cm at 806 nm, a molecule will fluoresce in a predictable fashion at 830 nm, which can be detected by a charged-couple device camera system (Spy Elite, Novadaq Technologies, San Jose, CA, USA) with florescence intensity proportional to the perfusion of a given area. The area of florescence can be viewed in gray scale or heat map mode, as relative quantification in percentages from 0% to 100% in relation to an internal anatomic reference that is positioned based on clinical judgment. In addition, various perfusion indices can be calculated by Spy software derived from the relation between pixel strength over time.

This case describes the use of a NIRF imaging system using ICG to evaluate the adequacy of bowel microcirculation after living-donor small bowel transplant. We believe this is the first report of such a kind in the field of living-donor small bowel transplant.

Case Report

A 55-year-old white woman with short bowel syndrome and severe malnourishment was offered bowel transplant because of near loss of central venous access, severe growth retardation, and total parenteral nutrition (TPN) dependence. Her 36-year-old daughter volunteered to donate. The blood type was ABO compatible, and HLA haplotypes revealed 3/6 antigen mismatch. The recipient had class I and class II panel reactive antibodies of 80% and 26%, respectively. She received desensitization therapy with plasmapheresis along with intravenous immunoglobulins and pre- and postoperative immunosuppression as per our hospital's protocol.

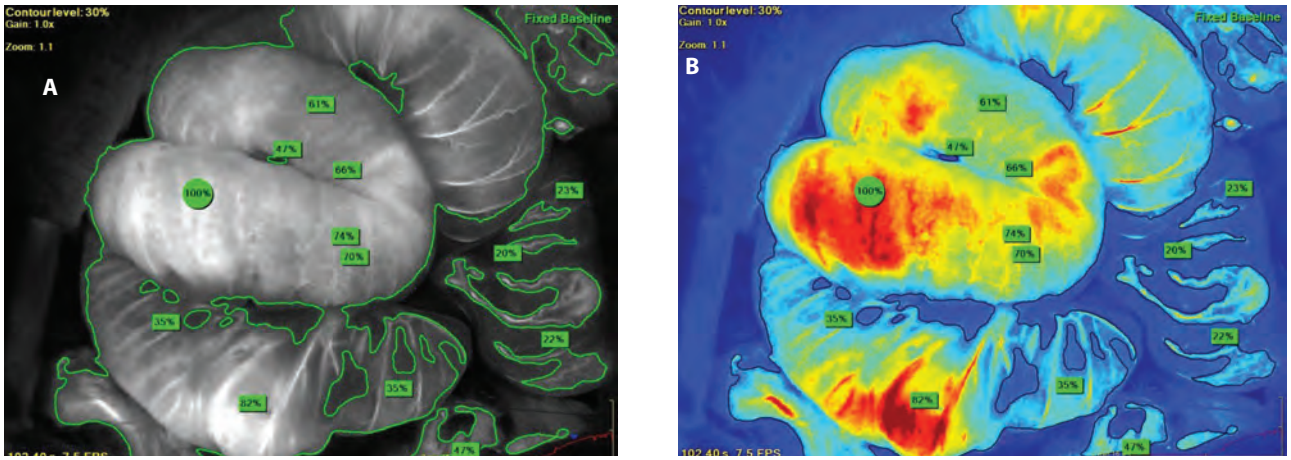
The intestine was procured from the donor by following a technique previously described by our group.[5] Abdominal exploration of the recipient revealed remnant distal colon with a distal third of transverse colon in situ and the fourth part of the duodenum at the ligament of Treitz. The recipient's aorta and inferior vena cava (IVC) were prepared. The graft artery was anastomosed to the infrarenal aorta using 6-0 Prolene, and venous anastomosis was done with the IVC. After reperfusion, the bowel remained cyanotic. Our evaluation of the bowel revealed diminished pulsations on the mesentery. Doppler evaluation showed the absence of diastolic flow over the mesenteric arcade, and signals from the intestinal wall could not be appreciated. Based on these signs, we decided to revise the arterial anastomosis. During reexploration, it was found that the arterial anastomosis had positional flow. The arterial anastomosis was then taken down and redone after altering the orientation of the artery to ensure that the flow was not position dependent. After the second reperfusion, there was a strong, palpable pulse and normal arterial Doppler signals over the mesenteric arcade, but there was still discoloration of the intestinal wall. Therefore, we decided to test the microcirculation of the bowel wall.

We elected to use for the first time NIRF using ICG for assessment of microcirculation. We have been using ICG in robotic kidney transplants as a matter of routine with good outcomes. The Spy Elite System was positioned over the bowel, and 0.16 mg/kg ICG (2.5 mg/mL, total dose of 10 mg) was administered intravenously. At the same time, the machine was buffered and the sequence was captured for 136 seconds as soon as a blush appeared on the screen without moving the area of interest. The fluorescence signals of the blood perfusion were visualized in the regions of interest by the Spy Elite software. The reference region of the measurement was the stomach, which was set at 100%. For comparative measurements, different areas of the mesentery and the small bowel wall were defined as other regions of interest. The software calculated the perfusion index in these regions as the steepness of the curve of fluorescence intensity over time (pixel intensity per second). Results were computed and given as a percentage of the perfusion index compared with the reference area.

After the second reperfusion, we observed suboptimal bowel microcirculation as indicated by fluorescent and heat map images (Figure 1, A and B). The bowel was rewarmed for about 15 minutes, and the test was repeated, which revealed good microcirculation (Figure 2, A and B). Furthermore, more different perfusion indices at these 2 time intervals were compared by focusing on a region of interest point with the stomach as a reference, lending credence to the adequacy of arterial anastomosis with good blood flow.

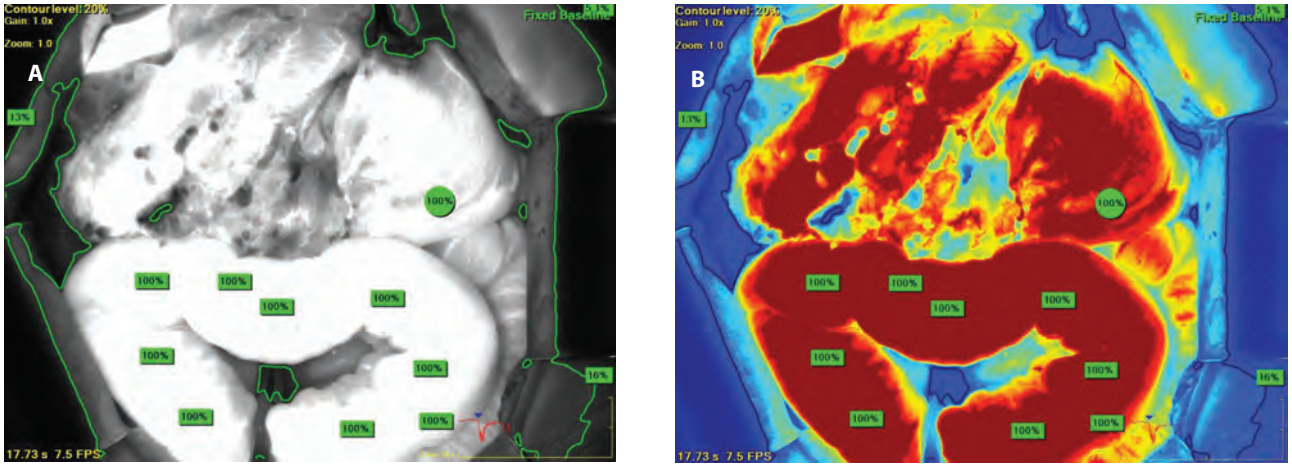
Bowel continuity was restored proximally to the duodenum and distally to the remnant colon. We always make a loop ileostomy to facilitate the postoperative monitoring of blood flow and surveillance

Figure 1. Indocyanine Green Gray Scale and Heat Map Images of Poor Microcirculation



(A) Indocyanine green (ICG) fluorescence gray scale image showing the differential intensity of uptake. **(B)** Heat map image revealing variegated color intensity with red identifying areas of good and yellowish and greenish areas of poor microcirculation.

Figure 2. Indocyanine Green Gray Scale and Heat Map Images of Optimal Microcirculation



(A) Indocyanine green (ICG) gray scale image revealed fluorescent intensity rising to 100% over the transplanted bowel, signifying good adequate perfusion using the stomach as a control. **(B)** Heat map image showing uniformly red color over the transplanted bowel, suggesting optimal microcirculation.

endoscopies for rejection. The postoperative course was uneventful. By the third postoperative week, a full diet was introduced, and TPN was gradually weaned. Graft surveillance for acute cellular rejection was accomplished by weekly zoom enteroscopy and biopsies, which did not reveal any rejection. She was discharged on postoperative day 41. Since discharge, she has recuperated well, has been weaned off TPN, and remains on an enteral diet.

Discussion

Adequacy of bowel perfusion cannot reliably be predicted for a number of reasons. These include patient factors, such as atherosclerotic disease, varied pathology location, and vascular variability.[6] In addition, several surgical factors such as overaggressive dissection, bowel manipulation, and anastomotic tension may further compound the problem. Visual inspection is a poor predictor of adequate blood flow.[7] There is, therefore, a pressing need for a technique that can accurately and consistently assess tissue perfusion in real time. Near-infrared fluorescence has proved useful in evaluating tissue perfusion in peripheral vascular procedures,[8] colorectal surgery,[9] kidney transplant,[10] plastic surgery,[11] and recently pancreatic transplant.[4]

In our center as a matter of routine, we had been using clinical assessment along with Doppler assessment of intestinal viability and circulation after implantation to assess the adequacy of bowel perfusion. In our present case, after successful

implantation of a bowel from a living donor on the recipient's aorta and IVC, clinical assessment was satisfactory, and the Doppler signals were normal over the vessels by the mesenteric border. Although Doppler examination of mesenteric vessels has been historically shown to be a reliable predictor of intraoperative bowel perfusion and viability compared with clinical assessment alone,[12] some authors contend that it adds little to the clinical judgment.[13] A study by Dyess and associates revealed that Doppler ultrasonography resulted in a high rate of false-negative and false-positive results.[14] The limitations of this technique have been widely discussed. The technique is considered to be vulnerable to signals from neighboring large vessels and requires an arterial exposure and a pulsatile blood flow; in addition, tissue contact is required, which can impair local blood flow.[15]

In the present case, clinical assessment and Doppler assessment of the mesenteric blood vessels were normal despite the bowel having a variegated appearance. Although the anatomic orientation of the artery was corrected on the revision of the anastomoses, the chances of vascular thrombosis were still high due to the case being T- and B-cell flow cytometric cross-match positive. Therefore, a robust method of establishment of microcirculation was required. Hence, NIRF using ICG was used for assessment of microcirculation. The initial films revealed different perfusion intensities in different segments of the transplanted small bowel with the absence of microcirculation in such segments as shown in Figure 1. However, repeat examination revealed the increased intensity of uptake to 100% matching the intensity of the control, which was the native stomach. Thus, it helped us to assess the bowel wall microcirculation, attesting to the overall good perfusion of the bowel. Based on this experience, we suggest that utilization of this technology is another step up in difficult transplant cases with questionable initial perfusion of the graft, which can provide real-time intraoperative assessment of bowel perfusion. In particular, surgeons and patients will benefit from the application of this technique as it can identify ischemia in tissues, allowing surgeons the opportunity to perform operative revisions and prevent ischemia-related complications.

Conclusions

Indocyanine green fluorescence measurement, which is now widely available, is a simple and reliable method to evaluate bowel wall microcirculation in vivo during bowel transplant. It can assist surgeons with critical decisions in the operating room, thus contributing to the overall procedural success and good patient outcomes.

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Diffuse Hepatic Arterioportal Fistulas After Liver Transplantation

Jérôme Dumortier,[1,2] Domitille Erard-Poinsot,[3] Alexie Bosch,[3] Pierre-Jean Valette[2,4]

Dear Editor:

Arterioportal fistulas (APF) are abnormal communications between the hepatic artery and the portal vein.[1,2] The clinical spectrum of presentation ranges from symptom-free individuals to patients with severe portal hypertension. Arterioportal fistulas could be congenital (for example, hereditary hemorrhagic telangiectasia and Ehlers-Danlos syndrome), could be idiopathic, or could be secondary to cirrhosis, hepatic neoplasm, hepatic trauma, hepatic parenchymal congestion, inflammatory or infective disease, or obstruction of hepatic vein or portal vein. In addition, APF could be iatrogenic following percutaneous liver biopsy or cholangiography. Therefore, APF can be divided into 3 different types: small peripheral intrahepatic, large central, and diffuse congenital intrahepatic.[2] Here, we report a patient who developed multiple diffuse hepatic APF after liver transplant (LT) and ascites and who required transarterial embolization.

Case Report

A 53-year-old male patient was listed for LT for end-stage alcohol-related liver disease and refractory ascites. In June 2017, he received a liver graft from a 66-year-old brain dead donor who died from intracerebral hemorrhage. A liver biopsy was made at the end of the procedure in the left lobe. Early postoperative complications included kidney failure and abdominal sepsis due to *Klebsiella pneumoniae*. Evolution was favorable under prolonged antibiotic therapy, and the patient was discharged home 4 weeks after LT. Thereafter, on day 45, an arterial anastomotic stricture was treated by angioplasty. Angiography and computed tomography (CT) scan showed no other abnormalities at that time (Figure 1, A and B). Concomitantly, ascites persisted, requiring repeat paracenteses. Ascites fluid was a transudate, without observations of infection criteria. Histological analysis of a liver biopsy disclosed significant portal fibrosis with septa, ductal proliferation, and cholestasis.

In September 2018, because of persistent ascites, a transjugular portosystemic shunt was discussed. Cavography revealed no evidence of venous outflow obstruction, and the portosystemic gradient was 9 mm of mercury. Ultrasonography confirmed the presence of ascites and showed inversion of portal flow, and CT scan revealed multiple APF with early opacification of the portal trunk, in particular of its right branches (Figure 1, C and D). The fistulas appeared diffuse, affecting the right anterior and posterior branches, whereas the left areas did not seem to be involved. There was no portal thrombosis.

Treatment options were discussed, including retransplant or transarterial embolization. It was considered appropriate to embolize the shunts as a first option. Subsequent angiography confirmed multiple intrahepatic APF within the right lobe, and a first session of embolization with coils was performed in December 2018 (Figure 1, E and F). The patient's postoperative course was uneventful.

In March 2021, a CT scan confirmed the absence of ascites without further significant modifications of APF; the patient did not require paracenteses.

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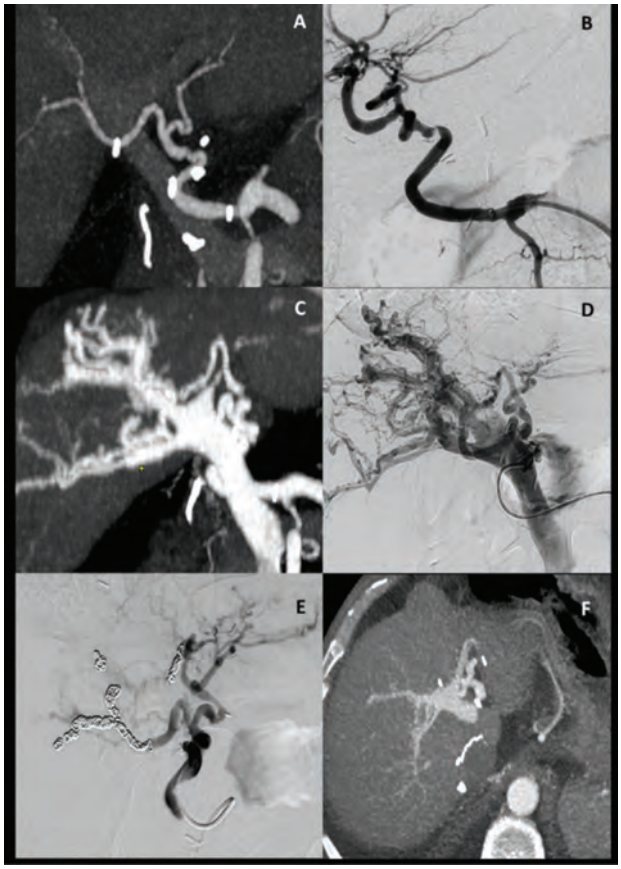
Figure 1. Hepatic Artery Appearance After Liver Transplant



Early after liver transplant (day 45), 3-dimensional (3D) maximum intensity projection computed tomography (MIP CT) scan **(A)** and arteriography **(B)** performed for balloon dilatation of an arterial anastomotic stenosis did not show any aspect of arteriovenous fistula. One year after liver transplant, 3D MIP scan image at arterial time **(C)**, as well as arteriography **(D)** performed for embolization, showed early opacification of the portal vein through diffuse right liver arterioportal fistulas. The portal flow was reversed. At the end of the embolization procedure, the angiographic image **(E)** with coils showed near disappearance of the right arterioportal fistulas. The portal vein is no longer opacified. One month later, CT scan **(F)** confirmed a decrease of the right periarterial fistulas.

Discussion

During and after LT, the liver graft undergoes a significant number of potential procedures that can cause APF, the most frequent being liver biopsy. Nevertheless, the appearance of APF in the posttransplant setting is not common. The precise incidence is unknown, but a large retrospective series that included 1992 patients disclosed 4 cases (0.2%) of hemodynamically significant APF, from which 2 were symptomatic; all cases were localized APF.[3] In the patient reported here, transplant of a liver graft with APF was ruled out by early initial radiological evaluation. The roles of arterial stenosis, prolonged sepsis, and liver biopsies were questionable, but we considered that development of multiple diffuse APF was of unknown cause. Morphological presentation was that of congenital APF (type 3), which are diffuse and intrahepatic and which can be the most difficult to manage.[2]

Only about 20 cases of APF after LT have been reported, all secondary to an interventional radiological procedure; the presence of multiple intrahepatic APF is very rare. Only Puri and colleagues reported a case quite similar to ours of multiple bilobar APF recognized 1 year after transplant.[4] The etiology was considered not clear. The patient was managed conservatively and has not required intervention. In the case of significant portal hypertension secondary to APF, percutaneous transarterial embolization has been increasingly performed in place of surgery, including, rarely, for LT recipients.[1,5,6]

In conclusion, multiple diffuse hepatic APF can develop after LT and can be hemodynamically significant, causing severe portal hypertension and requiring embolization.

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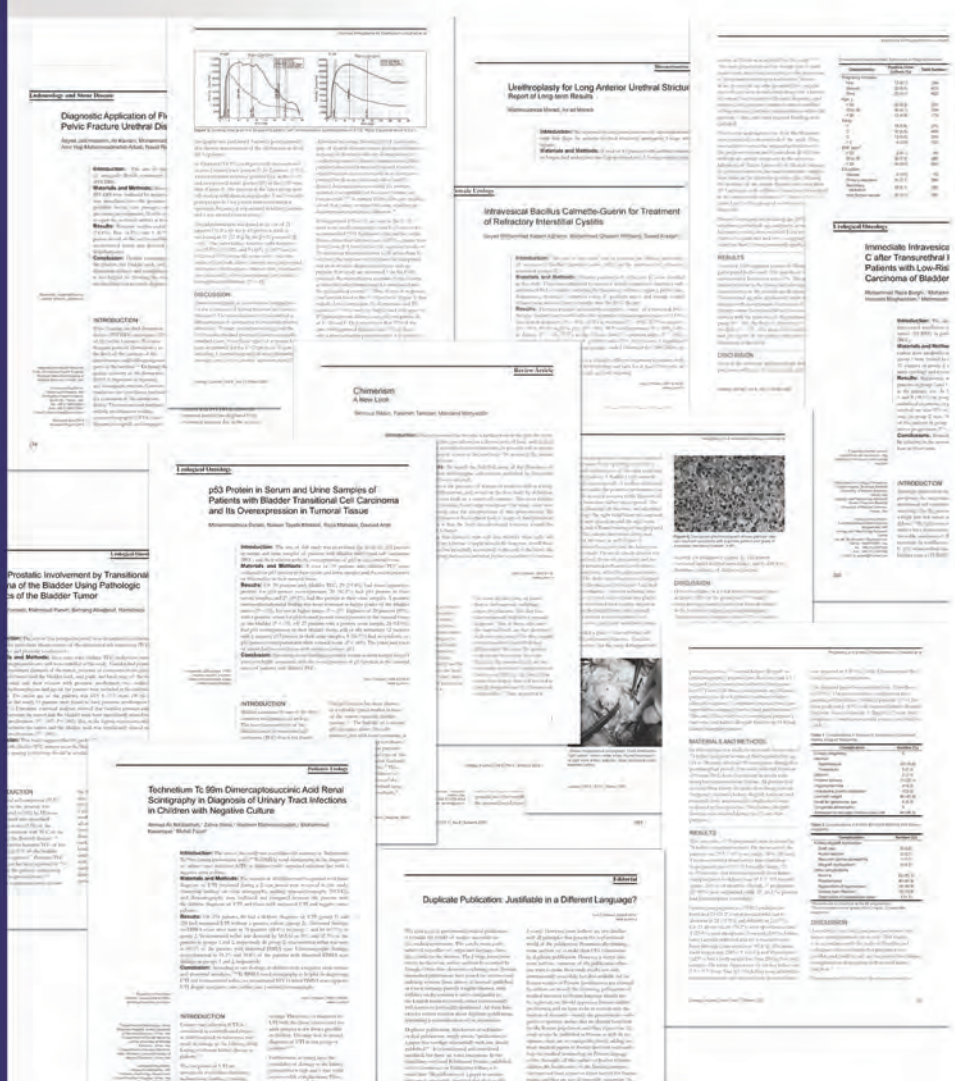

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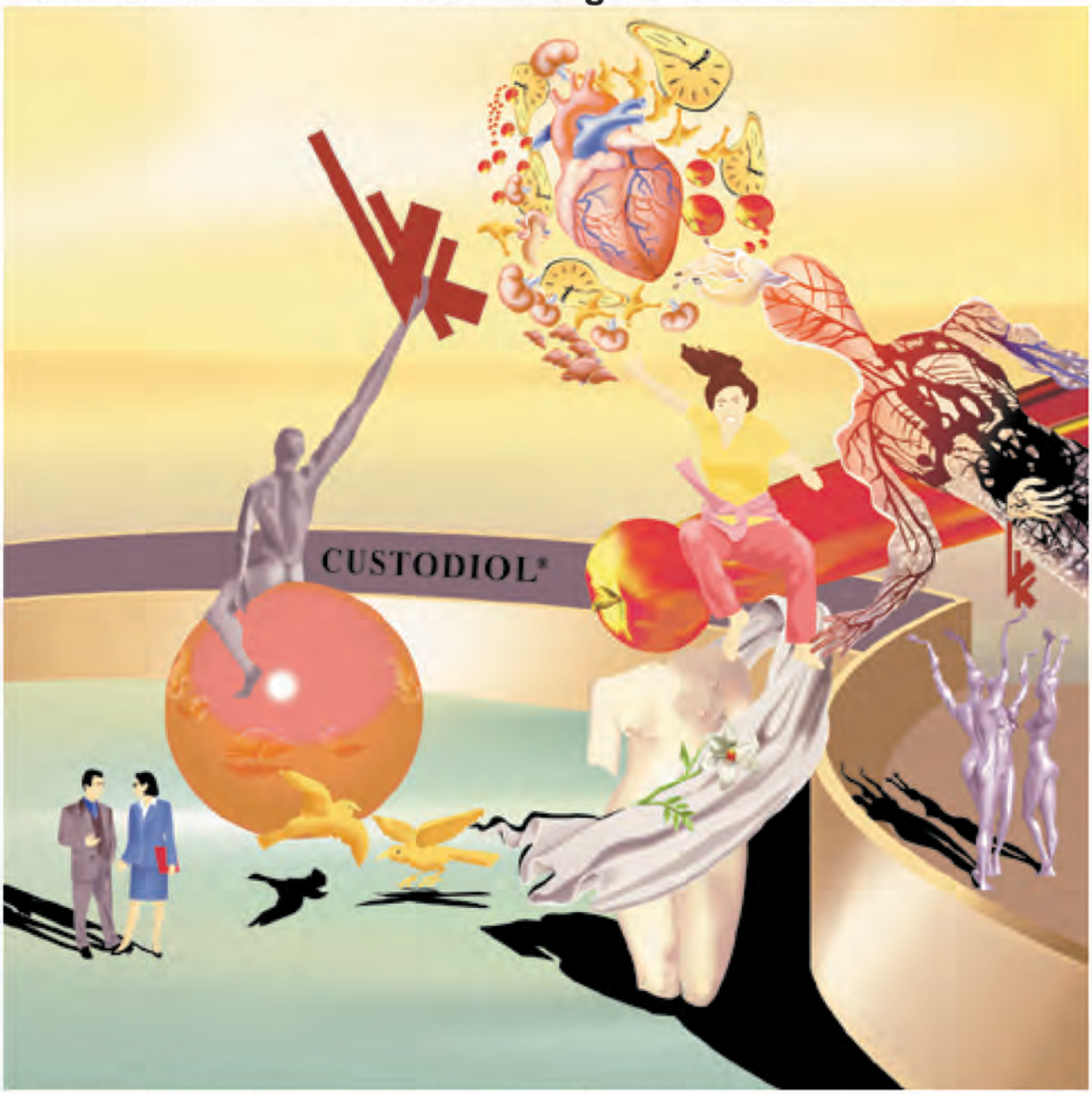

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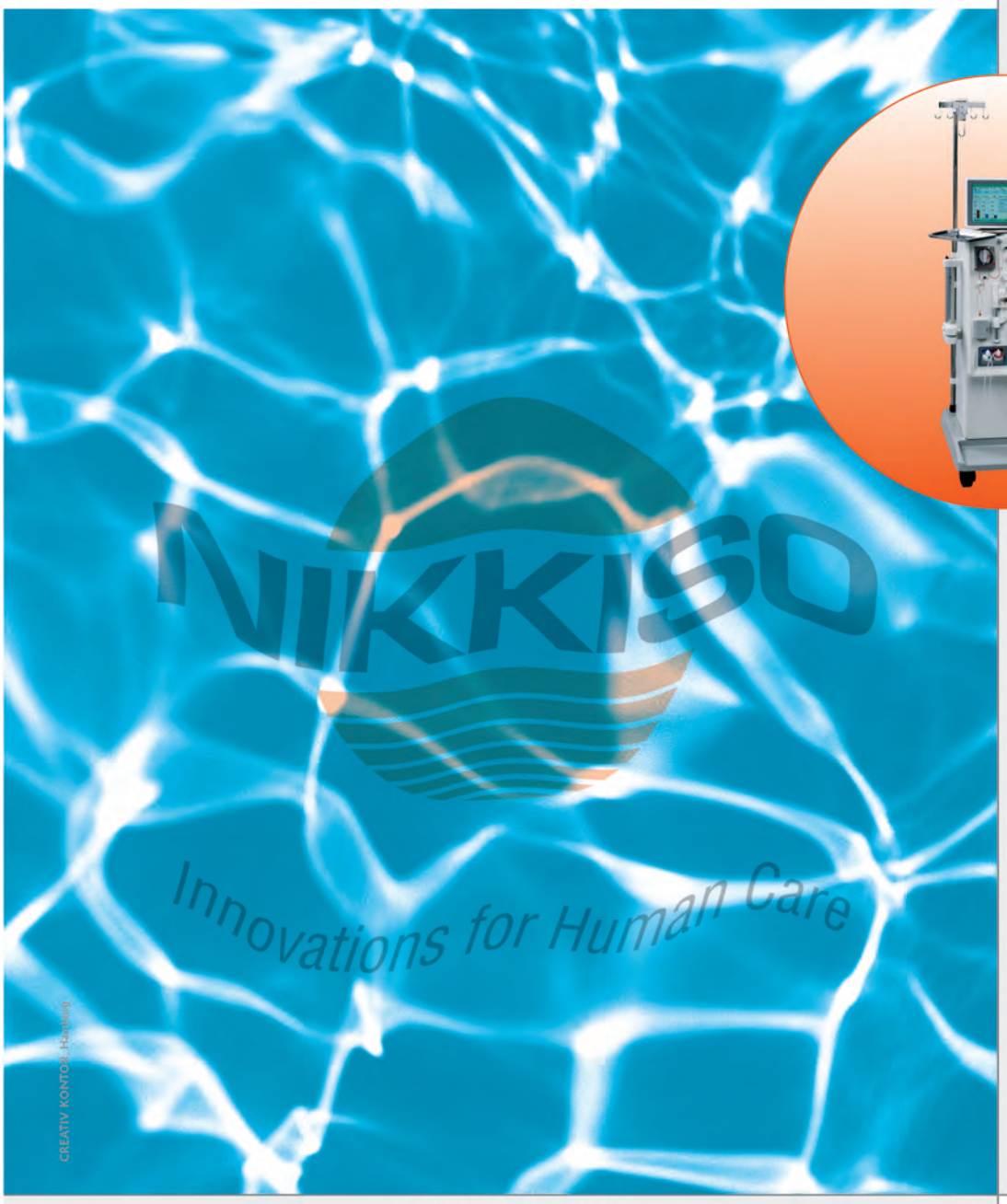

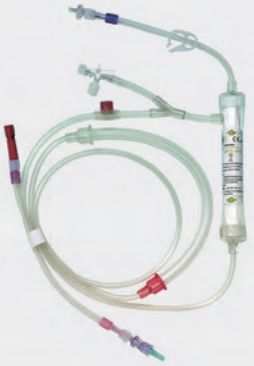

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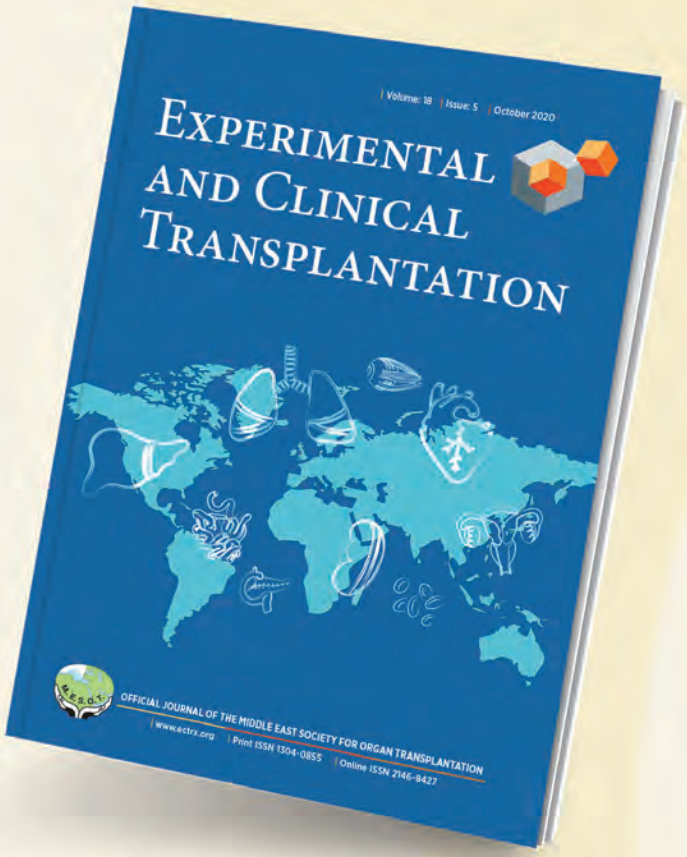

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