| Volume: 20 | Issue: 12 | December 2022

Experimental and Clinical Transplantation

OFFICIAL JOURNAL OF THE MIDDLE EAST SOCIETY FOR ORGAN TRANSPLANTATION

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

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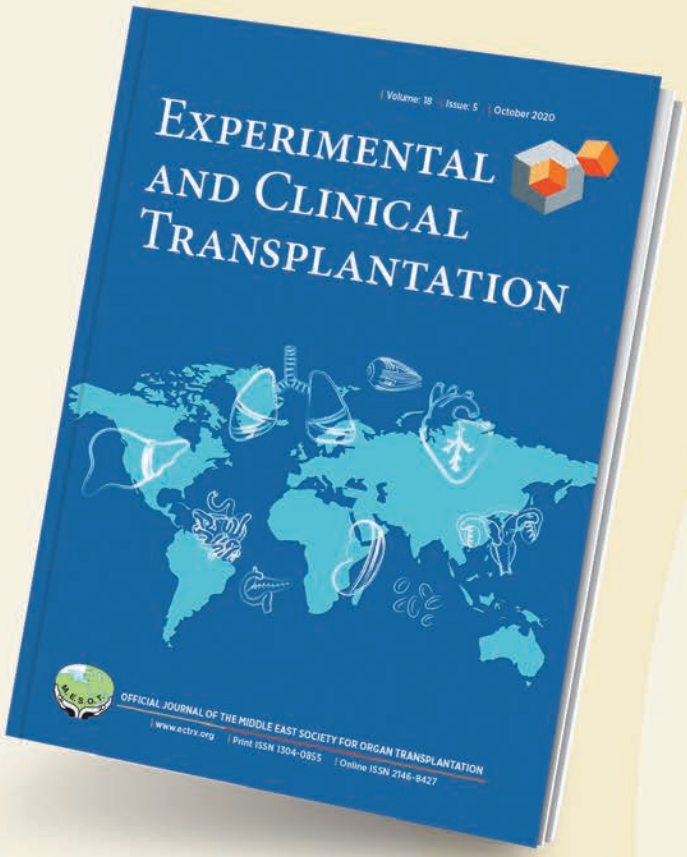

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publisher
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Printed by Elma Teknik Basım Matbacılık Tic. Ltd. Şti.
İvedik OSB Matbaacılar Sitesi 1516 / 1 Sokak No: 35
Yenimahalle 06378, Ankara-Türkiye (+90.312) 229 92 65

Date Printed 25 December 2022

Print ISSN 1304-0855
Online ISSN 2146-8427

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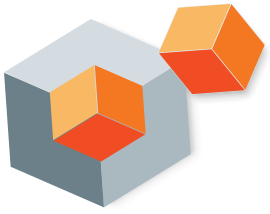

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Yayın Adı: Experimental and Clinical Transplantation
Yayın Türü: Yerel süreli yayın
Yayın Şekli: Aylık-İngilizce
Yayın Sahibi: Başkent Üniversitesi adına Mehmet Haberal
Sorumlu Yazı İşleri Müdürü: Mehmet Haberal
Yayının İdare Adresi: Taşkent Cad. No:77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

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

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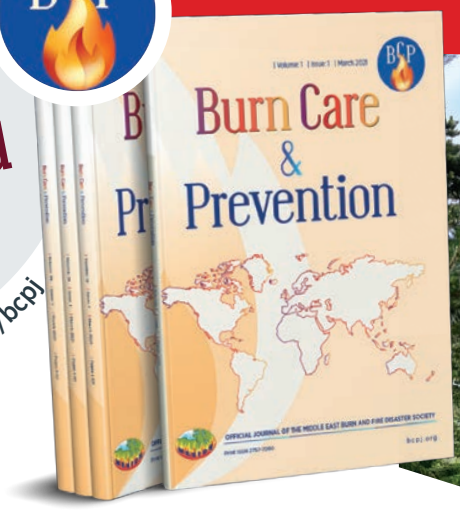

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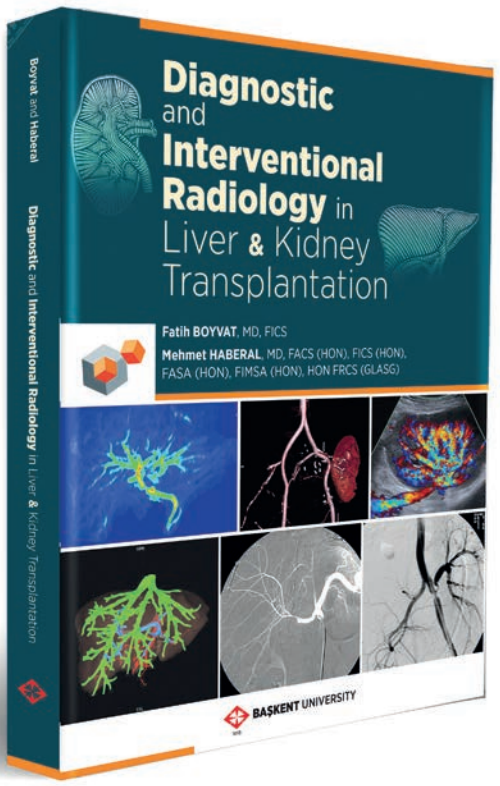

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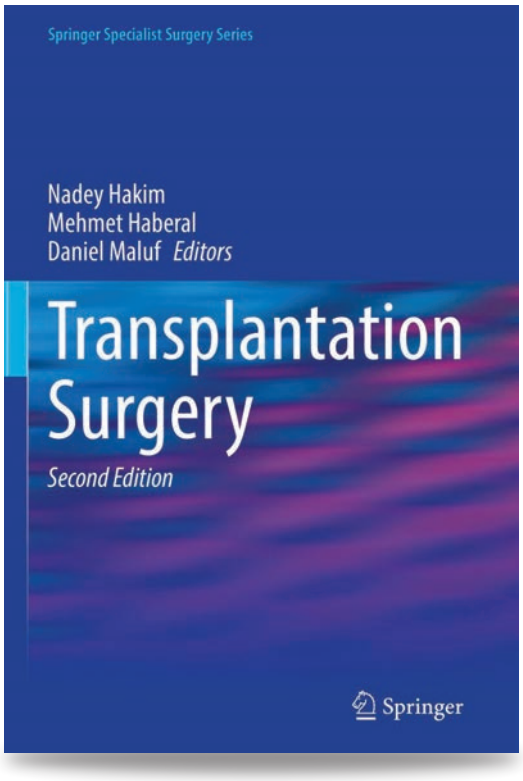

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Volume: 20 Issue: 12 December 2022

Contents

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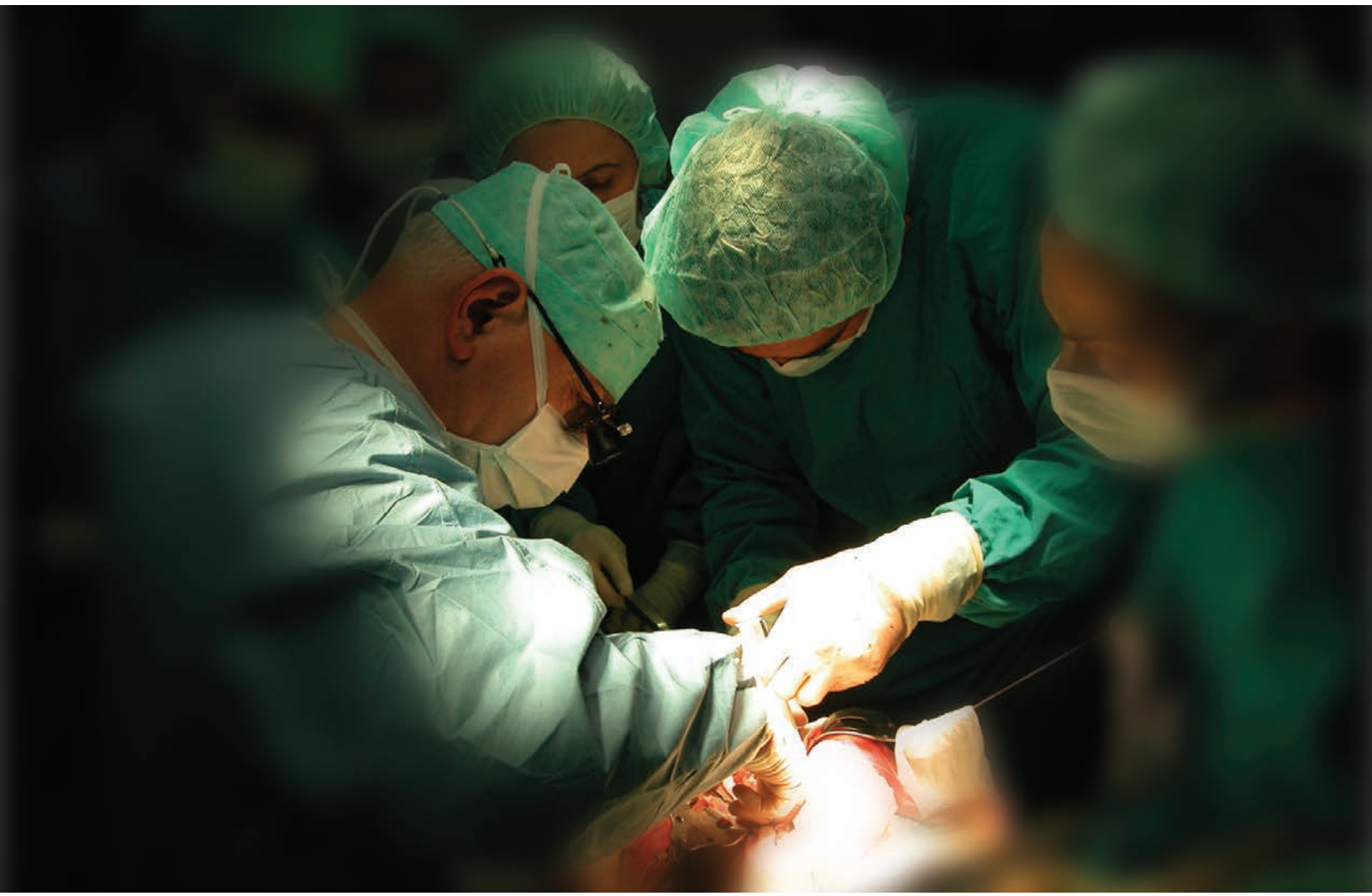
Kidney & Liver Transplantation Observer & Fellowship Programs



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Transplant Renal Artery Stenosis: Current Concepts

Thilina Gunawardena, Hemant Sharma

Abstract

Transplant renal artery stenosis is an increasingly recognized vascular complication after renal transplant. It can result in posttransplant hypertension and graft dysfunction and eventually in graft loss. Timely diagnosis and appropriate intervention for clinically significant transplant renal artery stenosis can potentially halt or reverse these adverse effects, leading to better outcomes. Duplex ultrasonography is the imaging modality of choice to screen suspected cases. Some patients with transplant renal artery stenosis will require invasive treatment with endovascular intervention or surgery, whereas others can be safely treated with blood pressure control and follow-up. In this review, we aimed to discuss the epidemiology, clinical presentation, pathogenesis, diagnosis, and management of this common posttransplant complication.

Key words: *Angioplasty, Renal transplant, Surgery, Vascular complications of kidney transplant*

Introduction

Transplant renal artery stenosis (TRAS) is the most common vascular complication following renal transplant. It has a reported incidence of 1% to 23%. This wide range has been attributed to variable definitions and imaging techniques used for its diagnosis.[1-4] At present, the condition appears to be more commonly diagnosed, probably as a result of more cases being discovered during surveillance ultrasonographic scans of transplanted kidneys. Transplant renal artery stenosis has been identified as a cause of posttransplant hypertension, graft dysfunction, and graft loss.[5] Contemporary use of advanced immunosuppression and protocol-driven management after kidney transplant has led to nonimmunological causes emerging as a leading cause of graft loss.[2] Among these causes, TRAS is a potentially curable entity; thus, early recognition and appropriate treatment are vital. This review presents an overview of the current diagnostic and therapeutic strategies available for the management of this condition.

Epidemiology

Transplant renal artery stenosis is considered the most common vascular complication after renal transplantation.[1,4,6] Hurst and colleagues, who analyzed the US Renal Data System registry, reported its incidence as 8.3 cases per 1000 patient-years.[7] In a case series involving 2594 kidney transplants, TRAS was diagnosed in 0.6% of the recipients.[8] After analysis of 1367 renal transplants, Dimitroulis and colleagues noted TRAS in 1.4%.[1] Another study that used duplex as a surveillance tool in 793 renal transplant recipients reported an incidence of 0.9%.[3] Wong and colleagues reported a 2.4% incidence of TRAS in the era before duplex ultrasonography. This increased to 12.4% once duplex ultrasonography was introduced to their practice.[9]

Clinical Presentation

The common time frame for the diagnosis of TRAS is between 3 months and 2 years after transplant surgery, but early or late presentations are possible.[1] Symptoms of TRAS have been reported as early as 1 week after transplant.[3]

The usual mode of presentation is with worsening or treatment-resistant hypertension and edema with

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

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Experimental and Clinical Transplantation (2022) 12: 1049-1057

DOI: 10.6002/ect.2022.0334

or without graft dysfunction. Rejection, obstructive uropathy, and infection should be excluded before graft dysfunction is attributed to TRAS.[2] Sudden, unexplained episodes of pulmonary edema in the absence of left ventricular dysfunction, also known as flash pulmonary edema or Pickering syndrome, is an uncommon presentation of TRAS.[2,10] The introduction of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensinogen receptor blockers (ARBs) in some patients with TRAS can precipitate acute graft dysfunction.[2] Asymptomatic cases may be discovered during routine surveillance duplex ultrasonography.[11]

The presence of a bruit over the renal allograft can be associated with TRAS, but this physical sign is nonspecific. Turbulent flow due to hyperdynamic flow at the renal artery anastomosis or stenosis of the iliac artery proximal to the anastomosis can result in such bruits over the iliac fossa. The presence of iatrogenic arteriovenous fistulas secondary to needle biopsy of the renal allograft is another differential diagnosis for a bruit over the transplanted kidney. Transplant renal artery stenosis may also exist without a bruit.[2]

Etiology and Risk Factors

Depending on the location and the pattern of the arterial narrowing, several morphologies of TRAS have been described: (1) at the site of the anastomosis, (2) proximal to the anastomosis, (3) distal to the anastomosis, (4) multiple stenoses at different locations, and (5) diffuse stenosis of the whole renal artery.[2]

Stenosis at the anastomosis site can be the result of faulty surgical techniques. Wide suture bites, extra stitches placed for hemostasis, and mechanical tucking of the artery have been identified as potential technical errors that can precipitate anastomotic stenosis. In contrast, techniques that allow a wide anastomosis under direct vision may lower the risk of TRAS.[12] End-to-end anastomosis of the donor renal artery to the recipient's inflow vessel has been reported as a risk factor for suture line stenosis, especially when there is a size mismatch between the 2 vessels.[13] In their case series, Sankari and colleagues did not note a difference in TRAS between end-to-end versus end-to-side anastomosis, but all patients who had an end-to-end anastomosis and TRAS were noted to have stenosis at the suture line.[14] According to Sutherland and colleagues, the incidence of TRAS was not different between patients who had an end-to-side renal artery anastomosis to the common or external lilac arteries versus those who had end-to-end anastomosis to the internal iliac artery. However, they identified endarterectomy of the internal iliac artery at the time of implantation as a significant risk factor for the late development of suture line stenosis. Intimal damage during endarterectomy or progression of atherosclerosis explains this phenomenon.[15] Another possible mechanism of stenosis at the anastomosis is endothelial damage and intimal flaps created during the donor organ procurement or backbench perfusion.[2]

Pre- or postanastomotic stenosis can be caused by progressive atherosclerosis of the donor's renal artery and recipient's inflow vessel.[2] Hurst and colleagues postulated that the increased incidence of TRAS noted in older donors and recipients was probably a consequence of ongoing atherosclerosis.[7] Transplant renal artery stenosis due to atherosclerosis tends to present relatively late in the posttransplant course.[2] Clamp injury to the recipient's iliac artery or the donor's renal artery is another mechanism for localized stenosis proximal or distal to the anastomosis.[13]

Diffuse stenosis of the allograft renal artery has been attributed to immune-mediated injury.[10] The histology of renal arteries affected by TRAS and acute rejection share some similarities.[13] As per the findings of Macia and colleagues, episodes of acute rejection were a critical predisposing factor for TRAS. They concluded that TRAS may be a vascular manifestation of acute rejection.[16] According to another study by Audard and colleagues, those with TRAS had more acute rejection episodes than those without TRAS, but this difference was not statistically significant.[17] Willicombe and colleagues have reported an association between postanastomotic diffuse TRAS and the presence of de novo donor-specific antibodies.[18]

Table 1 summarizes the predisposing factors for TRAS depending on the location of the stenosis. Delayed graft function (DGF) has been recognized as a significant risk factor for TRAS by several authors.[7,19] Kidneys from extended criteria donors have a higher chance of developing TRAS, which is probably secondary to the higher incidence of DGF associated with such transplants. Increased expression of vascular endothelial growth factor by kidneys that show DGF may cause intimal hyperplasia of the

donor renal artery, which can progress to stenosis.[7] The relationship between prolonged cold ischemia times and TRAS is controversial.[7,17,19]

Table 1. Risk Factors for Transplant Renal Artery Stenosis Depending on the Location of the Stenosis

Anastomotic stenosis	Faulty technique (wide suture bites, extra bites for hemostasis) End-to-end anastomosis to the internal iliac artery with endarterectomy of the recipient vessel End-to-end anastomosis with a size discrepancy between the donor and recipient arteries Intimal flaps/ intimal damage to the donor artery sustained during organ retrieval and perfusion
Preanastomotic stenosis	Progressive atherosclerosis of the recipient artery Clamp injury to the recipient artery
Postanastomotic stenosis	Focal Progressive atherosclerosis of the donor renal artery Intimal damage to the donor artery during retrieval/ perfusion/ clamping Diffuse Acute rejection

Cytomegalovirus (CMV) infection has been identified as a risk factor for TRAS. In the study from Pouria and colleagues, TRAS was significantly more common in patients with definitive evidence of CMV infection.[20] Audard and colleagues have also reported CMV infection as a significant and independent risk factor for the development of TRAS.[17] A case report by Ardalan and colleagues illustrated a case of TRAS associated with acute CMV infection. Antiviral therapy for the CMV disease led to spontaneous resolution of the stenosis of the renal artery.[21] The mitogenic effects of CMV gene products on the vascular smooth muscle may explain the association between CMV infection and the development of TRAS.[18,21]

Kinking or mechanical compression of the renal artery can mimic TRAS. Kinking of a redundant renal artery is possible in a right kidney transplant from a deceased donor where the donor renal artery tends to be longer than the renal vein.[2] External compression on the transplant renal artery by a polycystic kidney and an anastomotic pseudoaneurysm have been reported as TRAS mimics.[10,22]

Pathophysiology

Stenosis of the renal artery reduces the perfusion of the transplanted kidney. Renal ischemia activates the renin-angiotensin pathway and upregulates angiotensin II production. Angiotensin II in itself is a potent vasoconstrictor and enhances sympathetic nervous system-mediated vasoconstriction. It stimulates the production of aldosterone, which in turn expands the circulatory volume by promoting sodium and water retention. Hypertension in TRAS is a result of this vasoconstriction and volume expansion.[2] According to animal studies, stenosis above 70% affecting a single renal artery is sufficient to provoke a rise in systemic blood pressure.[23]

Differential Diagnosis

Calcineurin inhibitor toxicity can present as hypertension and graft dysfunction. Atherosclerotic narrowing of the recipient iliac arteries can be a mimicker of TRAS. This entity is termed "pseudo TRAS," and affected individuals are likely to have concomitant ischemic symptoms of the ipsilateral lower limb such as claudication, rest pain, and tissue loss. Hypertension in renal transplant recipients may be mediated by the contracted native kidneys or segmental infractions of the transplanted kidney due to thrombosis of polar arteries.[2]

Diagnosis

Duplex ultrasonography

Duplex ultrasonography is the first-line imaging modality used in suspected TRAS as it is readily available, noninvasive, and avoids nephrotoxic contrast or radiation exposure. Operator dependability is its main disadvantage. Compared with digital subtraction angiography (DSA), duplex has a sensitivity of 87% to 94% and a specificity of 86% to 100% for the diagnosis of this condition.[2] In addition to diagnosis, duplex ultrasonography is the preferred option for following up patients with TRAS.[24]

The typical duplex wave from distal to a hemodynamically significant narrowing in the transplanted renal artery will show a "tardus parvus" pattern, with a slow systolic upstroke and a diminished amplitude of the entire waveform (Figure 1). Additional extrarenal and intrarenal duplex indices have been described that can be used to suspect, diagnose, and assess the severity of TRAS. Peak systolic velocity (PSV) of the renal artery (Figure 2) and the ratio of PSV in the renal artery to the PSV in the external iliac artery are "extrarenal" duplex measurements that can be used to confirm

TRAS. Acceleration time (AT) in intrarenal arteries and intrarenal resistive index (RI) are helpful "intrarenal" indices.[25,26]

Figure 1. "Tardus Parvus" Wave Pattern on the Duplex Ultrasonograph in a Patient with Transplant Renal Artery Stenosis

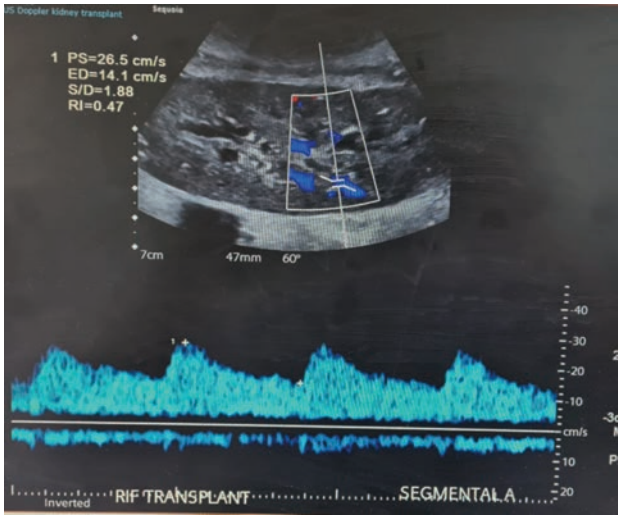
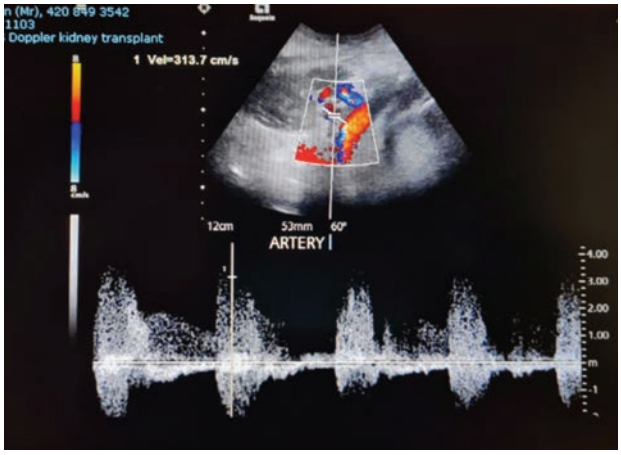


Figure 2. Elevated Peak Systolic Velocity



As reported by de Morais and colleagues, a PSV of 200 cm/s in the renal artery was 90% sensitive and 87.5% specific for the diagnosis of >50% stenosis. When the PSV cutoff was increased to 250 cm/s, the specificity increased to 96.8% but the sensitivity dropped to 70%. According to the same authors, when the PSV ratio between the renal artery and external iliac artery was >2, it showed 80% sensitivity and 100% specificity for detection of significant (>50%) TRAS.[25] Baxter and colleagues have reported that a PSV of >250 cm/s had 100% sensitivity and 95% specificity to indicate >50% narrowing of the transplanted renal artery.[27]

As reported by de Morais and colleagues, an AT of >0.09 seconds in the intrarenal arteries was the best single duplex criterion for the diagnosis of >50% stenosis.[25] Another study by Saarinen and colleagues, who used RI cutoff of ≤0.6 for the diagnosis of 50% or more stenosis of the transplant renal artery, reported a specificity of 100% and a sensitivity of 67%.[28] Gottlieb and colleagues have reported an AT of >0.1 second or the subjective interpretation of a dampened waveform in intrarenal arteries as more accurate indicators compared with elevated PSV.[29]

The accuracy of the PSV measurements can be affected by the Doppler angle and the tortuosity of the renal artery. Currently, there is no consensus on the use of duplex parameters such as AT and RI for the diagnosis of TRAS.[27,30] As described by Fananapazir and colleagues, the use of multiple duplex parameters in combination can increase the sensitivity and specificity of Doppler ultrasonography.[31] In the presence of Doppler ultrasonography showing features suspicious for TRAS, the diagnosis should be always confirmed by a more objective imaging modality, especially if the clinical circumstances dictate that TRAS should be confidently excluded.

Contrast-enhanced ultrasonography

Contrast-enhanced ultrasonography (CEUS) has been explored as an imaging option for the detection of TRAS. It involves intravenous injection of microbubbles, which act as the ultrasonography contrast agent. The nonnephrotoxic nature of such contrast material is an advantage. During CEUS, a longer time for contrast inflow indicates stenosis, and it is possible to directly identify such lesions on enhanced images. According to Pan and colleagues, who compared CEUS against standard duplex in patients with TRAS, CEUS emerged as the more superior imaging technique.[30] However, more experience in imaging with CEUS is required before it can be recommended as a preferred imaging option for TRAS.

Computed tomography angiography

Computed tomography angiography (CTA) is noninvasive but requires intravenous iodinated contrast, which has nephrotoxic properties. Hence, it is not a favored imaging modality in patients suspected of TRAS. Additionally, computed tomography exposes the patient to ionizing radiation. Despite these disadvantages, CTA can provide

excellent images of the allograft vasculature, especially after 3-dimensional (3D) reconstruction.[32] According to a study published by Sun and colleagues, 3D CTA had comparable sensitivity to DSA for the detection of TRAS.[33]

Magnetic resonance angiography

Magnetic resonance angiography (MRA) is noninvasive and does not involve ionizing radiation. Gadolinium can be used as a contrast agent, but in those with poor renal function, gadolinium can precipitate nephrogenic systemic fibrosis. Noncontrast-enhanced magnetic resonance imaging has also been reported to generate excellent images of transplanted renal arteries with an accurate depiction of stenoses.[34] Claustrophobia and incompatible metallic implants can preclude MRA in certain individuals. Magnetic resonance imaging has a reported sensitivity and specificity for the diagnosis of TRAS that ranges from 67% to 100% and 75% to 100%, respectively.[2] Combination of 3D contrast-enhanced MRA and 3D phase-contrast MRA can increase the specificity and accuracy of detecting TRAS. The presence of metallic clips close to the transplant artery is a common cause for false positive diagnoses or overestimation of the stenosis with MRA.[35]

Renal scintigraphy

Because of its poor sensitivity and specificity, renal scintigraphy is no longer recommended as an imaging modality to diagnose renal artery stenosis.[36]

Digital subtraction angiography

Digital subtraction angiography is considered the gold standard of imaging in TRAS. It is invasive, and arterial puncture can result in complications such as hematoma, pseudoaneurysm, dissection, and thromboembolism. Patients undergoing DSA are exposed to iodinated contrast material and ionizing radiation. Because of these drawbacks, DSA is generally not used as a first-line diagnostic tool, but it has a place when information gained from noninvasive imaging techniques tends to be nonspecific. In addition to confirming the diagnosis, DSA allows simultaneous treatment of the identified lesion by balloon angioplasty with or without stenting. Carbon dioxide, which is a nonnephrotoxic contrast medium, can be used as an alternative to reduce the volume of iodinated contrast required during diagnostic and therapeutic angiography.[37]

Fractional flow reserve (FFR) is a technique utilized during coronary angiography to identify hemodynamically significant stenosis that warrants treatment. This involves intra-arterial instillation of papaverine to induce vasodilatation and end-organ hyperemia. During peak hyperemia, the ratio of mean distal and proximal pressures to the stenosis is used to calculate the FFR.[38] According to De Bruyne and colleagues, a distal-to-proximal pressure ratio of <0.9 across a renal artery stenosis was associated with the upregulation of renin production.[39] Gomes Junior and colleagues used FFR in patients who underwent endovascular revascularization for TRAS and reported that it had a good correlation with other hemodynamic parameters such as the translesional pressure gradient.[38]

Table 2 summarizes the advantages and disadvantages of different imaging modalities used for the diagnosis of TRAS.

Treatment

Medical management

Those with stable renal functions and imaging parameters suggestive of <50% stenosis of the renal artery can be managed conservatively with antihypertensives. The preferred first-line antihypertensive agents are ACEIs or ARBs.[2] These drugs should be introduced with careful monitoring of the renal function, and any deterioration should prompt immediate drug withdrawal. In addition to controlling blood pressure, ACEI or ARB therapy limits cardiac mortality and morbidity in patients with renal artery stenosis. These effects are explained by their ability to mitigate the adverse cardiac effects of elevated angiotensin II and aldosterone.[40]

Although solid evidence for the use of antiplatelets and statins in patients with TRAS is unavailable, experts agree that these drugs should be given to patients affected with the condition, as well as for patients with atherosclerosis-related narrowing of the native renal arteries.[2]

Patients with adequate blood pressure control while on antihypertensive drugs, stable serum creatinine, and nonprogressive lesions on imaging can be continued on conservative management. Invasive treatment is indicated when there is resistant hypertension, graft dysfunction, or evidence of progressive disease.[2]

Table 2. Comparison of Imaging Modalities Used for Diagnosis of Transplant Renal Artery Stenosis

Imaging Modality	Advantage	Disadvantage	Sensitivity and Specificity
Duplex ultrasonography	1. Availability 2. No radiation of contrast exposure 3. Multiple duplex parameters that can be combined for increased sensitivity and specificity	1. Operator dependable 2. Findings can be confounded by factors such as tortuosity of the renal artery and the complexity of its anatomy 3. The value of duplex parameters such as AT and RI are debatable	87%-94% and 86%-100%
CTA	Excellent images especially after 3D reconstruction	1. Need for iodinated contrast which itself is nephrotoxic 2. Exposure to ionizing radiation	No data available as CTA is not a first-line imaging choice for TRAS
MRA	1. No ionizing radiation 2. Noncontrast MRI can provide acceptable images	1. Poor availability 2. Gadolinium can cause nephrogenic systemic fibrosis in patients with severely depressed GFR 3. Not compatible with certain metallic prosthesis 4. Claustrophobia is a contraindication 5. Metallic lips within the vicinity of the renal artery can lead to false positives/overestimation of stenosis	67%-100% and 75%-100%
DSA	Ability to intervene in addition to diagnosis	1. Need for iodinated contrast 2. Exposure to ionizing radiation 3. Arterial dissection, hematomas, embolic phenomena, and puncture site pseudoaneurysm	Gold standard imaging modality against which other methods are compared for sensitivity and specificity

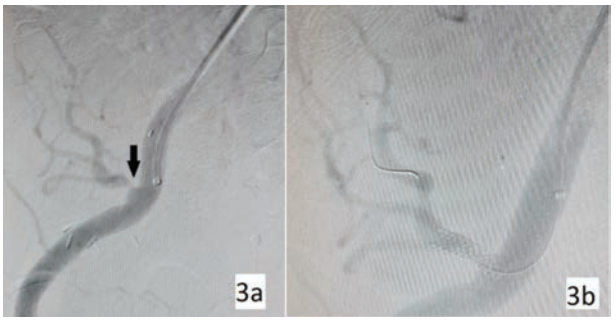
Abbreviations: AT, acceleration time; CTA, computed tomography angiography; DSA, digital subtraction angiography; GFR, glomerular filtration rate; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; TRAS, transplant renal artery stenosis

Endovascular treatment

Endovascular intervention is the preferred first-line treatment option for patients with TRAS with an indication for invasive intervention (Figure 3). A technical success rate of >90% has been reported.[41] Balloon angioplasty has a restenosis rate of 5% to 30% at 6 to 8 months.[42] Stenting reduces the incidence of restenosis.[43] According to Ngo and colleagues, the overall patency rates for angioplasty alone versus angioplasty combined with stenting for TRAS were 73% and 90.4%, respectively.[41] These findings were replicated by Biederman and colleagues, who noted better patency rates for stented lesions compared with nonstented lesions after angioplasty.[44] Drug-eluting stents appear superior to bare metal stents in preventing in-stent restenosis, especially for small-diameter renal arteries and postanastomotic TRAS.[44,45] Percutaneous intervention for TRAS has a 10% risk of complications. Major complications such as arterial dissection, rupture, or thrombosis occur in <4% of patients.[2]

Endovascular treatment for TRAS leads to better control of blood pressure and long-lasting improvement of renal function.[46] According to the findings of Beecroft and colleagues, at the end of 1 month after percutaneous intervention for TRAS, patients had significant improvement of their systolic and diastolic blood pressures with a reduction in the number of antihypertensive agents required to achieve target blood pressure values. Furthermore, they noted a significant drop in serum creatinine after successful revascularization.[47] A different study by Gunawardena and colleagues noted significantly better serum creatinine values and diminished antihypertensive drug requirements at the end of 4-year follow-up in patients who had endovascular treatment for clinically significant TRAS.[46] Halimi and colleagues reported good long-term outcomes following balloon angioplasty for TRAS, with sustained improvements in blood pressure and renal function. According to their study, at the end of 8 years, patients with TRAS who were treated had patient and graft survival rates similar to those shown in patients without TRAS.[19] Patel and colleagues followed up 41 patients with TRAS who underwent angioplasty with or without stenting for 21 years and compared them against a matched sample of renal allograft recipients without TRAS; their results showed that long-term

Figure 3. Digital Subtraction Angiography Images



(a) Anastomotic transplant renal artery stenosis (arrow) on digital subtraction angiography. **(b)** After balloon angioplasty and stenting.

patient and graft survival rates were not different between the 2 groups. According to their findings, successful intervention for TRAS was associated with a 12% improvement in systolic blood pressure, a 7.4% improvement in diastolic blood pressure, and a 27% improvement in serum creatinine values.[48]

Surgery

Surgery is indicated for TRAS when percutaneous intervention fails or when there are lesions that are not amenable to angioplasty. Surgical options include revision of the anastomosis, bypass of the stenosis using the saphenous vein graft, patch angioplasty, and endarterectomy.[49] Shames and colleagues reported excellent outcomes with the use of ABO blood group-matched deceased donor iliac artery grafts.[50] The success after surgical therapy ranges from 67% to 92%. However, morbidity and mortality are much higher compared with that shown with angioplasty, with a 20% risk of graft loss and a 5% risk of death.[2]

Surgery versus angioplasty for transplant renal artery stenosis

A publication by Benoit and colleagues describes the comparative outcomes between surgery and angioplasty for TRAS. According to their results, the immediate and long-term success for surgery was 92.1% and 82.5%. The corresponding figures for angioplasty were 69% and 40.8%. The angioplasty group had a higher procedure-related morbidity (28% vs 7.6% for surgery).[51] These findings are of historical interest only, as advances in technology and operator skills have drastically improved the outcomes of percutaneous interventions.

Comparison of conservative vs interventional therapy for transplant renal artery stenosis

Invasive management of TRAS has been compared against medical therapy in a few studies. The case-control study by Zupunski and colleagues is an example. They studied and compared patients with >50% TRAS who received medical therapy versus those who had some form of invasive treatment, either percutaneous or surgical. The indication for intervention was an increase in serum creatine of 20% or more from baseline. They did not note a significant difference in blood pressure, serum creatinine, and the number of antihypertensive agents between the 2 groups. Among patients, 21% of those who had been conservatively managed had a spontaneous improvement in the severity of stenosis to <50%. The authors concluded that, on long-term follow-up, TRAS seemed to be nonprogressive and stable in the majority, irrespective of intervention.[52]

Another study by Geddes and colleagues compared long-term outcomes of those with TRAS who either received percutaneous intervention or medical therapy. The majority of the study population had >50% stenosis affecting the transplant renal artery. Angioplasty with or without stenting was indicated for patients with severe renal impairment, rapid deterioration of their graft function, or treatment-resistant hypertension. At the end of follow-up, there was no significant difference in terms of blood pressure, serum creatinine, or the number of antihypertensives required between the 2 groups. The authors concluded that conservative management is a reasonable option in patients with TRAS who have stable renal functions and adequate blood pressure control.[53]

Conclusions

Transplant renal artery stenosis is a treatable cause of graft dysfunction and posttransplant hypertension. Medical treatment with blood pressure control, antiplatelet drugs, and statins can be attempted when the graft function is stable and the stenosis is <50%. Transplant renal artery stenosis associated with resistant hypertension, graft dysfunction, or progression of the severity of stenosis on imaging requires revascularization. At present, endovascular techniques are preferred to open surgery. New evidence indicates that selected patients with >50% TRAS can be managed conservatively with outcomes similar to those who undergo invasive treatment. Further studies with larger patient numbers and randomization will be required to identify patients who are candidates for such conservative treatment.

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Current Scenarios of Pediatric Transplants of Kidney, Liver, Heart, and Lung in India: Systematic Review and Meta-Analysis

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Abstract

Objectives: **There is no systematic review and meta-analysis for pediatric solid-organ transplants in India. The objective of the study was to collect high-evidence data in this regard.**

Materials and Methods: **A systematic review and meta-analysis was performed for pediatric solid-organ transplants in India. We used the search engines of PubMed, Google Scholar, PubMed Central, Embase, and MEDLINE from beginning of data availability until April 26, 2022. Data from 2 participating centers were also used. Analyses were performed by the DerSimonian random model.**

Results: **Of 50 000 primary searches, only 31 studies were included for analysis. In total, data for pediatric kidney (n = 1057), liver (n = 914), and heart (n = 117) were reported. For the pediatric kidney, the 1-year, 5-year, and 10-year patient survival rates were 96% (range, 93%-99%; $I^2 = 91.17\%$, $H^2 = 11.33$, $P < .01$), 90% (range, 85%-94%; $I^2 = 93.54\%$, $H^2 = 15.47$, $P < .01$), and 75% (range, 62%-88%; $I^2 = 97.36\%$, $H^2 = 37.82$, $P < .01$), respectively. The 1-year, 5-year, and 10-year renal graft survival rates were 93% (range, 90%-96%; $I^2 = 63.82$, $H^2 = 2.76$, $P < .01$), 83% (range, 76%-89%; $I^2 = 86.39\%$, $H^2 = 7.35$, $P < .01$), and 66% (range, 57%-75%; $I^2 = 81.68\%$, $H^2 = 5.46$, $P < .01$), respectively. The acute rejection rate was 23% (range, 20%-27%; $I^2 = 5.44\%$, $H^2 = 1.06$, $P = .39$). For the pediatric liver transplant, the 1-year and 5-year survival rates were 92% (range, 89%-95%; $I^2 = 49.96\%$, $H^2 = 2$, $P < .04$) and 88% (range, 85-90%; $I^2 = 0$; $H^2 = 1$, $P = .72$), respectively.**

Conclusions: **The outcomes of pediatric solid-organ transplants in India are comparable to those of the Western world. However, cause of graft loss and patient death is largely attributed to infections, unlike the experiences reported in the West. An effective registry is a primary pillar to expand pediatric solid-organ transplants in India.**

Key words: *Kidney transplants, Living donor, Liver transplants*

Introduction

Pediatric solid-organ transplantation (SOT) remains an evolving field in the Indian context, as facilities and specialization are at an incipient stage at many of the centers with adult organ transplant programs. Moreover, compared with the developed world, where deceased donation is firmly established, progress in this part of the world is lagging behind. This worsens the situation of expanding the donor pool, especially in pediatric SOT. As of April 12, 2022, the Organ Procurement and Transplantation Network registry[1] reported a total of 888 209 SOT performed, of which 9216, 16 200, 9618, and 24 071 SOT belonged to age groups <1 year, 1 to 5 years, 6 to 10 years, and 11 to 17 years, respectively. Unfortunately, in the Indian context, no updated national registry exists to account for the actual number of pediatric patients on the wait list or scheduled for transplant. In India, the transplant programs are mostly dominant in private sectors, and their contribution in national registry is unsatisfactory, which partly explains the reason for the problems in maintaining a robust registry. Largely, the data regarding the pediatric SOT originate from the premier institutes and premier

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

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Experimental and Clinical Transplantation (2022) 12: 1058-1068

DOI: 10.6002/ect.2022.0207

transplant centers. At this point, it is clear that this area of medical care has many hurdles, from the access to care for organ failure to posttransplant care. Hence, we conducted a systematic review and meta-analysis, by digging into the published literature on the outcomes of pediatric SOT performed in India. This report will help to assess the current status and help to better align future goals to fill the deficit in this less-developed field of medical care.

Materials and Methods

Literature search strategy

The search engines we used in our study were PubMed, PubMed Central, Bookshelf, MEDLINE, Embase, and Google Scholar. We excluded non-peer-reviewed publications, works that included only abstracts, or any other gray literature. We used English language publications only. Other languages were excluded from the search results. We used the following Medical Subject Headings (MeSH terms) "pediatric", or "child" and "transplant", and "India", and "liver", or "kidney", or "renal", or "heart", or "lung". We have not used selective key words such as graft survival" or "patient survival" or "living donation" or "deceased donation" so as to avoid missing any reports. The search was performed by 2 authors (HSM and VBK), individually and separately. The manual search was performed according to forward and backward snowballing methods. Two authors (SC and VBK) independently assessed the validity of the titles, abstracts, or full texts of each published article.

Inclusion and exclusion criteria

After completion of the search process, any conflict or dilemma for inclusion or exclusion of items in the search results was discussed in-person between HSM and VBK, and the final decision was reserved until after consultation with SC. The eligible articles in our report were any pediatric SOT performed in Indian settings that included outcome data. We accepted the definition of pediatric population according to the description in each respective study, and there was no cutoff age for inclusion. Also, all of these articles were included irrespective of the organ transplanted. We included heart, lung, liver, kidney, and pancreas. Hemopoietic stem cell transplants were excluded from our report. The search was performed from April 12, 2022, through April 26, 2022. There were some restrictions to type of publications, and we included only original articles. Review articles, correspondence, letters to editor, meta-analyses, case reports, commentaries, and editorials were excluded from the search results. We have contacted an individual participating center (Dr. Rela Institute and Medical Centre; and Institute of Kidney Diseases and Research Center and Dr. H. L. Trivedi Institute of Transplantation Sciences [IKDRC-ITS], Ahmedabad, Gujarat, India) for updated data on pediatric SOT, and we included the data in the systematic review and meta-analysis.

Ethics

Our methods in this systematic review and meta-analysis were reported in accordance with the guidelines of the Meta-Analyses Of Observational Studies in Epidemiology checklist. Most of the pediatric SOT performed in an Indian scenario are living related (85%) and deceased donors (15%). The relationship of the living donors to the recipients was either parent or grandparent. The transplants performed in all the centers are in accordance with the Declaration of Istanbul, Helsinki, and the Transplantation of Human Organ and Tissue Act, India.

Data extraction

Data extraction was done by 2 authors (VBK and SC) in an open-ended approach, and no fixed parameters were prerequisite, as the data concerned are sparse and heterogenous. This method was used with a goal toward creation of a more comprehensive review of the available literature. Data assembled in the report included year of publication, authorship, setting, year of publication, age, etiology of organ failure, sex, and survival rates for patient and graft.

Outcome measurement

The primary outcome for the meta-analysis was the patient and graft survival at 1 year, 5 years, and 10 years for kidney transplant, as well as the patient and graft survival for the available follow-up of other organs. The other relevant data were accumulated and reported as a part of this systematic review, and no statistical analyses were performed, because the data were highly heterogeneous across the published literature.

Statistical analyses

The analyses were performed by HSM with Stata statistical software (release 16, StataCorp). The data were checked by VBK before analysis. In Table 1 and Table 2, categorical outcomes were reported as frequencies and percentages, whereas continuous outcomes were reported as median, mean, standard deviation, interquartile range, and range as appropriate. To perform meta-analyses of proportion (the primary outcome), metaprop commands were used, which are an extension of the Stata software. The metaprop extension allows computation of 95% confidence intervals (CI) from the score statistic and the exact binomial method. We used the DerSimonian-Laird random effect model as the statistical method. The outcomes were reported as effect size with the 95% CI. We reported the I statistic to assess the heterogeneity of the pooled estimate, in which a value >0.5 indicated substantial heterogeneity.[2] Data visualization for the primary outcomes was completed by forest plots with reference line and triangle effect size and bubble plots. Publication bias

Table 1. Summary of the Studies Published With Reports of Pediatric Kidney Transplant

Reference	Setting	No. of	Male Patients No. (%)	Duration Sex,	NKD	Dialysis, No. of patients	Age (range)	Survival, %			Graft survival, %			AR
								1 y	5 y	10 y	1 y	5 y	10 y	
Mehrotra 2002[29]	CMC, Vellore, Tamil Naidu	64	48 (75%)	1984 and 1996	RN	NR	12.86 ± 2.52 y	92	90*	NR	88	86*	NR	9 (14%)
Joshi 2002[30]	Jaslok Hospital, Mumbai, Maharashtra	81	63 (77.8%)	1979-2002	CGN	73 HD, 2 CAPD	3-16 y	96.4	92**	55.6	96.4	92**	55.6	NR
Gulati 2004[31]	SGPGI, Lucknow, Uttar Pradesh	39	32 (82%)	1994-2004	CGN	34 HD, 3 CAPD	15.6 ± 1 y	89	70	NR	89	50	NR	17 (45.8%)
Phadke 2006[32]	Manipal Hospital, Bangalore	47	30 (63.8%)	1988-2004	TIN	33.3% Presumptive, 57.2% HD, 9.5% CAPD	120 mo	78.2	44.6	36.2	80	45.8	37.5	23.40%
Chacko 2007[33]	CMC, Vellore, Tamil Naidu	90	59 (65%)	1991-2005	CGN	76 HD (84%), 6 CAPD (7%)	15 y (6-18 y)	95	87	79	98	84	76	46.70%
Vasudevan 2008[34]	St. John's Medical College Hospital, Bangalore	33	26 (78.7%)	1998-2008	TIN	18 HD, 5 CAPD, 6 both	12.5 ± 3.86 y	100	94	NR	94	90	NR	18.20%
Sinha 2010[35]	AIIMS, New Delhi	45	37 (86%)	1995-2008	CGN	39 HD, 5 CAPD	13.3 ± 4 y	95.3	87.9	76.9	91.1	80.4	75.1	31%
Kute 2012[36]	IKDRC-ITS, Ahmedabad, Gujarat	37	25 (67.5%)	1998 and 2011	CAKUT	NR	13.8 ± 3.1 y	NR	72	NR	NR	83.7	NR	8 (21.6%)
Srivastava 2015[37]	SGPGI, Lucknow, Uttar Pradesh	70	57 (82%)	1995-2011	CGN	56 HD, 10 PD	14 ± 1.4 y	95.7	96.4*	94.1	94.3	89.2*	66.8	20 (28.5%)
Bijalwan 2017[38]	Amrita, Kochi, Kerala	32	14 (43.75%)	2003-2014	CGN	29 HD, 2 CAPD	14.5 y (10-17 y)	100	100*	NR	96.7	85*	NR	4 (12.5%)
Meena 2018[39]	AIIMS, New Delhi	116	97 (84%)	1995-2017	RN	82 HD, 11 CAPD, 14 both	13 ± 3.8 y	95.5	91.7	89.2	92.2	85.6	77.6	30 (24%)
Parikh 2019[40]	MPUH, Nadiad, Gujarat	100	77 (77%)	2001 and 2018	CGN	90 HD, 6 PD	15.7 ± 2.3 y	100	100	100	98	80	73	26 (26%)
Kinnari 2020[41] (LDKT)	IKDRC-ITS, Ahmedabad, Gujarat	151	129 (85.4%)	1998-2011	CAKUT	106 HD, 45 CAPD	14.7 ± 2.7 y	92.5	80.9	75.1	87.4	72.1	72.1	30 (19.8%)
Kinnari 2020[41] (DDKT)	IKDRC-ITS, Ahmedabad, Gujarat	37	25 (67.5%)	1998-2011	CAKUT	26 HD, 11 CAPD	13.8 ± 3.1 y	83.4	67.9	67.9	90.4	86.4	73.3	8 (21.6%)
Mohapatra 2021[42]	CMC, Vellore, Tamil Naidu	139	93 (66.9%)	1991-2005 and 2006-2016	UE	123 HD, 8 CAPD	15.2 ± 2.9 y		89 and 94			92.1 and 95.3		26 (28.6%) and 13 (27.1%)
Kute 2022***	IKDRC-ITS, Ahmedabad, Gujarat	439	NR	1998-2020	CAKUT	NR	NR							

Abbreviations: AIIMS, All India Institute of Medical Sciences; AR, acute rejection; CAKUT, congenital abnormalities of kidney and urinary tract; CAPD, continuous ambulatory peritoneal dialysis; CGN, chronic glomerulonephritis; CMC, Christian Medical College; DDKT, deceased donor kidney transplant; HD, hemodialysis; IKDRC-ITS, Institute of Kidney Diseases and Research Center and Dr. H L Trivedi Institute of Transplantation Sciences; LDKT, living donor kidney transplant; MPUH, Muljibhai Patel Urological Hospital; NKD, native kidney disease; NR, not reported; RN, reflux nephropathy; SGPGI, Sanjay Gandhi Post Graduate Institute of Medical Sciences; TIN, tubulointerstitial nephritis; UE, undetermined etiology

*3 years. **2 years. ***Individual center data (unpublished).

Table 2. Pediatric Liver Transplant Studies From India

Reference	Setting	No. of Patients	Patient Sex	Duration	Most Common Etiolog	Recipient Age (range)	Recipient Weight, kg (range)	Outcome	Follow-up
Safwan 2008[43]	Chennai, Tamil Naidu	58 BA of 132 PLT	27 M (46.5%)	August 2010 to June 2015	BA for entire cohort	13.5 mo (5-117 mo)	9.2 ± 4.5	Patient survival: 96.6% at 1 mo and 91.4% at 1 y	1 y
Kaur 2011[44]	Apollo, New Delhi	5	3 M (60%)	January 2008 to June 2009	BA	8.2 ± 0.4 mo	6.8 ± 0.4	Patient and graft survival:both 100%	11 mo
Vijay 2013[45]	Army Hospital, Delhi	12	M:F = 6:9	April 2007 to June 2011	BA	5 mo to 12 y	5.4 to 30	Patient survival: 73.4% at 1 y	1 y
Malhotra 2015[46]	Apollo, New Delhi	20	M:F = 1.8:1	2008-2013	BA for entire cohort	8 mo (6 mo to 18 y)	7.3 (5.8-48)	Patient survival: 90% at 2.5 y follow-up	2.5 y (2-62 mo)
Mohan 2017[47]	Medanta, Gurugram	200	120 M (60%)	September 2004 to July 2016	Cholestatic liver disease (46%) followed by metabolic liver disease (33%)	60 mo (17-112 mo)	16 (9-27)	Patient survival: 94% at 1 y and 87% at 5 y	5 y
Pandey 2020[48]	ILBS, New Delhi	Total, 109; <10 kg, 45; and > 10 kg, 64	66 M (77.3%)	May 2017 to April 2020	Indication for LT was biliary atresia in 53 patients (49%), and 17 patients (16%) had primary genetic liver disease.	47 mo (1-192 mo)	15.2 (2.5 to 68)	Similar outcomes in 77% (<10 kg) or 86% (>10 kg) of children	NR
Raghunathan 2020[49]	Medanta, Gurugram	23 of 150	13 M (56.5%)	Until 2020	BA: HPS cohort of 23, compared with 150 non-HPS cohort	6.5 ± 6.08 y	NR	HAT, 4/23 (17.3%); PVT, 1/23 (4.3%); acute rejection, 2/23 (8.6%); 1-y survival, 20/23 (86.9%). Similar outcomes with non-HPS.	1y
Varma 2021[50] (2019 data)	ILBS, New Delhi	8	NR	March 24, 2018, to June 7, 2019	BA	38.8 mo (11-132 mo)	11.4 (5-23)		
Varma 2021[50] (2020 data)	ILBS, New Delhi	7	M:F, 4:3	March 24 to June 7, 2020	BA	56 mo (6-180 mo)	20 (5.2 to 50)	Similar outcomes.	1 y
Present study, 2022*	Dr. Rela Institute and Medical Centre, Chennai	500	NR	2010 to April 2022	BA			Patient survival: 93% at 1 y and 88% at 5 y	5 y

Abbreviations: BA, biliary atresia; F, female; HAT, hepatic artery thrombosis; HPS, hepatopulmonary syndrome; ILBS, Institute of Liver and Biliary Sciences; LT, liver transplant; M, male; NR, not reported; PVT, portal vein thrombosis
*Individual center data.

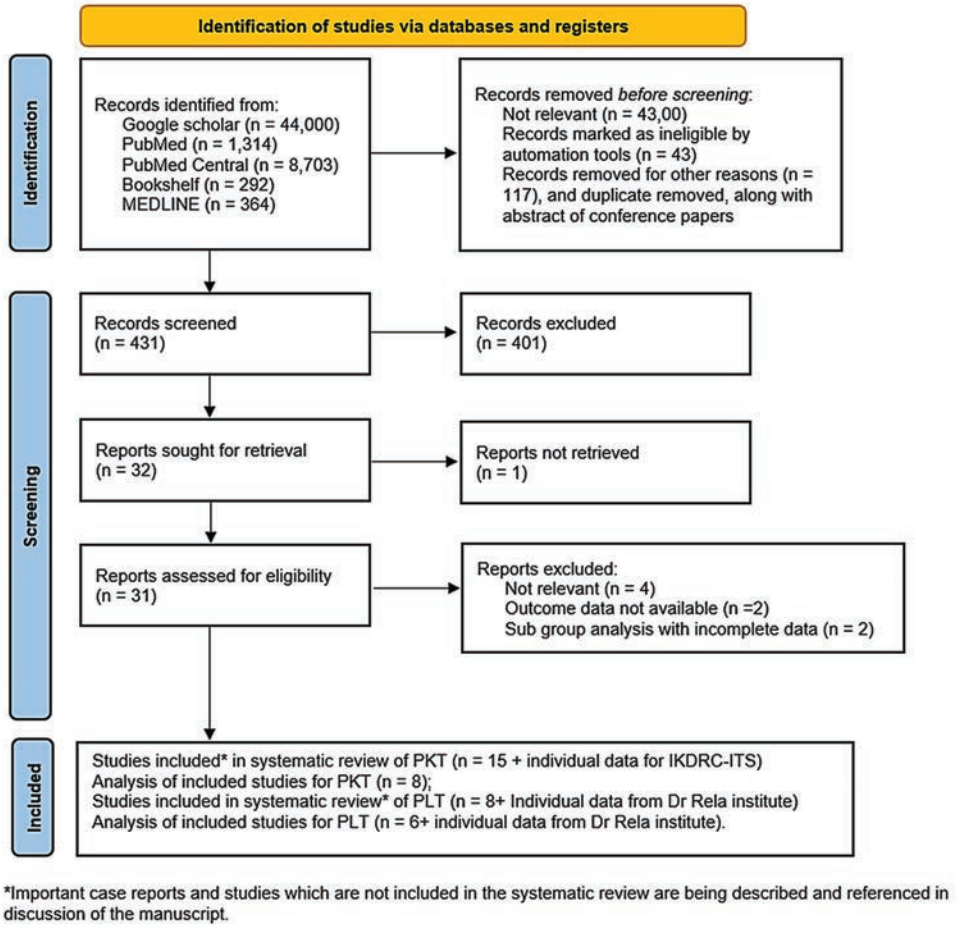
was depicted with funnel plots and Egger tests. Sensitivity analysis was performed according to a fixed model with some studies, instead of a random model. $P < .05$ indicates statistical significance.

Results

In this systematic review and meta-analysis, last searched through April 26, 2022, from search engines of Google Scholar, PubMed, PubMed Central, Bookshelf, Embase, and MEDLINE, we screened more than 50 000 articles. After initial survey and screening, 431 records were identified, which were further refined for data of interest. Thirty-one reports were finally included for inclusion in the study. Fifteen studies were included for pediatric kidney transplant (PKT) and 8 for pediatric liver transplant (PLT) in the statistical calculation. Only 2 studies were found for pediatric heart transplant. We collected data from PKT (n = 1057), PLT (n = 914), and pediatric heart transplant (n = 117). In this report the individual data contributions were 439 PKT cases from IKDRC-ITS, Ahmedabad, and 500 PLT cases from the Dr. Rela Institute and Medical Centre, Chennai, India.

Figure 1 shows a flow chart summary of the study process. Table 1 shows the published studies with PKT. We have included all possible important studies. For the analysis, we have selected the most recent studies, which have already been included in previous published results.

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses Flow Chart Diagram for the Study



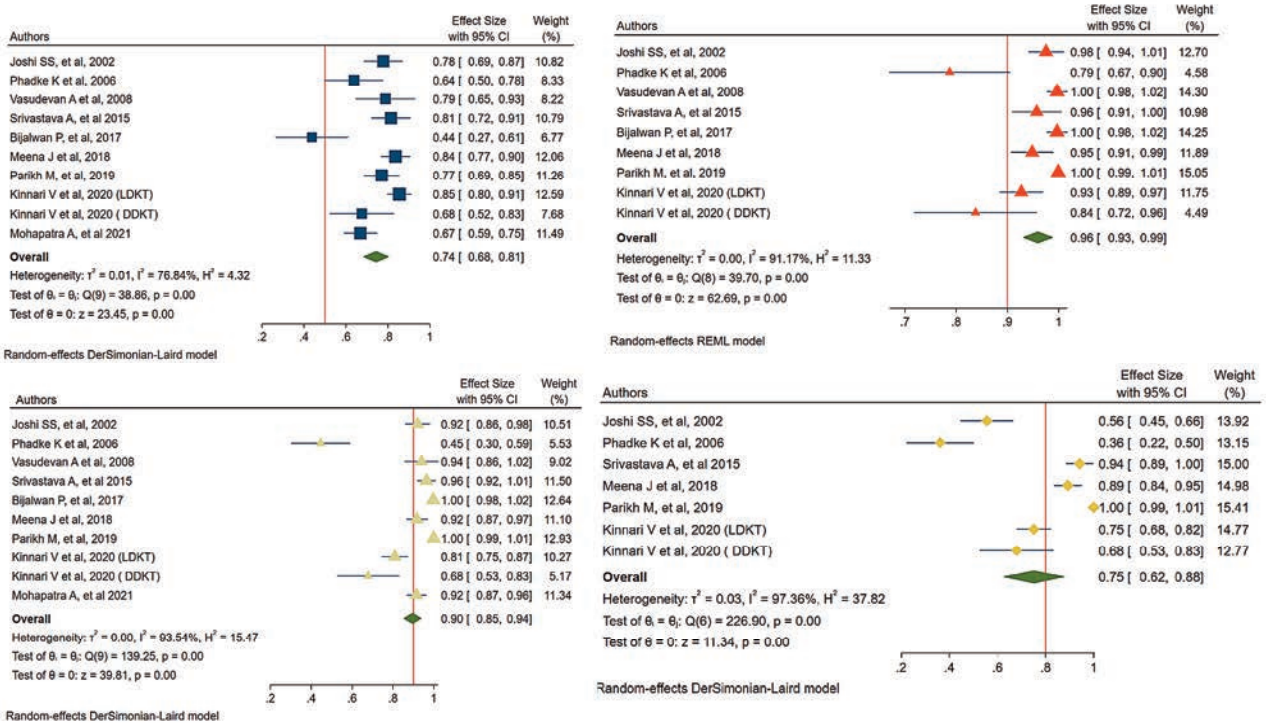
Abbreviations: IKDRC-ITS, Institute of Kidney Diseases and Research Center and Dr. H. L. Trivedi Institute of Transplantation Sciences; PKT, pediatric kidney transplant; PLT, pediatric liver transplant

Figure 2 (top right) shows the sex disparity in PKT recipients. The proportion of male recipients in PKT was 74% (range, 68%-81%; I^2 = 76.84%, H^2 = 4.32). The heterogeneity in the study was also not exceedingly high. Figure 2 depicts the forest plot for outcomes of patient survival in PKT from Indian studies. The 1-year, 5-year, and 10-year patient survival rates were 96% (range, 93%-99%; I^2 = 91.17%, H^2 = 11.33, P < .01), 90% (range, 85%-94%; I^2 = 93.54%, H^2 = 15.47, P < .01), and 75% (range, 62%-88%; I^2 = 97.36%, H^2 = 37.82, P < .01), respectively. It is notable that the individual center data from IKDRC-ITS as of 2020 for outcomes were not analyzed, because the center had not reported official statistics, but the outcome was roughly above 90% for the first year and above 80% for the 5-year survival rates for patients and grafts.

Figure 3 shows the forest plot for outcomes of graft survival in PKT. The 1-year, 5-year, and 10-year graft survival rates were 93% (range, 90%-96%; I^2 = 63.82, H^2 = 2.76, P < .01), 83% (range, 76%-89%; I^2 = 86.39%, H^2 = 7.35, P < .01), and 66% (range, 57%-75%; I^2 = 81.68%, H^2 = 5.46, P < .01), respectively. Figure 3 (bottom left) shows the acute rejection rate in PKT. The rejection rate on cumulative basis from the centers was observed as 23% (range, 20%-27%; I^2 = 5.44%, H^2 = 1.06, P = .39). The reported heterogeneity was not significant, which shows the uniform protocol of immunosuppression drugs and acute rejection rate in the transplant centers.

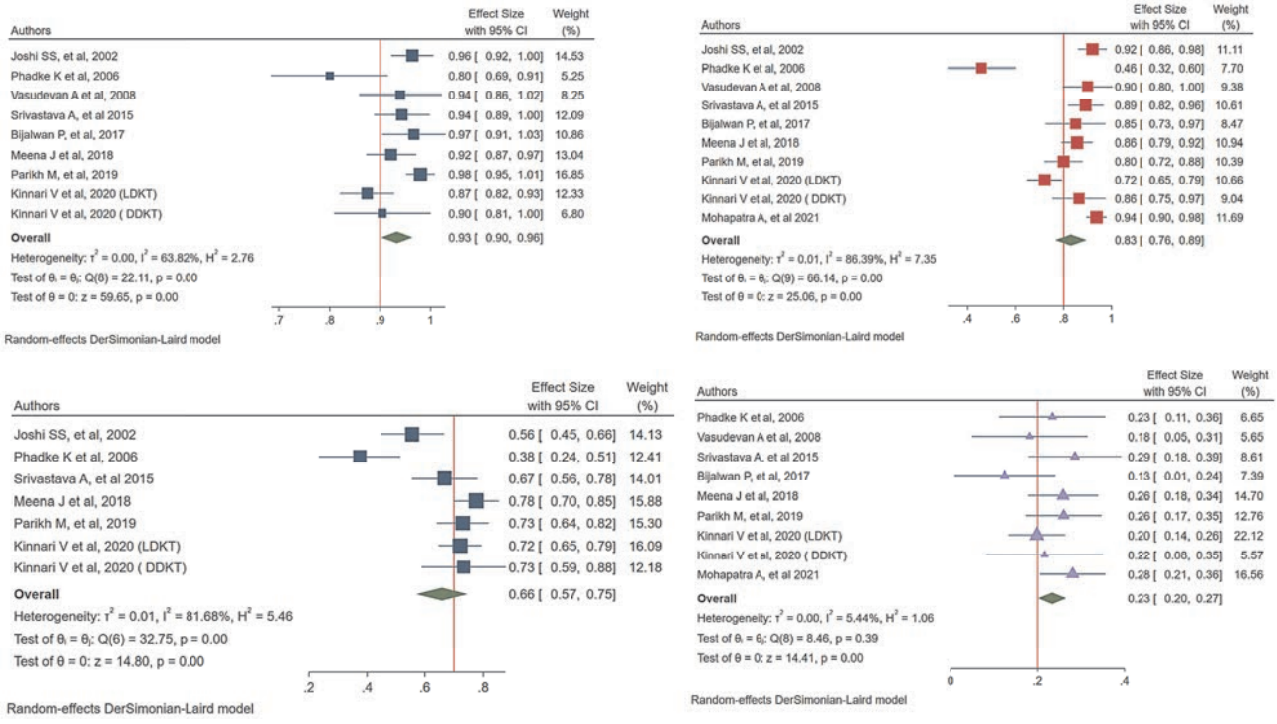
Figure 4 shows the publication bias for patient and graft survival in PKT. The presence of bias was expected with the small number of studies with diverse timelines and remote geographic areas. Table 2 summarizes the PLT data from India. The most common etiology for PLT in many centers was biliary atresia. The follow-up data were reported for 1 year, and a very few studies reported long-term data for PLT.

Figure 2. Forest Plots for Sex Distribution and Patient Survival in Pediatric Kidney Transplant



Abbreviations: DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant
Forest plots show pediatric kidney transplant recipient **(top right)** sex distribution and pediatric kidney transplant recipient survival at **(top left)** 1 year, **(bottom right)** 5 years, and **(bottom left)** 10 years.

Figure 3. Graft Survival and Acute Rejection in Pediatric Kidney Transplant



Abbreviations: DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant
Forest plots show pediatric kidney transplant recipient **(bottom left)** acute rejection, as well as graft survival at **(top left)** 1 year, **(top right)** 5 years, and 10 years.

Figure 5 shows the forest plot for patient survival after PLT at 1 year and 5 years. The 1-year and 5-year survival rates were 92% (range, 89%-95%; $I^2 = 49.96\%$, $H^2 = 2$, $P < .04$) and 88% (range, 85-90%; $I^2 = 0$, $H^2 = 1$, $P = .72$), respectively. The uniform outcomes in the PLT centers are depicted by the low heterogeneity in the studies. Figure 6 shows the publication bias for the PLT studies. We have also conducted the sensitivity analysis for both PKT and PLT from a fixed model, and the results were similar.

Discussion

To the best of our dedicated literature search, we report the first systematic review and meta-analysis on outcomes of pediatric SOT in Indian settings. From the results of our study, it is evident that pediatric SOT in India is in a very early stage. India represents the second leading nation with respect to living donor SOT numbers in the world. However, pediatric SOT is considerably less developed. There is no adequate registry for pediatric transplant in India. The extent of deficiency in this field can be estimated by comparing the Indian scenario, where there is no active registry, versus the North American Pediatric Renal Trials and Collaborative Studies of America, which was established in 1987 and currently has 108 participating centers with 21 332 pediatric patients.[2] Also, in our analysis, the cumulative transplant data are from 1200 pediatric

Figure 4. Publication Bias for the Pediatric Kidney Transplant Analyses

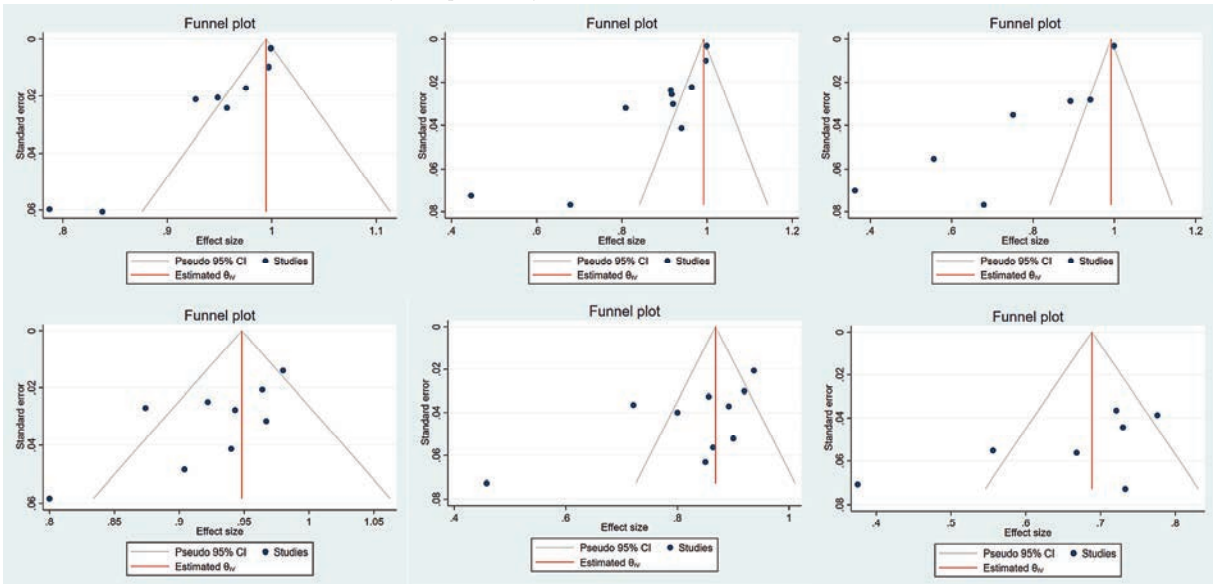
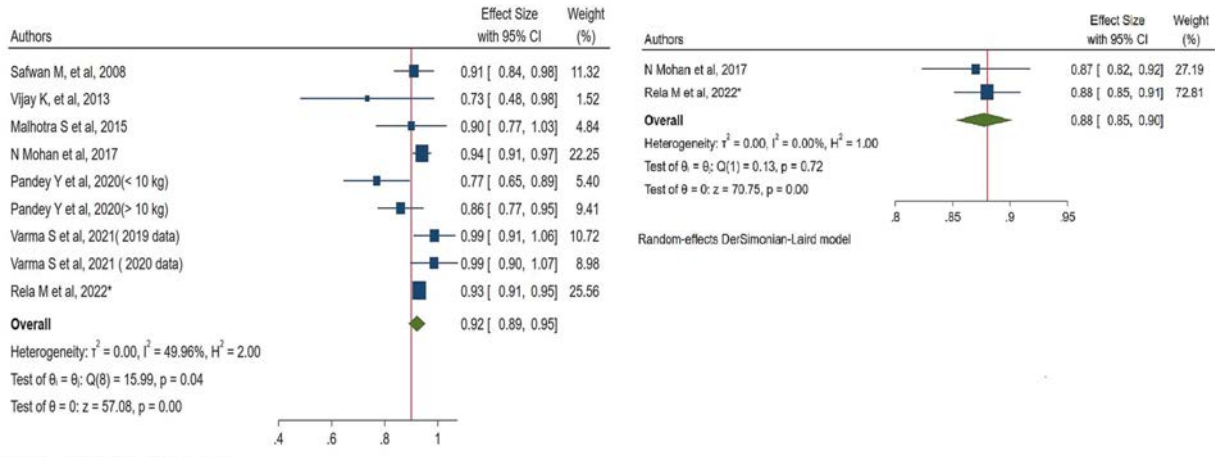
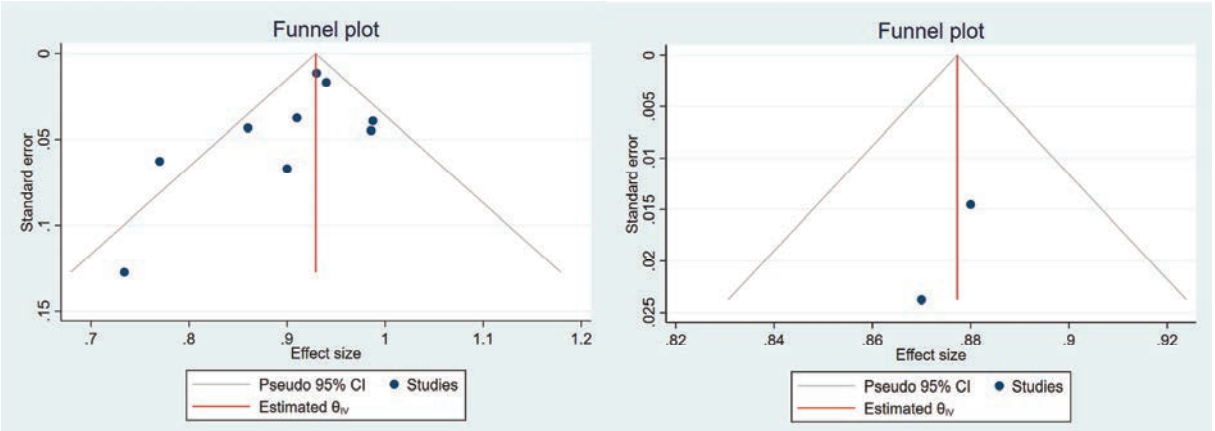


Figure 5. Pediatric Liver Transplant Outcomes in Indian Centers



Forest plots show patient survival after pediatric liver transplant at **(right)** 1 year and **(left)** 5 years.

Figure 6. Publication Bias in the Studies



Publication bias for Pediatric liver studies at 1 year **(right)** and 5 years **(left)**.

patients. As such, if we closely scrutinize the data, exclusion of the individual participation data from 2 institutes (IKDRC-ITS, Ahmedabad; and the Dr. Rela Institute and Medical Centre, Chennai, India) would reduce the transplant numbers by half. Hence, the real-world situation is grossly underrepresented for India. In our analysis, there was a clear preponderance of male patients in almost all of the studies, especially for PKT. This disparity is slightly less for PLT. The reason for this disparity is a complex amalgamation of various factors, which requires deep understanding and integrated efforts for solutions.

In our report, the outcomes of PKT are quite excellent, and this holds true for 5-year and 10-year outcomes as well. On close inspection, the patient and graft survival rates have only improved over the decades. In most cases in India, deaths are caused by infections, which is dissimilar to the experiences reported in the Western world. Malignancy, which is common to the Western world, is exceptionally rare in the Indian scenario. In the 21-year experience of PKT at the All India Institute of Medical Sciences, New Delhi, there were only 4 cases of posttransplant lymphoproliferative disorder reported.[3]

The incidence of rejection in the report is not homogeneous for the studies, and the rates are acceptable and did not result in graft losses. In this systematic review, a concerning observation is the involvement of only a few PKT centers in the data set. This provides a glimpse of the whole story of PKT in India. In a study that highlighted barriers of PKT in a low-resource setting, it was shown that the problem begins at the onset of end-stage kidney disease itself.[4] Another important observation was that the lost to follow-up patients were those who withdrew from care and mostly of low socioeconomic status. They also reported that medical challenges and negative attitudes toward transplant and organ donation are still prevalent.

In our analysis, the median age of transplant is higher, which reflects the late referral and problem in initial care and management. The state of PKT can be grossly understood from the results of a recent survey of 26 adult nephrologists.[5] Only 16 nephrologists (61.5%) were involved in pediatric transplantation. Most of the centers performing PKT were private institutions, with only 3 government sectors undertaking it. There were only a few centers in the country routinely doing >5 pediatric transplants per year. Also, preemptive transplants and protocol biopsies were rare. In the survey, however, induction and the immunosuppressant use were quite similar to the Western registry. Thus, the survey identified a prime deficiency present in PKT. Still, there is ongoing progress in PKT. In the world literature, the first report of a patient with anti-complement factor H antibody-associated hemolytic uremic syndrome who underwent living-related PKT from an Indian center[6] was characterized with a thrombosed inferior vena cava, so splenic vein was used.[7] Stem cell therapy in PKT was reported as early as 2002 by a tertiary transplant center in Western India.[8] An Indian center has reported good outcomes in robotic PKT.[9] The first case series of immuno-adsorption in a case of ABO-incompatible PKT was reported from an Indian center.[10]

Liver transplantation in India has definitely shown an exponential growth in past decades, but only 10% of these transplants comprised PLT.[11] The first PLT was performed at the Institute of Liver and

Biliary Sciences (New Delhi, India) in 2001. The youngest 6-month-old[12,13] with extrahepatic biliary atresia was performed in 2009, with 2 at the Institute of Liver and Biliary Sciences. In our analysis, the survival rates in most of the reports are above 90% for the first year, which is a remarkable achievement with respect to the fact that there are resource limitations, as well as other psychosocial and financial issues in the targeted population. In our report, biliary atresia remains the major indication for PLT. However, with advancement, the centers are including more patients with liver-based metabolic disorders and other difficult-to-treat conditions. In a similar trend, the Dr. Rela Institute and Medical Centre has recently reported successful PLT for nitisinone tyrosinemia type 1 among 9 of their 203 PLT cases in the year 2021.[14] There are reports of PLT in cases that were considered unsuitable for transplant, such as nonstandard hepatic venous reconstruction (NHVR) and hepatopulmonary syndrome (HPS), and these patients are now being successfully treated with innovations and dedicated team efforts. In 2021, PLT for NHVR was reported by an Indian PLT center.[15] This was a successful case series of 15 cases among the 304 PLT procedures performed at the center. No differences in patient and graft survival rates were reported compared with non-NHVR cases. Chaubal and colleagues reported PLT in a patient who weighed less than 6 kg in an Indian experience.[16] The ABO-incompatible barrier was once considered to be an important hurdle in this field but is now successfully tackled. Mohan and colleagues[17] reported 8 ABO-incompatible PLT among the 203 PLT performed at the Medanta, Gurugram, center for liver transplantation. Living donor or split-liver transplants are safe with short-term or long-term outcomes per the data from the United Network for Organ Sharing.[18] Also, the first case in the world literature of monosegment robotic hepatectomy for a child less than 6 kg body weight with extrahepatic biliary atresia was reported from the Dr. Rela Institute and Medical Centre.[19] Hepatopulmonary syndrome is highly associated with morbidity and mortality, and 25 PLT recipients with HPS were reported by the Dr. Rela Institute and Medical Centre in cooperation with a liver transplant center in the United Kingdom.[20] The recent report from the Organ Procurement and Transplantation Network registry also showed similar outcomes with HPS.[21] Infectious encounters in the field of pediatric SOT require special attention, because otherwise rare entities are relatively common in this part of the world. For example, the first case of fascioliasis in a PLT recipient was reported from an Indian center.[22] Another exceptional example is a case of varicella infection in an immunized PLT recipient, also reported from an Indian center.[23] The report of uncommon presentation in PLT, such as recurrent diaphragmatic hernia, from an Indian center highlights that there is unparalleled scope for further research and expansion.

Solid-organ transplants with organs like lung and heart are performed in low numbers of patients in India, because a robust deceased donor program is not present.[24] The first lung transplant unit in India reported that deceased donor organ donation is a viable option in patients; if patients are referred in early phase of illness, the outcome of transplant is better even in low-resource settings like India.[25] Similarly, the MGM Hospital, Chennai, has reported outcomes of 257 HT procedures and proved that successful midterm outcome is possible even with resource restrictions typical in India.[26] There are only a few published studies of pediatric heart transplant, with 20 done in Mumbai[27] and 97 one in Chennai,[28] India.

In brief, the initiation of even a small pediatric transplant program would require a multidisciplinary action and involvement of a team of experts, as well as long-term care services after transplant, in view of financial burden in the lower middle-income nations like India. In this part of the world, an important goal is the establishment of a pediatric transplant program that would be available, affordable, acceptable, and sustainable.

There are some limitations in this report. The most important limitation is the possibility of exclusion of some important studies or data that may have not been discovered in our searches (for example, if such reports were vague with regard to the adult vs pediatric nature of a transplant program). Also, many reports are published without peer review or in publications that are not included in the standard research indexes. We have excluded conference papers and studies for which only an abstract has been published; also, because the data about SOT from India are not robust compared with the Western world, the chances are low for a study to published as a complete article in an indexed journal. Only 2 individual center data analyses were done. Addition of other data from other centers could improve the cumulative analysis.

Conclusions

In summary, we have shown that persistence is required to maintain an active and successful pediatric SOT program with transparent and updated pediatric registry. In our report, although there are fewer studies, the medical outcomes reported are comparable to the Western world, which is an encouraging sign, considering the limited manpower, logistics, and infrastructure. Any successful SOT program for the pediatric population should ensure the availability of inexpensive immunosuppressive drugs, establish additional transplant centers, whether partially or completely dedicated to pediatric patients, and include a robust deceased donor program. We emphasize that the efforts of medical professionals would not be sufficient for success, and involvement of both state and national government officials, as well as other nongovernmental agencies, would be required to establish a successful national-level pediatric SOT program.

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Controlled Donation After Circulatory Death Using Normothermic Regional Perfusion Does Not Increase Graft Fibrosis in the First Year Posttransplant Surveillance Biopsy

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Abstract

Objectives: **The number of kidney transplants obtained from controlled donations after circulatory death is increasing, with long-term outcomes similar to those obtained with donations after brain death. Extraction using normothermic regional perfusion can improve results with controlled donors after circulatory death; however, information on the histological impact and extraction procedure is scarce.**

Materials and Methods: **We retrospectively investigated all kidney transplants performed from October 2014 to December 2019, in which a follow-up kidney biopsy had been performed at 1-year follow-up, comparing controlled procedures with donors after circulatory death and normothermic regional perfusion versus donors after brain death. Interstitial fibrosis/tubular atrophy was assessed by adding the values of interstitial fibrosis and tubular atrophy, according to the Banff classification of renal allograft pathology.**

Results: **When we compared histological data from 66 transplants with donations after brain death versus 24 transplants with donations after circulatory death and normothermic regional perfusion, no differences were found in the degree of fibrosis in the 1-year follow-up biopsy (1.7 ± 1.3 vs 1.7 ± 1.1; *P* = .971) or in the ratio of patients with increased fibrosis calculated as interstitial fibrosis/tubular atrophy >2 (18% vs 13%; *P* = .522). In our multivariate analysis, which included acute rejection, expanded criteria donation, and the type of donation, no variable was independently related to an increased risk of interstitial fibrosis/tubular atrophy >2.**

Conclusions: **The outcomes of kidney grafts procured in our center using controlled procedures with donors after circulatory death and normothermic regional perfusion were indistinguishable from those obtained from donors after brain death, showing the same degree of fibrosis in the 1-year posttransplant surveillance biopsy. Our data support the conclusion that normothermic regional perfusion should be the method of choice for extraction in donors after circulatory death.**

Key words: *Delayed graft function, Extended criterial donor, Interstitial fibrosis/tubular atrophy*

Introduction

With the persistently high number of patients on wait lists for solid-organ transplants, organs from donors who were previously considered unsuitable for transplant are now used. These organs include those from donors after circulatory death (DCDs), including controlled donors after circulatory death (cDCDs) and uncontrolled donors after circulatory death (uDCDs).[1] The use of DCDs has made it possible to increase the number of available organs and thus limit the number of patients on kidney transplant wait lists. Recently, in several countries such as the United Kingdom, the Netherlands, and Spain, the number of kidney transplants performed from DCDs has increased considerably.[2,3]

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Acknowledgements: *This work was sponsored by the Ministry of Science and Innovation, the Carlos III Health Institute and the European Regional Development Fund Research Network for Kidney Diseases ("RedInRen" RD16/0009/0027), and Networks for Cooperative Research Health Results ("RICORS2040" RD21/0005/0010). The authors have no declarations of potential conflicts of interest.*

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Experimental and Clinical Transplantation (2022) 12: 1069-1075

DOI: 10.6002/ect.2022.0171

The ischemia time with use of organs from DCDs has raised concerns that the results obtained with kidneys from DCDs are significantly worse than results with kidneys from donors after brain death (DBDs). However, although the frequency of primary nonfunction and delayed graft function (DGF) is higher in DCDs (especially in uDCDs), long-term renal graft survival rates are comparable to rates for DBDs.[4,5] To limit the ischemic damage, improve graft results, and increase the number of usable abdominal organs, the use of normothermic regional perfusion (NRP) has been advocated instead of super-rapid extraction, not only for uDCDs but also for cDCDs. Normothermic regional perfusion allows "in situ" perfusion of abdominal organs with oxygenated blood, restoring the energy substrates of the cells and hence limiting ischemic damage. The use of NRP also allows the organ extraction procedure to be performed less urgently, reducing surgical injuries of the kidney that commonly occur in rapid laparotomy.[6] In addition, the use of NRP does not hinder combined retrieval of abdominal and thoracic organs, allowing for maximum utilization of donor organs.[7,8]

In a preliminary noncomparative study, the use of NRP increased the number of organs transplanted effectively in our center, with excellent survival rates.[9] A recent Spanish multicenter study involving 770 kidney transplants from DCDs confirmed that the use of NRP reduced the risk of DGF and improved graft survival at 1 year posttransplant compared with super-rapid extraction.[10]

Apart from short- and long-term data on function and survival of renal grafts from DCDs, information is scarce on the histological changes that develop in these grafts. The degree of fibrosis and inflammation in the first year posttransplant can provide relevant prognostic information on the subsequent evolution of kidney grafts.[11,12] Therefore, an analysis of how DCDs affect the development of fibrosis in the graft is of the utmost interest to better understand its subsequent evolution. In addition, this histological information may allow identification of subgroups of patients with different evolution of fibrosis.

To our knowledge, there are no studies on the effects of ischemia on the degree of long-term renal fibrosis in DCDs treated with NRP. A recent US study of cDCDs using super-rapid extraction showed that this practice increases the risk of interstitial fibrosis and tubular atrophy (IFTA) in the first year posttransplant compared with results shown with DBDs.[13] A previous French study found increased progression of IFTA at 1 year posttransplant in renal grafts obtained from uDCDs.[14] Our hypothesis is that the degree of chronic damage at the first year posttransplant in renal grafts from cDCDs that used NRP, an emerging technique that offers good results in terms of renal graft survival in cDCDs, would be comparable to that shown in grafts from DBDs.

Materials and Methods

All transplants performed in our center from October 2014 to December 2019, in which a follow-up biopsy had been performed in the first year posttransplant and an adequate sample had been obtained for histological study, were considered candidates for inclusion in this study. Living-donor transplants, uDCD transplants, cDCD transplants that used the super-rapid technique, and hypersensitized recipients were excluded. Since 2012, our center has routinely performed surveillance biopsies at 1 year after transplant for all kidney transplant recipients who agree to the procedure. This study was conducted following the guidelines of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Cantabria (2020.388).

Renal graft biopsies were reviewed and classified according to the Banff classification system by 2 expert pathologists.[15] We assessed IFTA by adding the ci + ct values, which ranged between 0 and 6. Patients with irreversible brain injury or heart disease, pulmonary involvement, or terminal neurodegenerative disease in which the treatment team had made the decision to withdraw life-sustaining measures were considered as potential cDCDs. Functional warm ischemia time was defined as the time from systolic blood pressure <60 mm Hg to the onset of NRP (including a 5-min standoff period). To accept the kidney grafts, the maximum period of functional warm ischemia was 60 minutes.

To perform the NRP, a Maquet Rotaflow extracorporeal membranous oxygenation system was used. After specific informed consent was obtained from the legal representatives of the potential donors to perform premortem interventions, 400 to 500 U/kg of heparin were administered and the femoral vessels were cannulated before treatment withdrawal using the Seldinger technique in the intensive care unit. To avoid cerebral and coronary perfusion during NRP, an endoaortic balloon occlusion was placed into the

groin. The target flows to be maintained during abdominal NRP were between 2 and 2.4 L/min. In addition, a pressure of 60 to 65 mm Hg in the femoral arterial cannula, a temperature of 37 °C, and a supply of bicarbonate to keep the pH between 7.35 and 7.45 were maintained.

At the judgment of the attending transplant team, a perfusion machine was used as a method of preservation to limit the damage associated with cold ischemia in those kidneys in which time was expected to be greater than 12 hours.

Immunosuppressive therapy for recipients in our center consisted of tacrolimus, mycophenolate mofetil, and prednisone. Until July 2016, all cDCD recipients received thymoglobulin induction treatment with delayed introduction of tacrolimus, and from August 2016 induction therapy was changed to basiliximab with immediate tacrolimus initiation. Demographic and clinical variables related to the recipient, donor, and transplant process were collected retrospectively from the prospectively maintained renal transplant database of the Nephrology Department. Delayed graft function was defined as the need for at least 1 dialysis treatment during the first week after kidney transplant. Renal function was recorded at day 10 and at month 1, 3, and 12 posttransplant; glomerular filtration rate at the first year was estimated by using the CKD-EPI equation.

Continuous variables are presented as means ± SD and compared by *t* test, and qualitative variables are expressed as percentages and analyzed by chi-square test. We compared patient and graft survival rates using Kaplan-Meier analysis. We analyzed the relationship between IFTA and continuous variables using Pearson correlation and logistic regression. Statistical analysis was performed using SPSS.

Results

Between October 2014 and December 2019, 246 renal transplants were performed in our center. Of the total number of nonhypersensitized DBD transplants (n = 125), 68 kidney biopsies were performed 1 year later (54.4%), with inadequate biopsy samples retrieved for 2 of them. Of the total number of nonhypersensitized asystole cDCD kidney transplants obtained with NRP (cDCD/NRP; n = 49), 24 recipients (49.0%) underwent renal biopsy 1 year later. We found no differences in 1-year patient survival (95.8% vs 95.6%; $P = .980$) and 1-year graft survival (94.3% vs 91.8%; $P = .903$) between the grafts obtained from DBDs and cDCDs/NRP.

The median follow-up time was 3.7 ± 1.4 years. Clinical variables and patient-related variables included in the analysis are listed in Table 1. In the cDCD/NRP group, there were fewer donors who died from stroke, donor creatinine levels were lower, and induction treatment and machine perfusion were used more frequently; no significant differences were shown with remaining variables.

Table 1. Variables of Renal Transplants From Donors After Brain Death and Controlled Donors After Circulatory Death

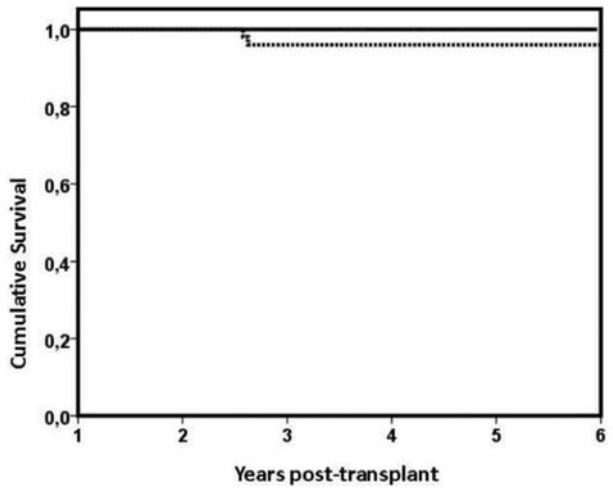
	DBD (n = 66)	cDCD (n = 24)	*P*
Recipient age, y	53 ± 11	53 ± 13	.979
Male recipient	60.6%	62.5%	.871
Diabetes as cause of ESRD	30.3%	16.7%	.196
Preemptive transplant	18.2%	8.3%	.254
Time on RRT	1.1 ± 1.4	1.5 ± 2.2	.301
Previous transplant	16.7%	25.0%	.372
Virtual PRA	6 ± 17	2 ± 8	.206
Donor age, y	50 ± 14	48 ± 14	.572
Male donor	64.6%	58.3%	.586
Donor terminal creatinine, mg/dL	0.89 ± 0.41	0.70 ± 0.33	.044
Cause of death			
Anoxia	15.2%	41.7%	
CVA	60.6%	33.3%	
Trauma	19.7%	8.3%	.003
Other	4.5%	16.7%	
ECD	33.3%	16.7%	.125
f-WIT, min		14.8 ± 8.3	
CIT, h	16.8 ± 5.8	13.7 ± 8.9	.126
HLA mismatching	4.3 ± 1.1	4.3 ± 1.4	.923
Machine perfusion	3.0%	50.0%	<.001
Induction therapy	54.5%	100.0%	<.001
Thymoglobulin	28.8%	45.8%	.129
DGF	12.1%	12.5%	.961
Creatinine day 10, mg/dL	2.09 ± 1.51	1.87 ± 1.36	.523
Creatinine month 1, mg/dL	1.31 ± 0.51	1.29 ± 0.37	.859
Creatinine month 3, mg/dL	1.32 ± 0.47	1.33 ± 0.33	.880
Creatinine year 1, mg/dL	1.29 ± 0.47	1.32 ± 0.39	.789
GFR year 1, mL/min	62 ± 18	59 ± 18	.521
Albuminuria, mg/g	78 ± 141	98 ± 161	.578
Acute rejection during year 1	21.2%	8.3%	.158
IFTA score	1.7 ± 1.3	1.7 ± 1.1	.971
IFTA >2	18.2%	12.5%	.522

Abbreviations: cDCD, controlled donor after circulatory death; CIT, cold ischemia time; CVA, cerebrovascular accident; DBD, donor after brain death; DGF, delayed graft function; ECD, expanded criteria donors; ESRD, end-stage renal disease; f-WIT, functional warm ischemia time; GFR, glomerular filtration rate; IFTA, interstitial fibrosis/tubular atrophy; PRA, panel reactive antibody; RRT, renal replacement therapy; WIT, warm ischemia time

In relation to the progression of renal graft, we did not observe any differences in DGF rates or creatinine levels throughout the first year between DBD and cDCD/NRP recipients. There were also no significant differences in patient survival (3-year survival 96.0% vs 94.4%; $P = .592$) and graft survival (3-year survival 96.5% vs 100%; $P = .382$) comparing DBD and cDCD/NRP recipients who had undergone

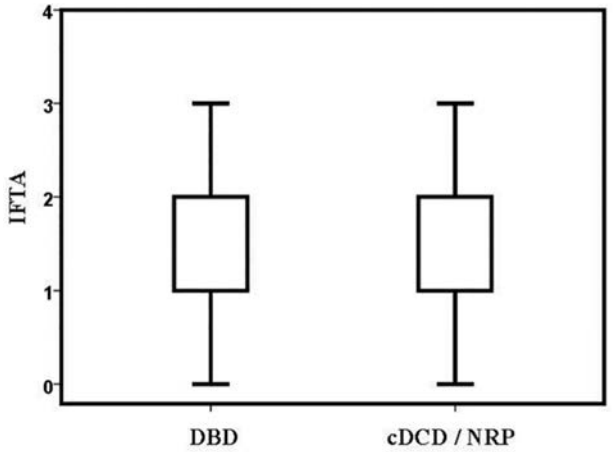
renal biopsy at 1 year included in the analysis (Figure 1). Moreover, no differences were found between groups on the degree of fibrosis in the 1-year follow-up kidney biopsy (Figure 2), nor in the rate of patients with increased fibrosis assessed as IFTA >2 (Table 1).

Figure 1. Renal Graft Survival Censored by Death



Solid line, recipient of controlled donor after circulatory death with normothermic regional perfusion; dashed line, recipient of donor after brain death. Log-rank *P* = .391.

Figure 2. Box Plot With Interstitial Fibrosis/Tubular Atrophy Values Comparing Recipient Groups (*P* = .971)



Abbreviations: cDCD/NRP, controlled donor after circulatory death with normothermic regional perfusion; DBD, donor after brain death; IFTA, interstitial fibrosis/tubular atrophy

Correlations between continuous variables and IFTA are listed in Table 2.

Table 3 shows the variables associated with a high degree of fibrosis in the first year posttransplant biopsy. Receiving a cDCD graft was not associated with increased risk of fibrosis. The donor's final creatinine level approached borderline statistical significance, as well as grafts from donors with expanded criteria, with the development of acute rejection throughout the first year posttransplant. In our multivariate analysis, which included acute rejection (odds ratio [OR] = 3.375; 95% CI, 0.874-13.028; *P* = .078), donor expanded criteria (OR = 3.102; 95% CI, 0.905-10.630; *P* = .072), and type of donation (DBD vs cDCD/NRP) (OR = .966; 95% CI, 0.597-1.563; *P* = .888), there were no independent variables related to higher risk of IFTA >2.

Table 2. Correlation Between Continuous Variables and Interstitial Fibrosis/Tubular Atrophy

	r	*P*
Donor age	0.111	.296
Donor terminal creatinine	0.200	.060
CIT	0.001	.992
HLA mismatching	0.049	.648
No. of dialysis treatments posttransplant	0.175	.098
Creatinine year 1	0.305	.003
GFR year 1	-0.334	.001
Albuminuria year 1	0.163	.127

Abbreviations: CIT, cold ischemia time; GFR, glomerular filtration rate

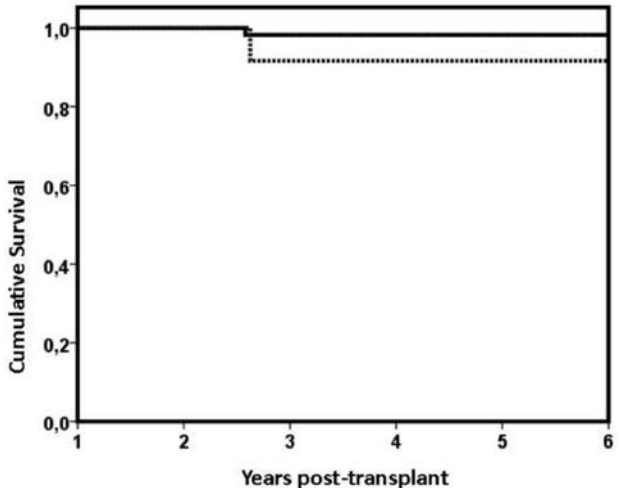
Table 3. Variables Related to Higher Risk of Fibrosis in the First Year Posttransplant (Interstitial Fibrosis/Tubular Atrophy >2).

	IFTA ≤2 (n = 75)	IFTA >2 (n = 15)	*P*
Recipient age, y	53 ± 11	53 ± 14	.922
Male recipient	62.7%	53.3%	.498
Diabetes as cause of ESRD	28.0%	20.0%	.522
Preemptive transplant	13.3%	26.7%	.193
Time on RRT	1.3 ± 1.7	0.7 ± 1.00	.159
Previous transplant	21.3%	6.7%	.185
Virtual PRA	5 ± 15	7 ± 19	.668
Donor age, y	49 ± 13	52 ± 15	.370
Male donor	62.2%	66.7%	.742
cDCD	28.0%	20.0%	.522
Donor terminal creatinine, mg/dL	0.79 ± 0.36	1.06 ± 0.52	.073
CVA as cause of death	52.0%	60.0%	.571
ECD	25.0%	46.7%	.092
CIT, h	16.2 ± 6.9	14.9 ± 6.6	.504
HLA mismatching	4.3 ± 1.2	4.4 ± 1.1	.568
Machine perfusion	16.0%	13.3%	.795
Induction therapy	66.7%	66.7%	1.000
Thymoglobulin	36.0%	20.0%	.230
DGF	10.7%	20.0%	.314
No. of dialysis treatments posttransplant	0.20 ± 0.64	0.53 ± 1.14	.283
Creatinine day 10, mg/dL	1.89 ± 1.34	2.79 ± 1.89	.123
Creatinine month 1, mg/dL	1.25 ± 0.46	1.56 ± 0.48	.028
Creatinine month 3, mg/dL	1.26 ± 0.37	1.68 ± 0.59	.024
Creatinine year 1, mg/dL	1.24 ± 0.41	1.60 ± 0.55	.004
GFR year 1, mL/min	64 ± 18	47 ± 14	.001
Albuminuria, mg/g	72 ± 130	147 ± 211	.218
Acute rejection during year 1	14.7%	33.3%	.084

Abbreviations: cDCD, controlled donor after circulatory death; CIT, cold ischemia time; CVA, cerebrovascular accident; DGF, delayed graft function; ECD, expanded criteria donor; ESRD, end-stage renal disease; GFR, glomerular filtration rate; IFTA, interstitial fibrosis/tubular atrophy; PRA, panel reactive antibody; RRT, renal replacement therapy

Patients with greater degree of fibrosis on follow-up biopsy at 1 year posttransplant showed poorer renal function from the first month throughout the first year posttransplant (Table 3) and slightly worse death-censored graft survival, although this did not reach statistical significance (3-year survival of 98.2% vs 91.7%; $P = .237$) (Figure 3).

Figure 3. Death-censored renal graft survival Comparing Patients With Interstitial Fibrosis/Tubular Atrophy ≤2 or >2



Solid line, interstitial fibrosis/tubular atrophy (IFTA) ≤2; dashed line, IFTA > 2. Log-rank $P = .237$.

Discussion

The cDCD procedure is a type of multiorgan procurement that allows simultaneous extraction of thoracic and abdominal organs with excellent results.[7,8] With experiences gained since the beginning of the asystole donation program in our center in 2014 and the successful results achieved, which are consistent with those found across Spain and other countries, this type of donation has expanded.[3]

No significant differences were found in terms of patient or graft survival when we compared outcomes between grafts from DBDs or cDCDs/NRP. These data support those published by British and Dutch registries.[16,17] The use of NRP has been promoted in our center from the beginning of the program; however, a few renal grafts were obtained by super-rapid extraction and were excluded from our study. Rates of DGF for cDCD/NRP recipients were not significantly higher than those for DBD recipients (12% vs 13%; $P = .961$). However, rates of DGF for cDCD recipients published by other groups have been higher (49% in the British study and 42% in the Dutch) and always significantly higher than rates for DBD recipients.[16,17] Previously published studies have suggested that those centers that used NRP showed a lower DGF rate (18%-40%) than those that used super-rapid extraction (48.5% in the British registry and >60% in the Dutch).[18] A Spanish study that retrospectively compared 865 cDCD/NRP renal transplants versus 1437 transplant with super-rapid extraction revealed that this technique increased the risk of DGF (OR = 1.97; 95% CI, 1.43-2.72; $P < .001$).[10] Compared with previous findings, the progression of renal function from day 10 and throughout the first year posttransplant was indistinguishable between groups (cDCD/NRP vs DBD) in our study (Table 1).

The limited follow-up time period (~3 years) may be the underlying cause as to why no differences were observed in the evolution of renal grafts between cDCD/NRP and DBD groups. Thus, having histological information at 1 year posttransplant may be useful as a substitute marker of later evolution. As referred to in the introduction, the degree of fibrosis in the first year posttransplant biopsy provides information on later renal graft outcome.[11,13] Accordingly, the most important finding of our study was that no significant differences were found in the degree of fibrosis on the follow-up surveillance biopsy in the first year posttransplant between renal grafts from cDCDs/NRP and DBDs. Our multivariate analysis confirmed that cDCD/NRP was not associated with an increased risk of fibrosis in the first year posttransplant. In light of these data and given that there is no higher subclinical fibrosis, we can speculate that cDCD/NRP grafts will have a long-term progression (beyond the 3 years of follow-up period that we recorded) comparable to DBD grafts.

This finding partially contradicts the recently published finding from van der Windt and colleagues.[13] This group at the University of Pittsburgh compared the histological results of follow-up biopsies at 1 year posttransplant between 87 cDCD recipients (with probable super-rapid extraction) and 246 DBD recipients, finding an IFTA significantly higher in the cDCD group (2.42 ± 1.26 vs 1.98 ± 1.19; $P = .004$).[13] However, we suggest that our results are different because of the protective effects of NRP in the kidney, which minimizes ischemic damage and enables a better preservation of the kidney after potential donor death. In fact, the

rate of DGF published by our group was lower than those reported by super-rapid extraction in the scientific literature.[2,9,10]

Furthermore, the association between cDCD and IFTA reported by van der Windt and colleagues[13] was independent of other donor-related variables such as rejection, cold ischemia time, and DGF. Despite the fact that their study is broader than ours, the data cannot be extrapolated to our population, since their cDCD recipients showed higher incidence of DGF (39% vs 19%; $P = .0001$) and worse renal function 1 year posttransplant (40 ± 16 vs 48 ± 19 mL/min; $P = .0005$), whereas, in our cDCD/NRP and DBD recipients, the incidence of DGF and renal function were indistinguishable. The difference in degree of fibrosis between our cDCD/NRP recipients (1.8 ± 1.4) and the cDCD recipients of the Pittsburgh study (2.42 ± 1.26) could be due to many factors, not only related to donor characteristics (slightly younger, but hypertensive in the Pittsburgh study) but also to posttransplant management. Nevertheless, the use of NRP by our team could explain, at least partially, both the better renal function and the lower degree of fibrosis at 1 year after transplant.[13]

Although donor characteristics, mainly age, determine the degree of fibrosis in preimplant or early posttransplant biopsies, fibrosis in the first year posttransplant is determined not only by the degree of prior fibrosis but also by events, primarily inflammatory, for graft experiences throughout the first year.[19] We did not find an independent relationship between fibrosis and donor-related variables such as age, renal function, and expanded criteria in our study. It is possible that a stricter donor selection procedure in our center could explain the lack of relationship between fibrosis and donor characteristics. The relationship between donor age and fibrosis at 1 year posttransplant has been reported in the literature,[14,20,21] although not by all.[22] Later events can influence the development of fibrosis at 1 year posttransplant.

Events occurring throughout the first year posttransplant can also influence subsequent fibrosis. The appearance of DGF has been reported as a risk factor for fibrosis by some authors, but not by others.[20,22,23] Other prior analysis have consistently shown than development of clinical[21,24] or subclinical[20,25,26] rejection during the first year posttransplant is an important factor for fibrosis progression.

Several limitations of our study should be noted. Because most of the organs obtained from cDCDs were extracted by NRP, it was not possible to compare these results with a super-rapid extraction group. The lack of sequential biopsies did not allow us to determine whether the degree of fibrosis of the early damage was related to donor characteristics and whether the NRP extraction method influenced subsequent fibrosis events. Finally, our study population was small, and a larger multicenter study would be needed to confirm these findings.

In conclusion, the posttransplant evolution of renal grafts procured in our center by cDCD/NRP was indistinguishable from those obtained by DBD. The fact that a surrogate marker, the degree of fibrosis in the first year posttransplant, showed the same results when we compared cDCD/NRP versus DBD confirmed that using NRP in cDCDs helps to minimize the damage induced by warm ischemia related to the asystole period. From our data, we can conclude that NRP should be the method of choice for extraction in cDCDs. It would be advisable to conduct a multicenter study that compares the degree of fibrosis in follow-up biopsies between cDCD grafts obtained by NRP and the super-rapid technique.

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Transplant of Kidneys From Hepatitis C Virus-Positive Donors To Hepatitis C Virus-Negative Recipients: A Retrospective Study and Systematic Review

Halinuer Shadekejiang, Jiefu Zhu, Xiongfei Wu

Abstract

Objectives: **Kidneys from hepatitis C virus-positive donors were often discarded due to the lack of an effective treatment for hepatitis C virus. However, the advent of direct-acting antivirals has facilitated great progress for treatment of hepatitis C virus, providing additional opportunities for patients waiting for kidney transplant. We explored the feasibility and safety of kidney transplant from hepatitis C virus-positive donors to hepatitis C virus-negative recipients in combination with direct-acting antiviral therapy.**

Materials and Methods: **This was a single-center retrospective study of 7 recipients of hepatitis C virus-positive kidneys from June 2018 to June 2021. All recipients were treated with sofosbuvir/velpatasvir for 12 weeks after kidney transplant. The primary recipients' outcome was achievement of sustained viral eradication at 12 weeks after treatment, and follow-up secondary outcomes were kidney function recovery, liver function, and adverse drug reactions. We reviewed previous studies, from 2017 to 2022, to analyze achievement of sustained viral eradication at 12 weeks after treatment, recipient and graft survival, and adverse event of kidney transplant from a hepatitis C virus-positive donor to a hepatitis C virus-negative recipient.**

Results: **Median follow-up time was 71 weeks (range, 56-183 weeks). All recipients achieved sustained viral eradication at 12 weeks after treatment, and their kidney function recovered without severe liver damage or adverse drug reactions. Previous studies suggested that transplant of hepatitis C virus-positive donor kidneys is safe and feasible when combined with direct-acting antiviral therapy. However, details regarding optimal duration of treatment and direct-acting antiviral regimen remain undetermined, so prospective randomized studies are warranted.**

Conclusions: **Our study further confirms that kidney transplant from hepatitis C virus-positive donors to hepatitis C virus-negative recipients is safe and feasible with direct-acting antiviral treatment. Grafts from hepatitis C virus-infected donors may be effective to resolve the problem of kidney shortage.**

Key words: *Direct-acting antiviral drugs, Graft shortage, Graft survival, Kidney transplantation, Patient survival*

Introduction

Kidney transplant is the most effective treatment for patients with end-stage renal disease.[1,2] The latest data show that, since 2015, the total number of kidney transplant patients in the United States has been increasing, and there were 24 273 transplants in 2020,[3] which leads to problems such as a shortage of kidney grafts and longer wait times for kidney transplants. Recent data show that, globally, an estimated 58 million people are chronically infected with hepatitis C virus (HCV), and approximately 290 000 people died from HCV in 2019.[4] About 10 million people were infected with HCV in China.[5] If HCV-infected patients could be used as extended criteria kidney donors, these problems should be effectively resolved. However, since the 1990s, numerous studies have shown that organ transplant can lead to the transmission of HCV, and liver disease caused by HCV infection is an important cause of death in kidney transplant recipients.[6-12]

Before 2013, the treatment of HCV infection was based on interferon-α and ribavirin. However, ribavirin is nephrotoxic, so it cannot be used in

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Acknowledgements: *We sincerely thank the patients who participated in this study and the medical staff at the Renmin Hospital of Wuhan University for their contributions to this work. This work was supported in part by National Youth Science Foundation of China (grant No. 82100803), the Natural Science Foundation of Hubei Province (grant No. 2021CFB101), and Fundamental Research Funds for the Central Universities (grant No. 2042021kf0097). Other than described, the authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

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Experimental and Clinical Transplantation (2022) 12: 1076-1084

DOI: 10.6002/ect.2022.0315

patients with glomerular filtration rate less than 50 mL/min.[13] In addition, in kidney transplant patients, interferon-α partly depends on the patient's own immune system and interferon treatment may lead to acute rejection and is not suitable for HCV-infected patients after kidney transplant. Therefore, kidneys from HCV-positive donors were often discarded.[14,15] However, since 2013, direct-acting antivirals (DAA) that directly act on the replication mechanism of the virus have been developed and gradually optimized, which has facilitated significant progress in the treatment of kidney disease complicated with HCV infection. In the THINKER-1 clinical trial (Transplanting Hepatitis C Kidneys into Negative Kidney Recipients),[16,17] 20 HCV-negative recipients received HCV-positive kidneys. Within 5 days after kidney transplant, HCV RNA was detected in all recipients (100%), so a 12-week regimen of elbasvir/grazoprevir was initiated for these patients. Eventually, all treated recipients achieved sustained viral eradication at 12 weeks after treatment (SVR12). Since then, there has been growing interest in kidney transplants from HCV-positive donors to HCV-negative recipients (HCV D+/R−). In this study, we also reviewed previous studies and retrospectively analyzed the safety and feasibility of HCV D+/R− kidney transplant when combine with DAA therapy.

Materials and Methods

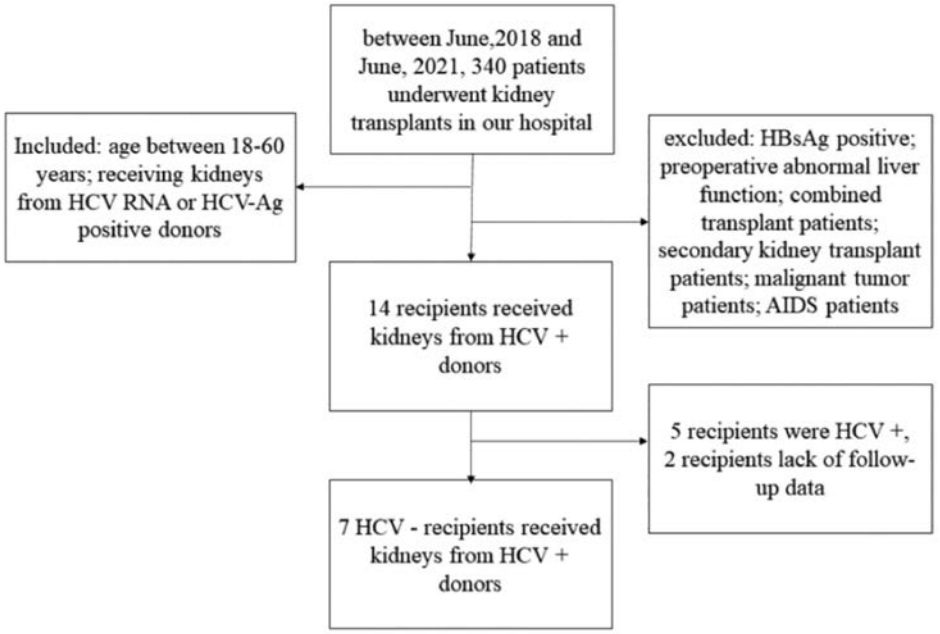
Study population

From June 2018 to June 2021, there were 340 people who underwent kidney transplant at the Renmin Hospital of Wuhan University. Among them, recipients aged 18 to 60 years who had received HCV-positive donor kidneys were included in our study, and we excluded patients who met the exclusion criteria (exclusion criteria are shown in Figure 1). We defined HCV-positive status as positive for HCV core antigen (HCV-Ag) or detection of HCV RNA above the minimum detection limit.[14] Fourteen recipients (4.11%) received HCV-positive donor kidneys. Seven of these 14 patients were excluded, 5 of whom showed HCV-positive test results and 2 others lacked follow-up data. A total of 7 HCV-negative recipients who received HCV-positive kidneys were eventually included in our study (Figure 1). All patients provided written informed consent for kidney transplant. This study was approved by the Ethics Committee of the Renmin Hospital of Wuhan University (No. WDRY2021-KS059).

Posttransplant treatment

All recipients received antithymocyte globulin or basiliximab/methylprednisolone immunosuppression induction after surgery and were started on a

Figure 1. Flow Chart of Patient Selection



Abbreviations: HBsAg, hepatitis B surface antigen; HCV-Ag, core antigen for hepatitis C virus; HCV+, positive test for hepatitis C virus; HCV-, negative test for hepatitis C virus

triple immunosuppressive regimen consisting of tacrolimus/cyclosporine, mycophenolate mofetil, and prednisone. Dosages of each medication were performed according to drug instructions and clinical guidelines. We chose a pan-genotypic DAA regimen, and all recipients started antiviral treatment 1 week after transplant. The sofosbuvir/velpatasvir regimen (fixed-dose combination of sofosbuvir 400 mg and velpatasvir 100 mg) was given orally once each day, 1 tablet at a time.

Outcomes

We recorded baseline clinical data, including pretransplant and posttransplant kidney and liver functions (including serum creatinine [SCr], urea, alanine aminotransferase [ALT], and aspartate aminotransferase [AST]), hepatitis virus-related testing, and blood testing.

The primary outcome was achievement of SVR12. The levels of HCV antibody (anti-HCV), HCV-Ag, and HCV RNA were recorded at 4 weeks, 8 weeks, and 12 weeks after transplant; 12 weeks after DAA treatment; and at the end of the follow-up period.

Secondary outcomes were kidney function recovery, liver function, and adverse drug reactions (including clinical symptoms such as headache, fatigue, nausea, and vomiting) in recipients during follow-up. Kidney and liver functions were assessed by SCr, ALT, and AST at 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 8 weeks, and 12 weeks after transplant and at each visit during follow-up.

Statistical analyses

Statistical analyses were performed with the SPSS (version 25.0, IBM) and Prism (version 8.0, GraphPad) software packages. Results are expressed as mean values ± SD for normally distributed variables or as median values (with IQR) for nonnormally distributed variables, depending on data type, and count data are expressed as number of patients (with percent).

Results

Patient characteristics

The 7 recipients (5 male, 2 female) had an average age of 42.43 ± 13.16 years (range, 19-57 years). They were negative for HCV and hepatitis B virus, and their liver function was normal before the kidney transplant. The primary diseases related to renal failure included chronic glomerulonephritis (42.8%), immunoglobulin A nephropathy (28.6%), lupus nephritis (14.3%), and diabetic nephropathy (14.3%). Six recipients had received hemodialysis prior to kidney transplant, and their median duration of dialysis was 22 months (IQR 3.3, 76.0). Seven recipients received kidneys from 6 donors after circulatory death. Six donors were male, with an average age of 47.67 ± 10.17 years (range, 31-55 years). The pretransplant liver and kidney functions of the donors are shown in Table 1.

Table 1. Characteristics of Recipients and Donors

Baseline Characteristic	Value
Recipients	
Age, mean ± SD, y	42.43 ± 13.16
Male sex, No. (%)	5 (71.4%)
BMI, mean ± SD	21.28 ± 3.83
Blood group, No. (%)	
O	2 (28.6%)
A	3 (42.8%)
B	2 (28.6%)
AB	0 (0)
PRA class I, mean ± SD, %	6.2 ± 1.9
PRA class II, mean ± SD, %	6.0 ± 1.5
Comorbidity, No. (%)	
Hypertension	5 (71.4%)
Diabetes	2 (28.6%)
Autoimmune disease	3 (42.8%)
Cause of kidney failure, No. (%)	
Chronic glomerulonephritis	3 (42.8%)
Diabetic nephropathy	1 (14.3%)
IgA nephropathy	2 (28.6%)
Lupus nephritis	1 (14.3%)
Dialysis pretransplant, No. (%)	
Hemodialysis	6 (85.7%)
Peritoneal dialysis	0 (0)
No dialysis	1 (14.3%)
Duration of dialysis, median (IQR), mo	22 (3.3, 76.0)
Pretransplant ALT, median (IQR), U/L	14.5 (11.0, 19.0)
Pretransplant AST, median (IQR), U/L	12 (10.0, 18.0)
Donors	
Age, mean ± SD, y	47.67 ± 10.17
Male sex, No. (%)	6 (100%)
DCD, No. (%)	6 (100%)
Pretransplant SCr, mean ± SD, μmol/L	89.67 ± 57.17
Pretransplant ALT, median (IQR), U/L	26.4 (22.7, 69.0)
Pretransplant AST, median (IQR), U/L	67.0 (32.5, 97.3)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DCD, donation after circulatory death; IgA, immunoglobulin A; PRA, panel reactive antibody; SCr, serum creatinine

Hepatitis C virus infection

The 7 recipients had a median of 71 weeks (range, 56-183 weeks) of posttransplant follow-up. Anti-HCV positive was detected in 3 recipients (42.86%) at 4 weeks, 4 recipients (57.14%) at 12 weeks, and 6 recipients (85.71%) at the end of the follow-up period, after DAA treatment. The presence of HCV RNA was undetected (ie, below limits of quantification) in all recipients until the end of

follow-up, and there were no DAA-related adverse drug reactions during the follow-up period.

Posttransplant kidney function

The renal function of all recipients is shown in Figure 2. Renal function recovered well in all but 1 recipient. At 12 weeks after kidney transplant, the mean SCr of the 7 recipients was 153.14 ± 46.10 μmol/L. One recipient developed delayed graft function (DGF, defined as at least 1 hemodialysis treatment within week 1 after surgery or SCr greater than 400 μmol/L on the postoperative day 7 without dialysis),[18,19] and SCr was 824 μmol/L on day 7 after transplant. Then, his kidney function gradually recovered, and SCr decreased to 139 μmol/L at 9 months after kidney transplant.

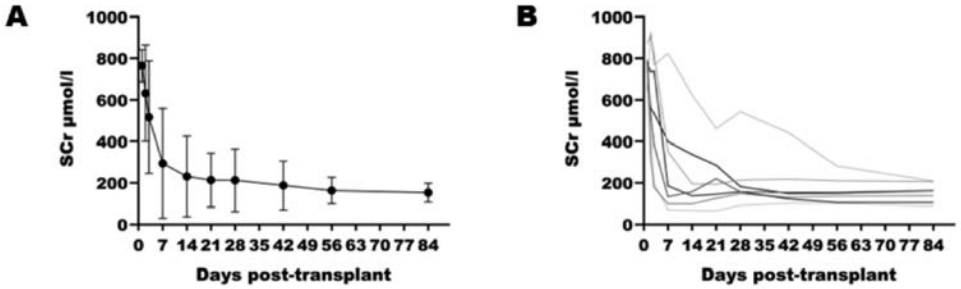
Posttransplant liver function

During the follow-up period, none of the recipients had any acute hepatitis clinical symptoms such as jaundice, fatigue, anorexia, nausea, and vomiting. At 12 weeks after kidney transplant, the 7 recipients showed the median ALT was 16 U/L (IQR 9, 22) and median AST was 17 U/L (IQR 13, 25). Two recipients had a transient elevation of ALT at 2 weeks and 8 weeks after transplant (peak 95 U/L, at week 2 after surgery), and ALT returned to the reference range 1 week later, with the AST levels within the reference range. Liver function results of all recipients within 12 weeks after kidney transplant are shown in Figure 3.

Posttransplant infection

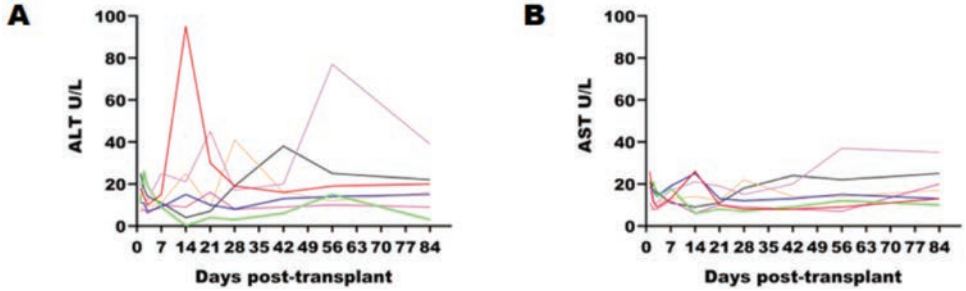
One recipient (the same patient who developed DGF) presented with clinical symptoms such as fever and anuria in week 2 after kidney transplant, and the condition improved after anti-infective treatment. The blood culture results suggested *Candida albicans* infection. Cytomegalovirus DNA was detected at 3060 copies/mL (low detection limit of 500 copies/mL) at week 8 after kidney transplant in 1 recipient, which improved after antiviral treatment. Also, BK virus DNA (low detection limit of 5000 copies/mL) and Epstein-Barr virus DNA (low detection limit of 400 copies/mL) were not detected. For the other 6 recipients, BK virus DNA, EB virus DNA, and cytomegalovirus DNA were not detected.

Figure 2. Posttransplant Kidney Function Among Recipients



Abbreviations: SCr, serum creatinine
(A) mean SCr level of recipients within 12 weeks after kidney transplant. **(B)** SCr levels of recipients within 12 weeks after kidney transplant.

Figure 3. Posttransplant Liver Function Among Recipients



Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase
Posttransplant **(A)** ALT levels and **(B)** AST levels among recipients.

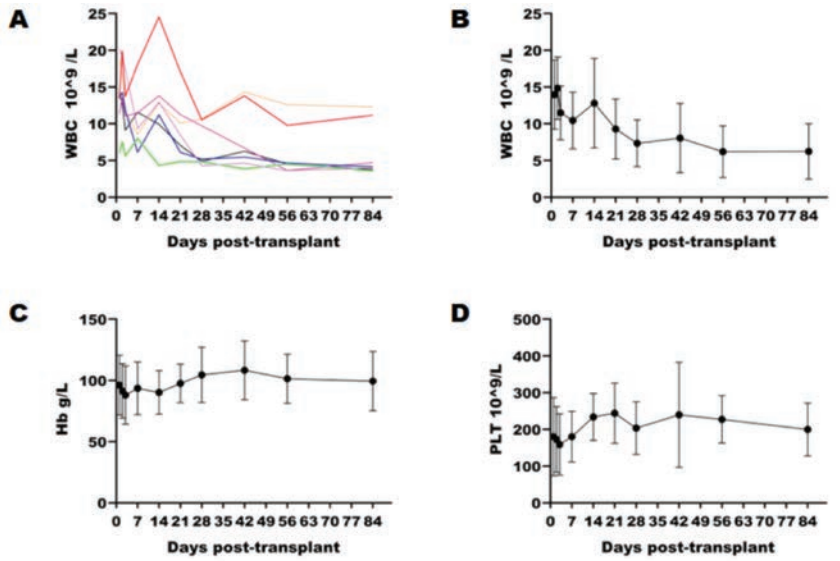
Posttransplant blood test

During the follow-up period, the white blood cell (WBC) counts of recipients gradually decreased and eventually stabilized. Five of the 7 patients (71.43%) experienced leukopenia during the follow-up period (detected at least once), the lowest WBC count was 2.65×10^9 cells/L, which appeared 4 months after transplant (leukopenia defined as WBC count $<4.0 \times 10^9$ cells/L). Hemoglobin and platelet levels of all recipients tended to be stable. Recipient blood cell results within 12 weeks after kidney transplant are shown in Figure 4.

Systemic review on hepatitis C-positive donors

To further evaluate the safety and feasibility of HCV D+/R− kidney transplant, we performed a systematic review of studies published from 2017 to 2022, as identified in the databases of PubMed, Embase, and the Cochrane Library. We collected a total of 14 clinical series, and these are listed in Table 2 in chronological order of the year of publication (comprises 8 pros-pective studies and 6 retrospective studies).[16,17,20-31]

Figure 4. Posttransplant Blood Cells Among Recipients



Abbreviations: Hb, hemoglobin; PLT, platelets; WBC, white blood cells
(A) WBC trends after transplant in 7 recipients. Mean values of **(B)** WBC, **(C)** Hb, and **(D)** PLT within 12 weeks after kidney transplant in 7 recipients.

Table 2. Some Studies Related to Kidney Transplant from Hepatitis C Virus-Positive Donors to Hepatitis C Virus-Negative Recipients (With Direct-Acting Antiviral Treatment)

Study Year and Country	Ref	Design	D+/R- (D-/R-)	DAA Regimen	DAA Start Time	Duration	Follow-up Time	SVR12 Achieved	DGF	AR	Death	Graft Survival	Hepatic Events
2017 USA	Goldberg[16]	PS	10 (0)	EBR/GZR (n = 10)	HCV RNA detected	12 wk	12 wk	10/10	1/10	0/10	0/10	10/10	2/10
2018 USA[a]	Reese[17]	PS	20 (0)	EBR/GZR (n = 17), EBR/GZR + RIB (n = 3)	HCV RNA detected	12 wk (n = 17), 16 wk (n = 3)	12 mo	20/20	5/20	0/20	0/20	20/20	5/20
2018 USA	Durand[20]	PS	10 (0)	EBR/GZR (n = 7), EBR/GZR + SOF (n = 3)[b]	Day of transplant	12 wk	24 wk	10/10	4/10	0/10	0/10	10/10	1/10
2019 USA	Kapila[21]	RA	64 (0)	GLE/PIB (n = 33), SOF/LDV (n = 24), SOF/VEL (n = 1), no DAA (n = 4)[c]	Median 72 d (range, 9-198 d)	12 wk	≤55 wk	44/45d	1/64	NR	1/64	NR	2/64

Table 2 (Continued). Some Studies Related to Kidney Transplant from Hepatitis C Virus-Positive Donors to Hepatitis C Virus-Negative Recipients (With Direct-Acting Antiviral Treatment)

Study Year and Country	Ref	Design	D+/R- (D-/R-)	DAA Regimen	DAA Start Time	Duration	Follow-up Time	SVR12 Achieved	DGF	AR	Death	Graft Survival	Hepatic Events
2019 USA	Molnar[22]	RA	53 (0)	GLE/PIB (n = 47) SOF/VEL (n = 5) SOF/LDV (n = 1)	Median 76 d (IQR 66, 88)	12 wk (n = 48), 16 wk (n = 5)	3-6 mo	53/53	3/53	4/53	0/53	53/53	1/53
2020 USA	Gupta[23]	PS	50 (0)	SOF/VEL (n = 50)	6 h before transplant and at POD 1 (n = 10), 6 h before transplant and daily for 3 d after transplant (n = 40)	12 wk (n = 6)[e]	8 mo	47/49[f]	24/50	2/50	1/50	49/50	0/50
2020 Canada	Feld[24]	PS	10 (0)	GLE/PIB + ezetimibe	6 h before transplant	8 d	16 wk	10/10	NR	NR	0/10	10/10	0/10
2020 USA	Graham[25]	RA	30 (30)	GLE/PIB (n = 29) SOF/VEL (n = 1)	Median 9[d] (range, 5-41 d)	90 d	6 mo	30/30	8/30	2/30	0/30	30/30	0/30
2020 USA	Sise[26]	RA	8 (0)	EBR/GZR (n = 8)	Day of transplant	12 wk	6 mo	8/8	3/8	0/8	0/8	7/8	0/8
2020	Sise[27]	PS	30 (0)	GLE/PIB (n = 30)	POD 2-5	8 wk	Median 35.6 wk (range, 24.6-49.7 wk)USA	30/30	7/30	3/30	1/30	30/30	0/30
2021 USA	Durand[28]	PS	10 (0)	GLE/PIB (n = 10)	Before organ perfusion	4 wk	12 mo	10/10	1/10	0/10	0/10	9/10	0/10
2021 USA	Terrault[29]	PS	11 (7)	SOF/VEL (n = 10)[g]	Median 16.5 d (IQR 9.8, 24.5 d)	12 wk	36 wk	11/11	2/10	0/10	0/11	11/11	2/10
2021 USA	Torabi[30]	RA	52 (0)	GLE/PIB (n = 48) SOF/VEL (n = 3) SOF/VEL + VOX (n = 1)	Median 11 d (IQR 7, 17 d)	12 wk	12 mo	39/39h	13/52	3/52	0/52	50/52	NR
2021 USA	Molnar[31]	RA	65 (59)	GLE/PIB (n = 59) SOF/VEL (n = 5) SOF/LDV (n = 1)	Median 76 d (IQR 68, 88 d)	12 wk	Median 390 d (IQR 367, 408 d)	65/65	5/65	3/65	1/65	65/65	1/65

Abbreviations: AR, acute rejection; DAA, direct–acting antiviral; DGF, delayed graft function; D+/R-, kidney transplant from HCV-positive donor to HCV-negative recipient; D-/R-, kidney transplant from HCV-negative donor to HCV-negative recipient; EBR/GZR, elbasvir and grazoprevir; GLE/PIB, glecaprevir and pibrentasvir; HCV, hepatitis C virus; LDV, ledipasvir; NR, not reported; POD, postoperative day; PS, prospective; RA, retrospective; RIB, ribavirin; SOF/VEL, sofosbuvir and velpatasvir; SVR12, sustained virologic response at 12 weeks after treatment; VOX, voxilaprevir

[a]Continuation of the THINKER trial by Reese and colleagues. [b]Addition of SOF for genotype 2 and 3 recipients. [c]Four recipients did not receive DAA treatment, including 3 patients who were persistently negative for HCV RNA and 1 patient who died. [d]The remaining recipients remain in treatment or had not reached 12 weeks of treatment by the time of this publication. [e]Six recipients with HCV transmission continued treatment for 12 weeks (EBR/GZR or SOF/VEL or other DAA regimens). [f]Forty-three recipients received only preventive treatment and were considered to have achieved SVR12 (not specified); 4 recipients of HCV-infected patients who received DAA treatment achieved SVR12, and 1 recipient had not yet reached the 12-week follow-up. [g]One recipient was consistently negative for HCV RNA and did not receive DAA treatment. [h]The remaining 13 recipients had no detectable HCV RNA, but they did not reach the 12-week follow-up.

Discussion

In our center, 4.11% of recipients received kidneys from HCV-positive donors, indicating that HCV-positive donors account for a certain percentage that reduced the wait times for kidney transplant candidates. Were these events to have occurred before the advent of DAA, these kidneys would have been discarded.

In this single-center study, we had a median follow-up time of 71 weeks, and the longest follow-up was 3.5 years. No HCV RNA was detected in any recipient up to 12 weeks after treatment, all recipients achieved SVR12, and none developed chronic viral

hepatitis C during the follow-up period. All recipients tested negative for anti-HCV before transplant, and 6 recipients were positive for anti-HCV during the follow-up period, which indicates that recipients had been infected with HCV but the virus had been eliminated.[32] None of the 7 recipients had any DAA-related adverse drug reaction, which is consistent with the results of many studies mentioned in Table 2.

Direct-acting antiviral regimen

In our study, all recipients were treated with sofosbuvir/velpatasvir. In the study by Terrault and colleagues,[29] 10 recipients of HCV-positive kidneys were given the sofosbuvir/velpatasvir regimen, and all of them achieved SVR12. This result was consistent with our study. In the research published by Goldberg, Reese, Durand, and colleagues,[16,17,20] they used elbasvir/grazoprevir. In addition, most of the other studies used glecaprevir/pibrentasvir. The elbasvir/grazoprevir regimen is recommended for recipients of HCV genotypes 1, 1b, and 4, according to clinical guidelines (evidence grading A). Because glecaprevir/pibrentasvir is not eliminated through the kidneys, it is suitable for patients at any stage of kidney disease.[33] However, drug-drug interactions must be considered when using DAA, especially in posttransplant patients.

The elbasvir/grazoprevir regimen is known to affect the concentration of some drugs such as cyclosporin, statins, enalapril, rifampin, digoxin, some angiotensin-receptor blockers, and phenytoin.[34] Therefore, combined use of elbasvir/grazoprevir with such drugs is generally not recommended. Elbasvir/grazoprevir treatment increases levels of tacrolimus; thus, levels need to be closely monitored so that the drug dosage can be modified, if needed. Glecaprevir/pibrentasvir also interacts with calcineurin inhibitors, so the levels of calcineurin inhibitors need to be monitored and adjusted as needed during treatment and after the end of treatment.

Sofosbuvir, a polymerase inhibitor, is the basis of several DAA regimens. Sofosbuvir is mainly eliminated by the kidneys (80%) and was previously not recommended for patients with glomerular filtration rate <30 mL/min/1.73 m^2. However, recent studies have shown that sofosbuvir is well-tolerated and safe for patients with end-stage renal disease and for patients on hemodialysis.[33] Sofosbuvir/velpatasvir is pan-genotypic DAA treatment, so it is not necessary to test the patient's HCV genotype before treatment. Sofosbuvir/velpatasvir treatment for HCV was approved by the National Medical Products Administration in China in 2018[35] and was added to China's National Reimbursement Drug List in 2020.[36] Studies have concluded that sofosbuvir/velpatasvir treatment has no significant drug interactions with cyclosporine or tacrolimus.[34] The European Association for the Study of the Liver recommends that the treatment of hepatitis C with sofosbuvir/velpatasvir does not require adjustment of the dose of immunosuppressive agents and so has become the preferred choice after transplant.[33]

The time to start direct-acting antiviral treatment

At present, the time to start DAA after transplant remains controversial. In our study, recipients started sofosbuvir/velpatasvir at 1 week after transplant. In studies from Kapila and colleagues and Molnar and colleagues, the median time to start DAA was extended to more than 2 months after transplant, and almost all recipients achieved SVR12.[21,22,31] In the THINKER-1 trial,[16,17] recipients started the DAA regimen after detection of HCV RNA, and 100% of the recipients achieved SVR12. In the EXPANDER trial (Exploring Renal Transplants Using Hepatitis C Infected Donors for HCV-Negative Recipients),[20] 10 recipients received DAA prophylactically at the preoperative stage, with 5 of them were positive for HCV RNA within 1 week after transplant, and all patients achieved SVR12. These results suggest that prophylactic use of DAA can reduce the rate of HCV transmission. However, in a prospective study, Gupta and colleagues[23] treated 50 recipients within 6 hours before surgery and on the first day of transplant (total 2 doses) and within 6 hours before surgery and daily for the first 3 days after surgery (total of 4 doses). Six of the 50 recipients were positive for HCV RNA (12%), and 4 achieved SVR12. Gupta and colleagues suggested that prophylactic regimens can reduce the rate of HCV transmission. In summary, prophylactic use of DAA can reduce HCV transmission, but the precise timing of the regimen remains uncertain and will require extensive prospective studies to determine.

Duration of direct-acting antiviral treatment

In our study, recipients received sofosbuvir/velpatasvir for 12 weeks, according to

clinical guidelines.[37] The latest guidelines from the European Association for the Study of the Liver suggest that glecaprevir/pibrentasvir treatment duration should also be for 12 weeks (the same as for sofosbuvir/velpatasvir). Recently, however, studies have begun to investigate whether the shorter periods of DAA treatment can be effective. In the study by Durand and colleagues,[28] 10 recipients were given glecaprevir/pibrentasvir before organ perfusion and maintained for 4 weeks. All recipients acquired SVR12, and 1 recipient developed DGF (10%). In the MYTHIC trial (Multicenter Study To Transplant Hepatitis C-Infected Kidneys),[26] 30 HCV-positive donor kidneys were transplanted into HCV-negative recipients, and 8-week glecaprevir/pibrentasvir treatment was started 2 to 5 days after transplant. All of the 30 recipients achieved SVR12, but DGF occurred in 7 patients (23.3%) and acute rejection in 3 patients (10%). Therefore, whether the duration of DAA treatment can be shortened from 12 weeks to 8 weeks or less remains to be proved by further experiments.

Adverse events related to direct-acting antivirals

In our study, 1 recipient developed DGF, and we believe this was not caused by HCV but rather may be related to factors such as the patients' autoimmunity status. Otherwise, the kidney function recovered well for the remaining 6 recipients. There were 2 recipients with transient elevation of ALT, but this elevation was absent on reexamination after 1 week. Also, AST levels were within the reference range. These details indicate that the elevation of ALT in patients was not associated with the HCV virus. Cytomegalovirus DNA was detected in 1 recipient at week 8 after kidney transplant, but this condition improved after antiviral treatment. We believe that this event was not related to HCV infection.

The WBC status of recipients fluctuated greatly after transplant, which may be related to factors such as recipient infection. In a study by Gupta and colleagues,[23] 5 of 6 HCV-infected recipients (83%) had significant posttransplant leukopenia. In our study, 5 of the 7 patients (71.43%) experienced leukopenia during the follow-up period. This proportion of leukopenia is higher than that reported in previous studies.[38-40] Leukopenia after kidney transplant can be caused by a single drug or a single factor, but this adverse effect can also be the result of the interaction of multiple drugs and multiple factors. Thus, it is possible that the high incidence of leukopenia is related to the transplant of HCV-positive donor kidneys, but there is no strong evidence, as yet.

In summary, our study complements previous studies and further confirms that HCV D+/R− kidney transplant is safe and effective when combined with DAA treatment. If HCV D+/R− kidney transplant were to be established as an expanded criteria option, then this should create a meaningful expansion of the donor pool and effectively solve the problem of kidney shortage. Prophylactic use of DAA is effective to reduce HCV transmission. Shortening the duration of DAA treatment would contribute to a reduction of the (1) financial expense and (2) adverse effects of a DAA regimen. However, the details of an optimum treatment regimen remain unclear; therefore, a large-sample, prospective study is needed. The results of our study suggest that a 12-week regimen of sofosbuvir/velpatasvir therapy is presently the best postoperative treatment option against post-transplant HCV infection for recipients of HCV D+/R− kidney transplants.

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Role of Interventional Radiology in the Management of Early Vascular Complications After Liver Transplant

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Abstract

Objectives: **A hepatic vascular complication after liver transplant is a critical situation, often resulting in graft failure and potentially leading to patient death. Early diagnosis and treatment of vascular complications can provide prolonged graft survival and prohibit further complications. This study presents our experiences with endovascular treatment during the first week after liver transplant.**

Materials and Methods: **Between January 2012 and February 2021, 240 liver transplants were performed, with 43 patients having early endovascular treatment (37 men; mean age 27 ± 2.9 years) at a single center. Early endovascular interventions were carried out 1 to 7 days (mean ± SD of 2.7 ± 0.24 days) after transplant. Patients with vascular complications were grouped by arterial, venous, and portal complications. In addition, arterial complications were subgrouped by occlusive (hepatic artery thrombosis) and nonocclusive (hepatic artery stenosis/splenic artery steal syndrome) complications. Patients had median follow-up of 47 ± 4 months.**

Results: **In the first week after liver transplant, vascular complications included splenic artery steal syndrome in 27 patients (62.7%), hepatic complications in 10 patients (23.2%) (7 with hepatic artery thrombosis, 3 with hepatic artery stenosis), hepatic venous outflow complications in 4 patients (9.3%), and portal vein complications in 2 patients (4.6%). Only 1 patient required revision surgery because of excessive arterial kinking; the remaining patients with arterial complications were successfully managed with multiple endovascular treatment attempts. Patients with splenic artery steal syndrome were treated by selective arterial embolization with coil devices. Resistivity index, peak systolic velocity of hepatic arteries, and portal vein maximal velocity significantly improved (*P* < .001). Patients with hepatic venous outflow and portal vein complications who had endovascular treatments and vascular structures maintained good results over follow-up.**

Conclusions: **Early endovascular intervention is feasible and safe for hepatic vascular complications following liver transplant, with high success treatment rates with advances in interventional radiology.**

Key words: *Hepatic arterial stenosis, Hepatic arterial thrombosis, Hepatic venous outflow obstruction, Liver transplantation, Portal vein stenosis*

Introduction

Vascular complications, especially in the early postoperative period, may cause catastrophic results, manifesting with graft failure after liver transplant (LT). Hepatic artery stenosis (HAS) and hepatic artery thrombosis (HAT), both well-recognized vascular complications, have an overall incidence varying from 2% to 15% and have higher rates with more complex surgeries in pediatric patients and living donor LT recipients.[1-6] Nonocclusive hepatic artery hypoperfusion syndrome is another critical cause of graft loss in the early period after LT. Another complication, hepatic venous outflow obstruction (HVOO), is uncommon in adults. The absence of early diagnosis and treatment of HVOO may result in irreversible hepatic congestion and graft loss.[7-9] Another critical and devastating vascular complication is portal vein complications associated with anastomotic portal vein stenosis (PVS) or portal vein thrombosis (PVT).[10] Early diagnosis and endovascular treatment of these complications in the early period posttransplant are critical for graft survival.

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

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Experimental and Clinical Transplantation (2022) 12: 1058-1093

DOI: 10.6002/ect.2022.0244

A review of the literature showed that most endovascular interventions for vascular complications were conducted a median of 1 to 4 months after LT.[1,2,11-13] To our knowledge, there are no data on use of endovascular treatments for vascular complications (arterial, venous, and portal) during the first week after LT. Here, we described the management of these early vascular complications and demonstrated the benefits of early endovascular treatments (in the first week) after LT.

Materials and Methods

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Between January 2012 and February 2021, 240 LTs were performed in a single center with 43 consecutive patients having early endovascular treatment (37 men; mean age of 27 ± 2.9 years). Early endovascular interventions were performed 1 to 7 days (mean of 2.7 ± 0.24 days) after LT. Among the patient group, 32 had living donor LTs and 11 had deceased donors LTs. In living donor LT, donors were related to the recipients, and the relationship between donor and recipient was up to the fourth degree. Patients with vascular complications were grouped by arterial, venous, and portal vein complications. In addition, arterial complications were subgrouped by occlusive (HAT) and nonocclusive (HAS and splenic artery steal syndrome [SASS]) complications. Early endovascular treatments included 27 SASS interventions, 10 arterial interventions, 3 hepatic vein interventions, 1 inferior vena cava (IVC) intervention, and 2 portal vein interventions. The median follow-up period was 47 ± 4 months (range, 1-96 months). Patient characteristics are listed in Table 1.

Doppler ultrasonography was a first-line imaging study performed every 12 hours in the first week after LT surgery. Arterial peak systolic velocity, end diastolic velocity, and resistive index were evaluated for each patient. For hepatic vein and portal vein evaluations, we measured velocities. Computed tomography angiography was performed when there was evidence of vascular insufficiency in the Doppler examination. After the Doppler or computed tomography angiography results were reviewed, if there was suspicion of a vascular complication, patients immediately underwent diagnostic angiography.

Table 1. Patient Characteristics

Characteristic	Results (N = 43)
Mean age, years	27 ± 2.9
Sex (male/female)	37/6
Living donor/deceased donor LT	32/11
Type of vascular complication	
Arterial (HAT, HAS)	10 (23.2%)
Splenic steal syndrome	27 (62.7%)
Venous (hepatic vein, IVC)	4 (9.3%)
Portal	2 (4.6%)
Reason for LT	
HBV cirrhosis	13 (30%)
HCV cirrhosis	3 (6%)
Budd-Chiari	5 (11%)
Alcoholic cirrhosis	2 (4%)
Biliary atresia	4 (9%)
Primary sclerosing cholangitis	1 (2%)
Cryptogenic	4 (9%)
Wilson disease	2 (4%)
Progressive familial intrahepatic cholestasis	2 (4%)
Urea cycle disorder	3 (6%)
Tyrosinemia	1 (2%)
Acute liver failure	1 (2%)
Congenital hepatic fibrosis	2 (4%)
Follow-up, months	47 ± 4

Abbreviations: HAS, hepatic artery stenosis; HAT, hepatic artery thrombosis; HBV, hepatitis B virus; HCV, hepatitis C virus; IFC, interior vena cava; LT, liver transplant

Statistical analyses

All statistical analysis was performed with IBM SPSS statistical software version 23. Patient demographics were analyzed using descriptive statistics. We used the Wilcoxon signed-rank test to compare the peak systolic velocity, resistance index, and portal vein velocity at the time of diagnosis of SASS and after selective arterial embolization. $P < .05$ was considered as statistically different.

Diagnostic angiography procedures

These procedures were performed under local anesthesia and intravenous sedation. For arterial intervention, a single femoral artery wall puncture was performed using ultrasonography for sheath insertion. After aortography with a 4F to 5F pigtail catheter, we performed selective catheterization of the celiac trunk or the superior mesenteric artery with 4F to 5F shepherd hook catheter or Simmons catheter. Hepatic artery stenosis was diagnosed when the angiography showed a 50% decrease in the luminal diameter. The absence of graft flow (total occlusion) was categorized as HAT.

Interventions for hepatic artery stenosis and hepatic artery thrombosis

After the diagnostic hepatic arteriogram, we attempted to advance a 0.014- to 0.016-inch soft tip guidewire (Terumo) in a 2.4F or 2.7F microcatheter (Fast Tracker, Target Therapeutics, or Renegade HI-FLO, Boston Scientific) through the stenotic or the occluded hepatic artery. After the stenotic/occluded segment was crossed, we performed a control angiography to demonstrate the true distal hepatic artery lumen. If the hepatic artery luminal diameter was more than 50% decreased, a percutaneous transluminal angioplasty (PTA) was performed. Before PTA, heparin (100 U/kg) was administered from the arterial sheath. If the PTA was insufficient, stents were inserted (coronary stent, Boston Scientific). For patients with HAT, catheter-directed thrombolytic therapy was performed through the 4F or 5F catheter (2-4 mg of tissue plasminogen activator [tPA]). For patients with insufficient thrombolysis, an infusion catheter was placed at the thrombosed hepatic artery, with tPA administration continued with 0.75 to 1 mg/hour for 24 hours. Control angiography was performed the next day; if we did not observe adequate hepatic artery flow, tPA infusion continued for up to 72 hours; afterward, PTA was performed if needed, and a stent was placed for the hepatic artery.

Interventions for splenic artery steal syndrome

Splenic artery steal syndrome was confirmed with findings from a diagnostic arteriography from the celiac trunk that showed a patent hepatic artery but sluggish flow, delayed filling of intrahepatic arteries, and poor peripheral parenchymal perfusion with an early filling of an enlarged splenic artery.[14,15] After confirmation of SASS, patients received a super-selective catheterization of the splenic artery with a 4F diagnostic catheter and a coil embolization from the proximal splenic artery (Figure 1). An angiogram after the embolization was performed to evaluate hepatic artery perfusion.

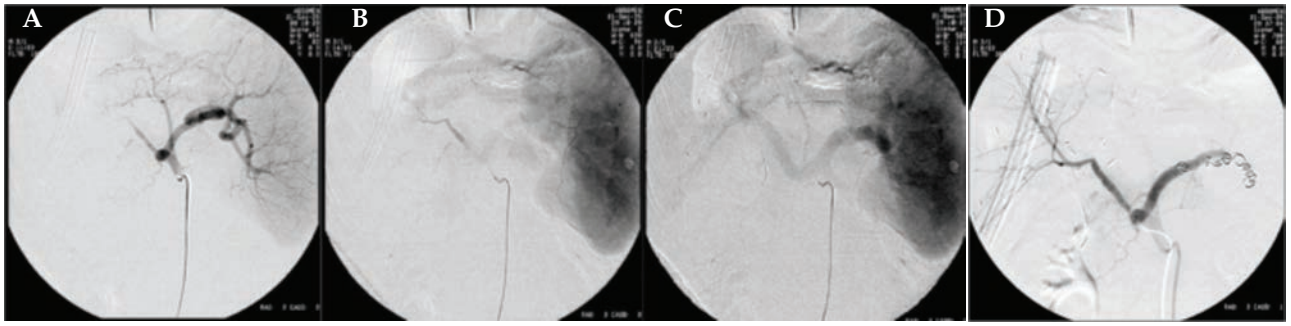
Interventions for hepatic vein and inferior vena cava complications

The right internal jugular vein was used to access for hepatic vein interventions. We inserted a 5F vascular sheath into the jugular vein and performed venography with a 5F pigtail catheter to define the HVOO. Angioplasty was performed over the 0.035-inch guidewire (Glidewire, Terumo) with balloons having diameters of 6 to 10 mm; if balloon angioplasty was insufficient, patients received stent placement. After endovascular interventions, hepatic venography and manometry were performed to determine the procedural success. For IVC interventions, the right femoral was accessed to achieve diagnostic venography with a 5F pigtail catheter. After the stenotic segment of the IVC was shown, balloon angioplasty was performed and a stent was placed if balloon angioplasty was insufficient (Figure 2).

Interventions for portal vein complications

Portal vein stenosis was managed with percutaneous transhepatic portal vein access by a biliary acoustic introducer system, and a 6F vascular sheath was inserted through the 0.035-inch guidewire. Afterward, the 0.018- to 0.035-inch guidewire with the 5F diagnostic catheter was passed through the stenotic segment of the portal vein, and spleno-portography was performed to determine the exact site

Figure 1. Patient with Splenic Arterial Steal Syndrome after Liver Transplantation .



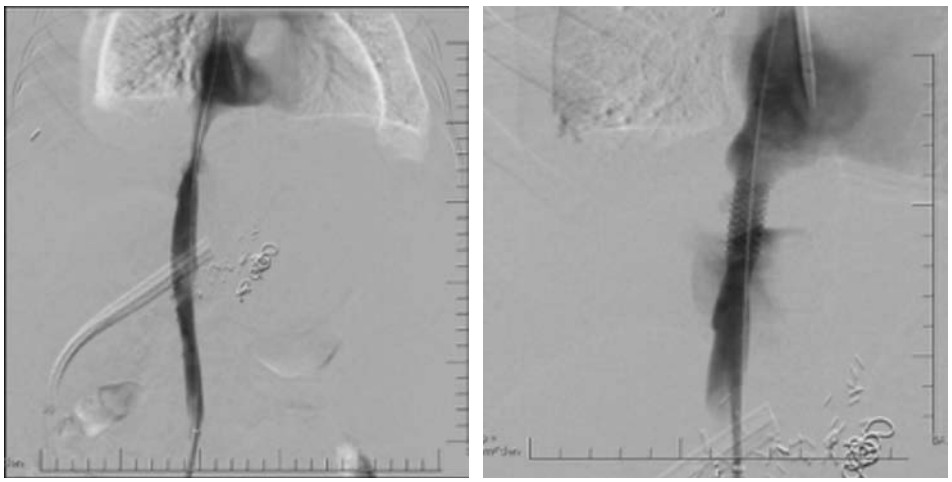
(A-C) Selective celiac angiography demonstrates the reduced hepatic artery flow with decreased distal perfusion; distal hepatic artery and intrahepatic portal vein branches are also seen. **(D)** After the selective proximal embolization of the splenic artery with coils, the angiogram shows increased hepatic artery flow.

of a stenotic segment of the portal vein. We then deployed an appropriately sized self-expandable stent (ev3, protage, Medtronic) at the stenotic segment of the portal vein (Figure 3). Balloon dilatation was performed if necessary. A control angiogram demonstrated sufficient flow on the portal vein. Before the procedure was completed, we embolized the portal access track with the coils to prevent track bleeding.

Results

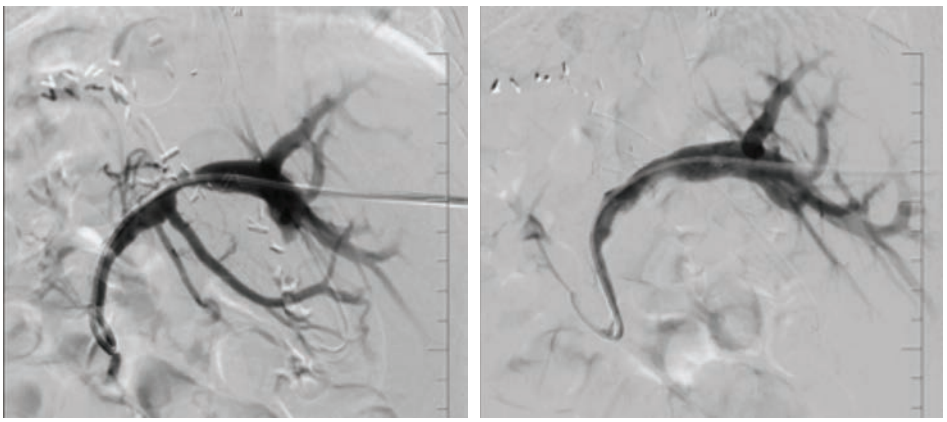
During the study period, 43 of 240 LT recipients (17%) received endovascular treatment in the first week after LT because of vascular complications. All HAS and HAT events were encountered in living donor LT recipients. Seven patients with HAT received tPA (0.25-0.75 mg/h) infusion for 4 to 72 hours. Five patients were successfully treated with balloon angioplasty after continuous tPA infusion. For 1 of the 5 patients, arterial reocclusion was encountered the day after catheter-directed thrombolytic treatment, and a stent was inserted into the hepatic artery to maintain patency. This patient's stent remained patent over follow-up. One patient had a HAS event 3 months after balloon angioplasty and received stenting with no further complications. The remaining 3 patients had no arterial complications during follow-up. One of the 7 patients with HAT received catheter-directed infusion of 2 mg of tPA and balloon angioplasty with unsatisfactory results; the patient then received stenting with good flow. Five days later, the stent was occluded, and tPA infusion was performed for 72

Figure 2. Patient With Inferior Vena Cava Stenosis After Liver Transplant



(A) Transfemoral venography demonstrates the stenosis of inferior vena cava. **(B)** A stent was deployed to relieve the stenosis; after stenting, venography demonstrates the normalization of inferior vena cava flow.

Figure 3. Patient With Portal Vein Stenosis After Liver Transplant



(A) Transhepatic splenoportography demonstrates the portal vein stenosis. **(B)** Primary stenting of the lesion with self-expandable stent; control portogram shows better filling of intrahepatic portal vein branches.

hours, with patency achieved without complications (Figure 4). Another patient with HAT received selective continuous intraarterial tPA infusion and balloon angioplasty, but this patient underwent surgical revision due to excessive arterial kinking. During follow-up, the patient received multiple arterial interventions due to reocclusion. Among 3 patients with HAS, 2 patients were treated with balloon angioplasty and 1 patient was treated with stenting because of insufficient arterial flow up. In 3 patients, the hepatic artery was patent during follow-up.

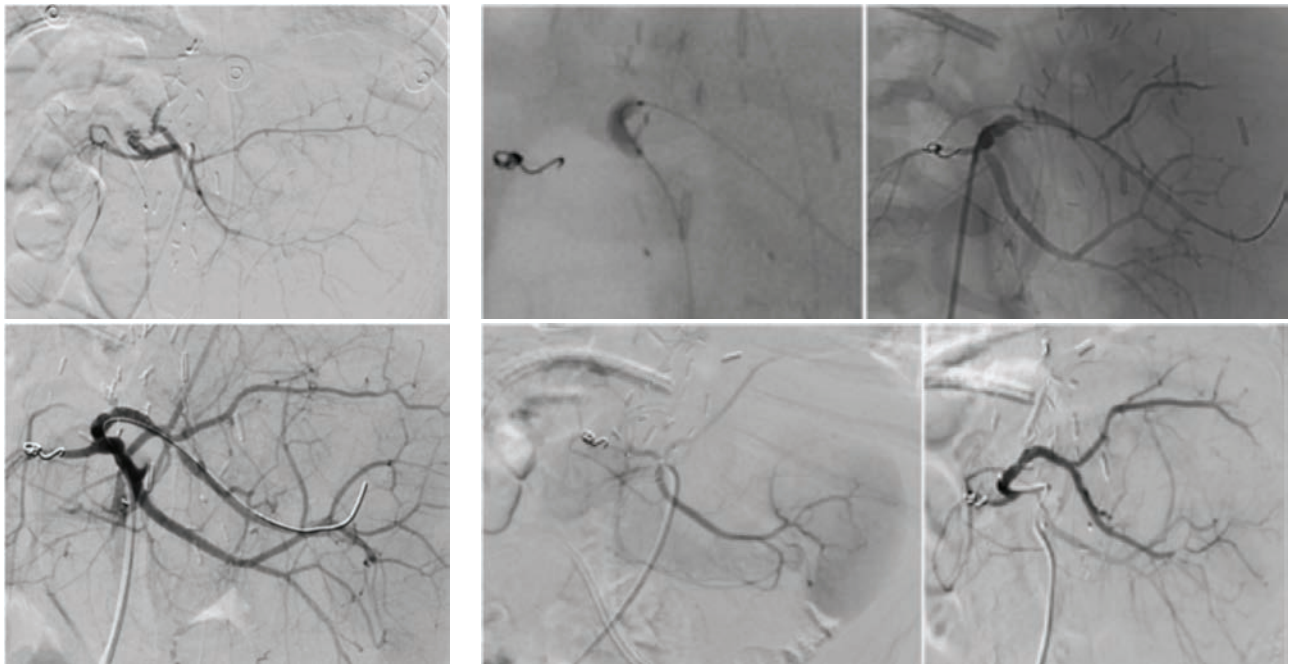
In addition to hepatic artery interventions, 2 of 10 patients received splenic artery embolization during the follow-up as a result of SASS. Four of the 10 patients with developed biliary complications, and those patients were HAT cases. Three patients had anastomotic stenosis and 1 had ischemic biliary stricture with biloma. Patients were treated with percutaneous anastomosis balloon dilatation and biliary drainage. During follow-up, 2 patients died. One of the patients had been administered an arterial revision and multiple arterial interventions, and the patient died as a result of multiple organ failure. The other patient died as a result of sepsis.

Twenty-seven of 240 LT recipients (11%) received a diagnosis of SASS angiographically. Patients with SASS consisted of 18 patients with living donor LT and 9 patients with deceased donor LT. After splenic artery embolization, control Doppler ultrasonography consistently showed normal hepatic artery flow, improved systolic amplitude, and increased diastolic flow in all treated patients. Resistance index values of the hepatic arteries showed a statistically significant decrease in posttreatment values compared with baseline measurements at SASS diagnosis ($P < .001$). Peak systolic velocity of the hepatic artery and portal vein maximal velocity improved significantly ($P < .001$) (Table 2). Local or systemic complications were not observed in any treated patient. Patients did not need any splenectomy during follow-up. Four patients with SASS had biliary complications on follow-up. Three had anastomotic site stenosis managed with a biliary drainage catheter and plastic stents. The other patient had an ischemic biliary complication with biloma. This patient died during follow-up as a result of sclerosing cholangitis.

Table 2. Median Values at Time of Splenic Arterial Steal Syndrome Diagnosis and After Selective Arterial Embolization

Test	Before Procedure	After Procedure
Resistance index	0.80	0.68
Peak systolic velocity, cm/s	46	78
Portal vein velocity cm/s	90	79

Figure 4. Patient With Hepatic Artery Thrombosis After Liver Transplant



(A) A celiac arteriogram demonstrates the occlusion of the hepatic artery. **(B-C)** Occlusion was crossed, 2 mg of tissue plasminogen activator was infused, and percutaneous transluminal angioplasty was performed with unsatisfactory result. **(D)** A stent was placed with good flow. **(E-F)** Five days later, angiography show reocclusion, and 72 hours of tPA was infused; control angiogram shows the recanalization of the hepatic artery.

Four patients (3 patients with HVOO and 1 patient with IVC stenosis) had venous complications in the first week after LT. All patients received primary balloon angioplasty. In 2 patients (1 with HVOO and 1 with IVC stenosis), stents were placed after insufficient results with balloon angioplasty, and stents were patent during the follow-up period. One patient with HVOO underwent stent placement 1 month after balloon angioplasty because of restenosis. The remaining 1 patient with HVOO and balloon angioplasty did not require an additional procedure during follow-up. Three biliary complications were observed during follow-up. Two were anastomotic stenosis, and one was biliary leakage treated with biliary drainage and plastic stent replacement. Four of the patient were still alive during the follow-up period.

Two patients with portal vein complications were encountered, and patients were treated with stent insertion for PVS. Both stents were patent during the follow-up period, with patients also alive over the follow-up period.

Discussion

Despite advances in LT surgical techniques and medications, vascular complications are still one of the main issues determining liver graft function. Early detection of vascular sufficiency and early treatment could prevent graft loss and retransplant.[16] In our review of the literature, endovascular procedures have been performed after a median or mean interval of 1 to 4 months after LT.[1,2,11-13] This shows us that most centers are still reluctant to perform endovascular procedures during the first days after LT due to the risk of arterial dissection and hemorrhage.[2] The reported complication rate was up to 23% after hepatic artery angioplasty procedures in LT recipients.[5-6,17] There are a few studies that reported results of endovascular interventions in the first week after LT.[18,19] Despite previous cases that have withheld from early endovascular interventions, we did not encounter arterial dissection or hemorrhagic complications in our LT recipients. In our opinion, avoiding aggressive wire manipulations at the juxta-anastomotic site and using nontraumatic soft wires in arterial intervention are critical steps. In addition, the covered stent has been a safe and effective treatment tool after hepatic artery rupture.[20]

Careful selection of paired arteries with microsurgical technique, intraoperative Doppler ultrasonography, and postoperative thrombosis prophylaxis are significant in preventing occlusive and nonocclusive arterial complications.[21-24] Despite the pre- and postoperative measures taken to avoid deterioration of graft arterial flow, arterial complications have been observed in 2% to 15% of patients.[1-6,25-27] In our study, the arterial complication rate was similar at 4%. Seven of the 10 arterial events consisted of thrombosis occlusion, with HAT contributing to acute flow deterioration in the hepatic artery, which supplies the hepatic parenchyma and bile duct. An early incidence of HAT can result in high mortality, with a rate of 34.3% in a study of adults after LT.[26] Hepatic artery stenosis may be an overlooked complication; HAS can cause persistent ischemic effects on the graft, leading to graft failure.[1] In our study, the benefits of frequent Doppler examinations can allow determination of arterial disruption in the graft and pave the way for early endovascular interventions that can prevent graft loss. One of the valuable findings in our study showed that early detection and treatment can aid in preventing ischemic biliary damage that may present with ischemic cholangiopathy and sepsis, which can result in graft and patient loss.

Arterial vascularization methods include surgical revision, endovascular revascularization, and retransplant. Retransplant has been the gold standard treatment method, but timely retransplant may not be feasible in regions such as Asia due to organ shortages. In a systematic review, the success rate in adult LT recipients with HAT who are treated with surgical revascularization has been reported as 50.9%, and 32.3% of patients required retransplant after surgical revascularization.[26] With these consideration, in our center, intra-arterial throm-bolysis is the first-choice therapy in early HAT after LT.

Various thrombolytic agents (urokinase, streptokinase, and tPA) have been used in hepatic artery complications, but there is no consensus on the use of thrombolytic agents, either temporarily or continuously. In our center, continuous tPA infusion (0.75-1 mg/h) is administered for 4 to 72 hours. If the patient has insufficient arterial graft flow due to residual thrombosis, tPA infusion is continued for 72 hours after stent replacement to overcome the residue thrombosis. The main concern in continuous thrombolytic therapy is hemorrhagic complications

(incidence rate up to 20%).[28] We monitored ACT levels and maintained ACT levels at between 140 and 180 to avoid hemorrhagic complications. We had only 1 local puncture site hematoma that presented with pseudoaneurysm, which was treated with ultrasonograph-guided thrombin injection. We did not have any life-threatening hemorrhage complications in our study patients; in our opinion, tPA is a safe and effective treatment for patients with early HAT.

Splenic arterial steal syndrome is a well-recognized cause of graft failure when left untreated.[29] The incidence of SASS varies from 0.6% to 8.4% as a posttransplant complication.[14-15,30] Differences in SASS incidence may be due to being unrecognized diagnosis in some institutions and overdiagnosis in others. In our study, we reported an 11% incidence of SASS in the first week after LT. We suggest that this high incidence of SASS was related to close follow-up with ultrasonography in the early period posttransplant. At the slightest suspicion of arterial flow, the patients are treated by further evaluation with angiography.

In a review from Pinto and colleagues, SASS was reported to develop from the early period after LT to 5.5 years later.[31] Another important aspect of our study was that the treatment of patients with SASS within the first week prevented complications that may lead to ischemic biliary damage and ultimately future graft loss. We encountered 4 biliary complications, with 3 nonischemic biliary complications (anastomotic stenosis and biliary leakage), which were treated with biliary drainage. At the beginning of splenic artery embolization treatment, distal embolization was the preferred method but had high morbidity and mortality because of splenic abscess and sepsis complications.[32] With gained experience, proximal splenic artery embolization became the preferred method, with significantly fewer complications. In our study, we did not encounter any complications in patients who underwent proximal embolization. Even in the early period after transplant, splenic artery embolization is a safe and effective treatment for SASS.

In our study cohort, we encountered 3 hepatic vein complications and 1 IVC stenosis in the first week post-LT. Venous complications can occur over a wide range of time, from the early posttransplant period to several months and years after LT.[7,33] Early venous complications may occur as a result of several factors, including tight suture line, kinking of a redundant hepatic vein, caval compression from a large graft, or donor-recipient size discrepancy. Late venous complications (≥3 months post-LT) usually occur due to fibrosis of the anastomotic site and intimal hyperplasia.[34] Treatment options for venous complications are angioplasty with or without stent placement, surgical reconstruction of the venous anastomosis, and retransplant. The existing literature has stated that early HVOO should be primarily treated with surgical revision; however, late venous complications should be treated with endovascular interventions because venous complications may cause liver and kidney function deterioration after a complicated surgical procedure.[35-38] The main hesitation of an early endovascular intervention for venous complications is the rupture of the anastomotic site during revascularization. In our study cohort, no vein ruptures occurred during the revascularization. In our opinion, it is crucial to be aware of harsh manipulation with wire and catheter at the anastomotic site, and selecting an undersized balloon is the key to preventing complications.

In addition to arterial and vein complications, we encountered portal vein complications in 2 patients in the early period after LT. Portal vein stenosis usually occurs ≥1 month after LT and more frequently occurs in pediatric patients.[39-42] As with early vascular interventions, the main concern for endovascular treatment for early PVS is anastomotic site rupture. Ko and colleagues[43] suggested primary stent placement in PVC versus balloon angioplasty to prevent the risk of the anastomotic site rupture. However, Kim and colleagues[40] treated PVS after LT with balloon angioplasty without rupture. In our 2 patients with PVS, we preferred primary stent insertion because of the possibility of rupture of the fresh anastomotic site. Sambommatsu and colleagues[39] reported early (1 month after LT) portal vein complications in 14 patients (3 patients with PVS, 11 patients with PVT), with endovascular treatment having 100% success for early PVS and 91% success for PVT. In addition, their PVS cases were identified within 1 week post-LT, and they did not encounter portal vein complications after treatment during follow-up. We also did not observe portal vein complications during follow-up; unfortunately, 2 patients with PVS were lost. Early portal vein complications can be treated with surgical revision because these complications are usually caused by

surgically correctable factors, including tension, twisting, kinking, or external compression of the portal vein. With the exclusion of surgically correctable cases, endovascular treatment is preferred. However, if PVS is left untreated, slow progression to portal vein occlusion can occur, which is more difficult to treat.[44]

Conclusions

The limitation of our study comes from the nature of its retrospective design. Nonetheless, this unique study included all types of vascular complications in the first week after LT. Despite advances in LT surgery and medications, vascular complications are still a significant cause of graft and patient loss in the early period after LT. With the consensus of the surgical team, intensive care, and interventional radiologists, the diagnosis and treatment of early vascular complications are essential in preventing graft and patient loss.

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Investigation of the Association Between the *ITPA* Gene 94C>A Gene Sequence Variant and Liver Transplant Rejection in Iranian Liver Transplant Recipients

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Abstract

Objectives: **Inosine triphosphate pyrophosphatase is an enzyme encoded by the *ITPA* gene and functions to prevent the incorporation of thiopurine nucleotides into DNA and RNA. Thiopurine drug metabolites such as azathioprine and 6-mercaptopurine have been included in the lists of inosine triphosphate pyrophosphatase substrates. Inosine triphosphatase gene alterations are other pharmacogenetic sequence variants possibly involved in thiopurine metabolism. This study aimed to evaluate the possible association between *ITPA* 94C>A gene sequence variant (C-to-A substitution at nucleotide 94) in liver transplant recipients.**

Materials and Methods: **The genotyping of *ITPA* 94C>A was evaluated by the polymerase chain reaction-restriction fragment length polymorphism method in 200 liver transplant recipients as well as 100 controls. Data were analyzed with SPSS statistical software.**

Results: **This study showed statistically significant associations between the CA genotype of the *ITPA* 94C>A sequence variant and liver transplant in the rejection and nonrejection groups. Moreover, the results reported in this study showed no significant differences between sex, age, and blood group in patients with liver transplant (with or without transplant rejection).**

Conclusions: **Our results indicated that there were statistically significant associations of the CA genotype of *ITPA* 94C>A sequence variant with liver transplant in the rejection and nonrejection groups. Further studies are recommended.**

Key words: *Gene sequence variation, Immunosuppressive drugs,* ITPA *gene variants, Orthotopic liver transplant*

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Acknowledgements: *This article was supported by the Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. The study team would like to gratefully acknowledge the staff of this center. Other than described, the authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

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Experimental and Clinical Transplantation (2022) 12: 1094-1098

Introduction

The orthotopic liver transplant is the final therapeutic procedure for end-stage liver failure. Despite the use of new immunosuppressive drugs and new methods, acute rejection is one of the main obstacles after liver transplant. Although patient survival has increased to 80% at 1 year posttransplant, incidence of acute liver rejection remains at 40% to 80%.[1,2] Acute liver rejection is an immune process in which lymphocytes play a major role. Immunosuppressor drugs such as azathioprine inhibit purine synthesis. Purines are needed to produce DNA and RNA. When purine synthesis is inhibited, less DNA and RNA are produced for the synthesis of white blood cells, thus causing immunosuppression. Azathioprine, a prodrug of 6-mercaptopurine, in combination with tacrolimus and prednisolone is the standard immunosuppression regimen after liver transplant.[3] Thiopurines are widely used in the treatment of diseases such as leukemia, inflammatory bowel disease, and severe rheumatic diseases and especially for immunosuppression following solid-organ transplant.[4]

An important enzyme in thiopurine metabolism is thiopurine *S*-methyltransferase, which determines the balance between 2 groups of active metabolites of potentially hepatic 6-methylmercaptopurine ribonucleotides and 6-thioguanine nucleotides of myelosuppressors.[5,6] Another enzyme is inosine triphosphate pyrophosphatase (ITPase), a deficiency of which is associated with thiopurine toxicity. During thiopurine nucleotide synthesis in cells,

DOI: 10.6002/ect.2022.0362

pyrophospho-hydrolysis of inosine triphosphate (ITP) is converted to inosine monophosphate (IMP) by the enzyme ITPase, which causes ITP to accumulate abnormally.[7,8]

The putative role of ITPase is to recycle purines trapped in the form of ITP and thus to protect the cell from the accumulation of "rogue" nucleotides, such as ITP, dITP, or xanthosine triphosphate, which may be deoxy forms of RNA or DNA.[9] Deficiency of ITPase is a common inherited condition characterized by the abnormal accumulation of ITP in red blood cells. There are pharmacogenomic implications with ITPase deficiency, and the possibility has been raised that metabolism of 6-mercaptopurine (azathioprine) in ITPase deficiency may lead to thiopurine drug toxicity in affected patients.[10]

The ITPase coding gene is on the short arm of chromosome 20, and 5 single-nucleotide variants of this gene have been identified so far, 2 of which are associated with ITPase loss of activity: 94C>A (C-to-A substitution at nucleotide 94) in exon 2, and IVS2+21A>C.[11] Three of the altered sequence variants are silent variants (138G>A, 561G>A, and 708G>A).[9] The most common single-nucleotide variant is 94C>A. Previous studies have shown that there is a significant relationship between *ITPA* 94C>A and the occurrence of adverse side effects such as flu-like symptoms, skin rashes, and pancreatitis in patients treated with thiopurine.[12-14] In extensive studies, the frequency of *ITPA* 94C>A has been investigated in different populations. In a study conducted on a group of White participants, it was shown that 2 *ITPA* gene sequence variants (94C>A and IVS2+21A>C) were associated with reduction of ITPase enzyme activity.[15]

In normal cells, ITP is formed by phosphorylation of IMP, and ITPase converts ITP back to IMP to prevent accumulation of ITP. Deficiency of ITPase interrupts this futile cycle and leads to the accumulation of ITP.[7,12] In the past, evidence has been published that sequence variations in the gene encoding ITPase may be associated with the occurrence of adverse drug reactions in patients treated with thiopurines.[16] Our study aimed to evaluate the possible association between the *ITPA* 94C>A gene sequence variant and liver transplant rejection in Iranian liver transplant recipients.

Materials and Methods

Patient information

Our study included 100 healthy participants and 200 recipients of liver transplants between 2011 and 2016. The transplant recipients (n = 200) were divided into 2 groups. The first group, without rejection (nonrejection group), consisted of 100 patients, and the second group was composed of 100 patients who had experienced rejection (rejection group). The patients were enrolled in the study according to the following inclusion criteria: the recipients had received a liver transplant from a deceased donor, and the recipients were treated with specific immunosuppressive drug regimens.[17]

Donors were selected on the basis of ABO blood group compatibility. Human leukocyte antigen matching was done as part of the standard work-up for liver transplant patients at this center. Rejection episodes were identified by an expert gastroenterology team according to accepted criteria such as increased serum levels of liver enzymes and total serum bilirubin level in the absence of biliary problems, histological findings after biopsy of the liver, and clinical and biochemical responses to high-dose steroids.

The ethics committee of Shiraz University of Medical Sciences approved the protocol, and written informed consent was obtained from all the participants. The study was conducted according to the Declaration of Helsinki. According to the rules of the transplant ward of Namazi Hospital, all transplant recipients received organs from deceased donors. Human leukocyte antigen typing and ABO blood compatibility were performed for all liver transplant patients.

Immunosuppression regimen

The routine immunosuppression regimen consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. Drug dosage was adjusted to maintain target therapeutic blood levels of 200 ng/mL for cyclosporine or 10 ng/mL for tacrolimus plus azathiopurine.

DNA extraction

Blood samples (3 mL, treated with EDTA) were collected from each participant. The serum and buffy coat from each sample were separated using a Ficoll gradient (Nycomed). Genomic DNA was extracted from the buffy coat using a commercial extraction kit

(DNG Plus DNA extraction kit, CinnaGen) according to the manufacturer's instructions.

Genotype analysis

The single-nucleotide variation of the study gene *(ITPA)* was evaluated by polymerase chain reaction-restriction fragment length polymorphism methods, using a thermal cycler (Techne Genius), as previously described.[18] The polymerase chain reaction products were analyzed by electrophoresis in 3% agarose gel stained with safe stain, and final detection was performed with an ultraviolet transilluminator. Details of primer, fragment size, and restriction enzyme are summarized in Table 1.

Statistical analyses

All statistical analyses were performed with SPSS software (version 16). The *t* test was used to compare continuous variables. In addition, the Pearson chi-square test or Fisher exact test was used to compare between-group differences in clinical findings and allele and genotype frequencies with and without acute rejection episodes. $P < .05$ was considered statistically significant. Odds ratios and 95% confidence intervals were calculated to estimate the risk of rejection.

Results

Descriptive characteristics

The nonrejection group consisted of 100 liver transplant recipients: 61 were male (age range, 14-69 years), and 39 were female (age range, 15-61 years). The rejection group consisted of 100 liver transplant recipients: 63 were male (age range, 6-68 years), and 37 were female (age range, 13-58 years). The mean ages in the nonrejection group and rejection groups were 42 ± 14.6 years and 42 ± 14.9 years, respectively.

Sequence variant rs1127354 and liver rejection

The results showed a statistically significant difference between rejection and nonrejection regarding rs1127354 genotype and allele frequency. The genotype frequency showed the rs1127354 sequence variant in both the rejection group (CC = 54, CA = 0, and AA = 46) and nonrejection group (CC = 65, CA = 4, and AA = 31). Also, the frequency of alleles C and A was 108 C and 92 A for the rejection group and 134 C and 66 A for nonrejection group.

The results revealed a statistically significant difference between the control and rejection group with regard to rs1127354 genotypes and allele frequency. The genotype frequencies showed the rs1127354 sequence alteration in both the rejection group (CC = 54, CA = 0, and AA = 46) and the control group (CC = 68, CA = 1, and AA = 31). Also, the frequency of alleles C and A was 108 C and 92 A in the rejection group and was 137 C and 63 A in the control group.

No significant associations were found between rs1127354 sequence variations with control and nonrejection groups. The genotype frequencies showed the rs1127354 sequence alterations in both the nonrejection group (CC = 65, CA = 4, and AA = 31) and the control group (CC = 68, CA = 1, and AA = 31). Also, the frequency of alleles C and A was 134 C and 66 A in the nonrejection group and 137 C and 63 A in the control group (Table 2).

The evaluation of the association of gene sequence variants, transplant patients, and underlying diseases revealed that there was a significant association with underlying disease type (autoimmune hepatitis and

Table 1. Primer, Fragment Size, and Restriction Enzyme for the 94C>A rs1127345 Variant

SNP	Primer	Sequence	Product Length, bp	Restriction Enzyme
rs1127345	Forward	CAGGTCGTTCAGATTCTAGGAGAAAAG	256	Xmn1
	Reverse	CAAGAAGAGCAAGTGTGGGACAAG		

Table 2 Distribution of rs1127354 Genotype and Allele in Rejection, Nonrejection, and Control Groups

Genotype	Rejection Group, %	Nonrejection Group, %	Control Group, %	*P*		
				R vs NR	R vs C	NR vs C
CC	54	65	68	.11	.04	.65
CA	0	4	1	.04*	.31	.17
AA	46	31	31	.02	.02*	1
C allele	108	134	137	.007*	.002*	.7
A allele	92	66	63			

Abbreviations: C, control group; NR, nonrejection group; R, rejection group

*$P < .05$, statistically significant difference.

viral hepatitis [HBV + HCV + HBV, and hepatocellular carcinoma]) in liver transplant patients (with or without transplant rejection) (Table 3). Also, the results revealed no significant differences in sex, age, and blood type in patients with liver transplant (with or without transplant rejection).

Table 3. Underlying Diseases in Recipients with Rejection and Nonrejection

Underlying Disease	Rejection Group (%)	Nonrejection Group (%)	*P*	Odds Ratio
Cryptogenic alcoholism and NASH	21 (21)	15 (15)	.2	1.51
Viral hepatitis (HBV + HCV + HBV and HCC)	21 (21)	34 (34)	.03	0.52
Autoimmune hepatitis	22 (22)	7 (7)	.002	3.75
PSC + PBC	13 (13)	16 (16)	.3	0.78
Congenital disorder (tyrosinemia biliary atresia + PSC and Budd-Chiari syndrome + acute liver failure + hypercholesterolemia + hyperoxalemia + Wilson disease)	23 (23)	28 (28)	.6	0.77

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis

Discussion

Inosine triphosphate pyrophosphatase is an enzyme that prevents nonconventional purine nucleotides from joining DNA and RNA and is encoded by the *ITPA* gene. In various tissues, such as bone marrow, skin, fibroblasts, amniotic fluid, and red blood cells, the activity of *ITPA* gene variants has been investigated. The sequence variations that have been identified to be associated with ITPase deficiency are *ITPA* 94C>A and IVS2+21A>C.[19,20] Marsh and colleagues studied the distribution of *ITPA* c.94C>A and stated that an association with the inosine triphosphate pyrophosphatase gene (ITPA) and azathioprine and 6-mercaptopurine intolerance has been defined. However, the allele frequency of *ITPA* c.94C>A varies dramatically between populations, being lowest in Central American and South American populations (1%-2%) and highest in Asian populations (11%-19%). In White, African American, and African populations, the allele frequency is 5% to 7%.[20,21] Previous studies revealed a significant relevance between *ITPA* 94C>A and the onset of noxious reactions such as flu-like symptoms, rash, and pancreatitis in patients treated with a thiopurine. Patients with the *ITPA* 94C>A variant are also known to experience significantly earlier onset of noxious reactions compared with patients who do not have the *ITPA* 94C>A variant.[22]

Our study showed statistically significant correlation between the CA genotype of the *ITPA* 94C>A sequence variant and liver transplant in the rejection and nonrejection groups ($P = .04$). Also, a meaningful association was observed between this sequence variant and allelic frequency in the liver rejection group and the control group ($P = .02$). Furthermore, the results reported in this study indicate no significant differences between sex, age, and blood group in patients with liver transplant (with or without transplant rejection).

In 2009, the prevalence of the *ITPA* gene in Asian populations was estimated to be 14% to 19%, which is the highest prevalence among the total 5% of global inhabitants that possess the *ITPA* c.94C>A variation.[20] Several other types of gene sequence variants with clinical manifestations have been observed in the *ITPA* gene of different populations, and the results of those studies indicated a reduction in the activity of the ITPase enzyme.[23] The *ITPA* c.94C>A sequence variant is associated with susceptibility to adverse drug reactions in patients treated with azathioprine and is responsible for the deletion of an exonic splicing silencer in exon 2 in peripheral blood leukocytes of these patients, which may eventually lead to a change in the structure of the ITPase and contribute to its deficiency (the *ITPA* c.94C>A and c.124+21A>C [g.IVS2+21A>C] sequence variants cause a misplacement of the *ITPA* gene).[11] In adult patients with hematological malignancy, the *ITPA* 94C>A variant has been reported to be connected to a notable surge in the total heteroplastic/homoplastic alterations in the mitochondrial DNA, which suggests that a reduction in the activity of the ITPase may cause incremental changes in the mitochondrial *ITPA* gene expression and suggests a possible connection with mitochondrial DNA defects.[23] Moreover, an *ITPA* genotyping study in one-quarter of the Tunisian population showed that gene sequence variants in that population had reduced the metabolic function of the enzyme.[24]

This study of the *ITPA* 94C>A sequence variant in transplant patients with and without rejection is the first study of its kind, to our knowledge, in Iran. Therefore, we cannot compare our results with other results.

In conclusion, the *ITPA* 94C>A variant is an important factor in liver transplant recipients treated

with thiopurine drugs. This study was performed on a few patients and controls. According to our research, this is the first report on the association between liver graft rejection and *ITPA* single-nucleotide variations; therefore, further studies are required to confirm and extend our results.

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Nomogram Prediction for Postoperative Mortality of Orthotopic Liver Transplantation

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Abstract

Objectives: **The purpose of this study was to assess and predict risk factors for death within 30 days after orthotopic liver transplant and to develop a nomogram to predict mortality after liver transplant.**

Materials and Methods: **We retrospectively studied 185 patients who underwent orthotopic liver transplant at Sichuan Provincial People's Hospital from January 1, 2010, to December 31, 2018. Multivariable logistic regression analyses were used to identify independent risk factors. A nomogram model was developed to predict mortality after liver transplant. The performance of the prediction model was assessed and validated by receiver operating characteristic curve and bootstrap methods (1000 replications).**

Results: **Multivariable logistic regression analyses revealed that tracheal extubation time, postoperative infection, and intraperitoneal hemorrhage post-transplant were independent risk factors for mortality after liver transplant. The receiver operating characteristic curve of the nomogram prediction model was 0.896 (96% CI, 0.803-0.989), and the mean absolute error of internal validation by bootstrap (1000 replications) was 0.019 (n = 184). These results showed that the nomogram model had an excellent prediction accuracy.**

Conclusions: **A nomogram model can provide clinicians with an individualized risk assessment of perioperative mortality in liver transplant recipients.**

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

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Experimental and Clinical Transplantation (2022) 12: 1099-1104

Key words: *Hepatic, Prediction model, Risk factors, Transplant*

Introduction

Liver transplantation (LT) is currently the most effective treatment method for end-stage liver disease, including end-stage acute liver failure, cirrhosis in adults, hepatic malignancies, and congenital biliary atresia in children.[1-4] With the continuous development of surgical techniques, perioperative management, and immunosuppressive therapy,[5] survival can now reach 80% to 90% after 1 year and 70% to 80% at 5 years.[6] However, as a result of shortages of donor organs, many patients die while on wait lists before undergoing LT.[7] Therefore, a major challenge to physicians is to improve the success rate of LT and to utilize the living donors more effectively.[8-10]

Mortality after LT is highly prevalent within 30 days after transplant.[11] Therefore, we retrospectively studied the risk factors associated with mortality within 30 days after LT. Although a number of studies have predicted the risk factors after LT,[12-14] because of numerous factors affecting LT outcomes, precisely predicting postoperative mortality is challenging.

The nomogram uses various clinicopathological parameters to generate specific information that can affect clinical management or inform the clinician of the patient's progress.[15] A nomogram can evaluate outcomes of high-risk patients through a total score calculation, is intuitive, and is easy to understand. Nomograms are widely used in all aspects of clinical practice to predict the disease process.[15,16] Although various nomograms have been established to estimate the incidence or risk of different diseases, as far as we know, nomograms are rarely used to predict mortality after orthotopic LT.

DOI: 10.6002/ect.2021.0431

Materials and Methods

The study cohort included patients who received orthotopic LT at Sichuan Provincial People's Hospital (Chengdu, China). All patient data were obtained from the hospital's electronic medical record system. The study was approved by the Ethics Committee of Sichuan Provincial People's Hospital (no. 2018-267) and has been registered with the Chinese Clinical Trial Registry (no. ChiCTR2000033742). Patient consent was not required for this study because it was a retrospective study without interventions that would affect patients' rights and health. Patient data were anonymous at the time of collection.

Study population

This study was a retrospective analysis of 185 patients who underwent orthotopic LT at Sichuan Provincial People's Hospital from January 1, 2010, to December 31, 2018. Exclusion criteria included blood group incompatibility between donor and recipient, patients who were pregnant or breastfeeding, those aged <18 years, patients with a previous history of liver surgery, multiple organ combination transplant recipients, patients with malignancy other than liver cancer, and patients with death in the operating room.

Variables analyzed

The preoperative variables selected for the study included recipient age, sex, body mass index (BMI), primary cause of the liver disease (liver cancer, severe hepatitis, chronic end-stage liver disease), nonhepatic phase duration, operative time, the volume of bicarbonate injection, the volume of saline injection, the volume of blood transfusions, total fluid volume, postoperative infection, postoperative intraperitoneal hemorrhage, Model for End-Stage Liver Disease (MELD) score, length of intensive care unit (ICU) stay, tracheal extubation (TE) time, and length of hospital stay.

Definitions in the model

This study examined mortality related to LT within 30 days after the operation, and the main prognostic outcome was survival or no survival. The diagnosis of postoperative infection was based on clinical manifestations and organism isolation.[17] Intraperitoneal hemorrhage after LT was diagnosed when there was a large amount of bloody drainage from the abdominal drainage tube, the hemoglobin level dropped rapidly, or there was instability in blood pressure.[18,19]

Statistical analyses

We performed all statistical analyses with IBM SPSS statistical software (version 25.0) and R software (version 3.4.6). Clinical features were summarized as categorical variables and presented as counts and percentages; continuous variables are presented as means and standard deviation or medians and interquartile range. Continuous variables were assessed by *t* tests, and categorical variables were assessed by chi-square tests. Statistical significance was defined as $P < .05$. Clinically relevant factors associated with nonsurvival ($P < .05$) in univariate analysis were included in subsequent multivariable logistic regression analyses to identify independent risk factors. The performance of the nomogram model was assessed by receiver operating characteristic curve (ROC)[20] and bootstrap methods (1000 replications).

Results

During the study period, 185 patients underwent LT. One patient died during LT. No primary nonfunction of the liver and no pulmonary embolisms occurred in the patients. In total, 184 patients were included, with 143 men (77.7%) and 41 women (22.3%) analyzed in the study who had median age of 47 years. Indications for LT included end-stage liver disease (n = 90, 48.9%), liver cancer (n = 88, 47.8%), and severe hepatitis (n = 6, 3.3%). The main complications after LT were postoperative infection (n = 25, 13.6%) and postoperative intraperitoneal hemorrhage (n = 7, 3.8%). Compared with the survival group, the study indicators were significantly more prevalent in the nonsurvival group. The significantly prevalent indicators in the nonsurvival group included TE time ($P < .001$), postoperative infection ($P < .001$), postoperative intraperitoneal hemorrhage ($P = .001$), ICU length of stay ($P = .003$), hospital length of stay ($P = .007$), 0.9% saline volume ($P < .001$), blood transfusion volume ($P = .029$), total fluid volume ($P = .016$), BMI ($P = .017$), and MELD score ($P = .012$) (Table 1).

Table 1. Clinicopathologic Characteristics of Liver Transplant Recipients

Parameter	All Patients (N = 184)	Survival Group (n = 164)	Nonsurvival Group (n = 20)	*P*
Median age (IQR), years	47 (40-55)	47 (45-56)	47 (40-55)	.346[a]
Sex, No. (%)				
Male	143 (77.7%)	125 (76.2%)	18 (90.0%)	.265[b]
Female	41 (22.3%)	39 (23.8%)	2 (10.0%)	
Primary cause of liver disease, No. (%)				
Liver cancer	88 (47.8%)	79 (48.2%)	9 (45.0%)	.882[a]
Severe hepatitis	6 (3.3%)	5 (3.0%)	1 (5.0%)	
Chronic end-stage liver disease	90 (48.9%)	80 (48.8%)	10 (50.0%)	
Operative time, h	9.0 ± 3.2	8.96 ± 3.32	9.56 ± 2.21	.432[c]
TE time, days	2.3 ± 1.59	2.08 ± 1.18	4.25 ± 2.83	<.001[c]
ICU length of stay, days	6.38 ± 3.78	6.09 ± 3.27	8.70 ± 6.28	.003[c]
Hospital length of stay, days	24.35 ± 12.97	25.24 ± 12.33	17.05 ± 15.92	.007[a]
Postoperative infection, No. (%)*				<.001[b]
Yes	25 (13.6%)	14 (8.5%)	11 (55.0%)	
No	159 (86.4%)	150 (91.5%)	9 (45.0%)	
Intraperitoneal hemorrhage, No. (%)				.001[b]
Yes	7 (3.8%)	3 (1.8%)	4 (20.0%)	
No	177 (96.2%)	161 (98.2%)	16 (80.0%)	
Sodium bicarbonate volume, L	0.42 ± 0.26	0.41 ± 0.22	0.50 ± 0.45	.140[c]
Saline volume, L	0.94 ± 0.51	0.90 ± 0.45	1.31 ± 0.76	<.001[c]
Blood transfusion volume, L	3.46 ± 2.21	3.34 ± 2.08	4.48 ± 2.94	.029[c]
Transfusion volume, L	7.71 ± 3.15	7.52 ± 2.90	9.31 ± 4.51	.016[c]
BMI	22.19 ± 3.54	21.99 ± 3.26	23.86 ± 5.15	.017[a]
MELD score	64.61 ± 7.31	64.50 ± 7.35	65.48 ± 7.09	.012[a]
Nonhepatic phase duration, h	1.52 ± 1.39	1.52 ± 1.46	1.52 ± 0.67	.981[a]
Coronary heart disease, No. (%)	1 (0.5%)	1 (0.6%)	0	
Diabetes mellitus, No. (%)	25 (13.6%)	24 (14.6%)	1 (5.0%)	
Renal failure, No. (%)	4 (2.2%)	1 (0.6%)	3 (15%)	
Cerebrovascular event, No. (%)	1(0.5%)	1(0.6%)	0	

Abbreviations: BMI, body mass index (in kilograms divided by height in meters squared); ICU, intensive care unit; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; TE, tracheal extubation

*Includes infections of abdominal cavity, respiratory tract, incision, and urinary system.

P < .05 was significant ([a]*t* test; [b]Pearson chi-square test; [c]likelihood ratio test.

Mortality risk factors

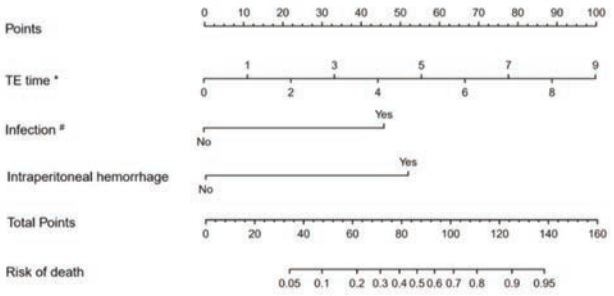
In the multivariable logistic regression analyses, risk factors for LT mortality included TE time, ICU length of stay, hospital length of stay, postoperative infection, postoperative intraperitoneal hemorrhage, saline injection volume, blood transfusion volume, and total fluid volume. Multivariable logistic regression analyses showed that TE time, postoperative infection, and postoperative intraperitoneal hemorrhage were independent risk factors for nonsurvival (Table 2).

Nomogram model development and validation

A nomogram to predict mortality after LT was developed based on 3 independent risk factors identified by multivariate logistic regression analysis (Figure 1). For example, for a patient with TE at 5 days after LT with postoperative infection and without postoperative intraperitoneal hemorrhage, the total score was 102 (56 + 46 + 0) and the corresponding risk of mortality post-LT was 70% (Figure 2).

A ROC curve was used to assess the predictive ability of the model for death in LT patients, and the result showed an area under curve of 0.896 (96% CI, 0.803-0.989) (Figure 3). The internal calibration was constructed using bootstrap resampling 1000 times, and the results showed high consistency in the nomogram (Figure 4).

Figure 1. Nomogram Prediction Model for Mortality After Liver Transplant



Abbreviations: TE, tracheal extubation

Table 2. Univariate and Multivariable Analyses of Mortality Risk Factors After Liver Transplant

Parameter	Univariate Analyses			Multivariable Analyses		
	OR	95% CI	*P*	OR	95% CI	*P*
Age	1.023	0.976-1.072	.345			
Sex (male/female)	0.356	0.079-1.603	.179			
Primary cause of liver disease (D1/D2/D3)	1.046	0.652-1.679	.851			
Operative time (hours)	1.151	0.960-1.380	.129			
TE time (days)	1.900	1.441-2.506	<.001	1.919	1.224-3.008	.004
ICU length of stay (days)	1.138	1.034-1.253	.008	0.989	0.830-1.179	.902
Hospital length of stay (days)	0.933	0.887-0.981	.007	0.955	0.903-1.009	.101
Posttransplant infection (yes/no)*	13.095	4.641-36.950	<.001	17.197	3.986-74.189	<.001
Intraperitoneal hemorrhage posttransplant (yes/no)	13.417	2.756-65.311	.001	12.860	1.187-139.34	.036
Sodium bicarbonate volume	3.335	0.664-16.754	.144			
Saline volume	3.971	1.704-9.257	.001	1.002	0.134-7.502	.998
Blood transfusion volume	1.212	1.014-1.449	.035	0.942	0.581-1.526	.808
Transfusion volume	1.162	1.024-1.318	.020	1.193	0.923-1.543	.177
BMI	1.161	1.022-1.319	.022	1.196	1.012-1.414	.035
MELD score	1.074	1.014-1.138	.015	0.981	0.892-1.078	.684
Nonhepatic phase duration	1.004	0.725-1.391	.981			

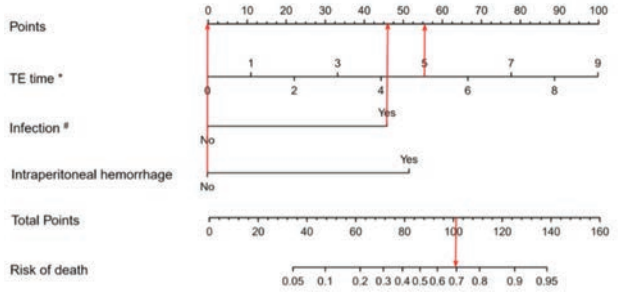
Abbreviations: BMI, body mass index (in kilograms divided by height in meters squared); D1, liver cancer; D2: severe hepatitis; D3, chronic end-stage liver disease; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; OR, odds ratio; TE, tracheal extubation

*Includes infections of abdominal cavity, respiratory tract, incision, and urinary system.

P < .05 was significant

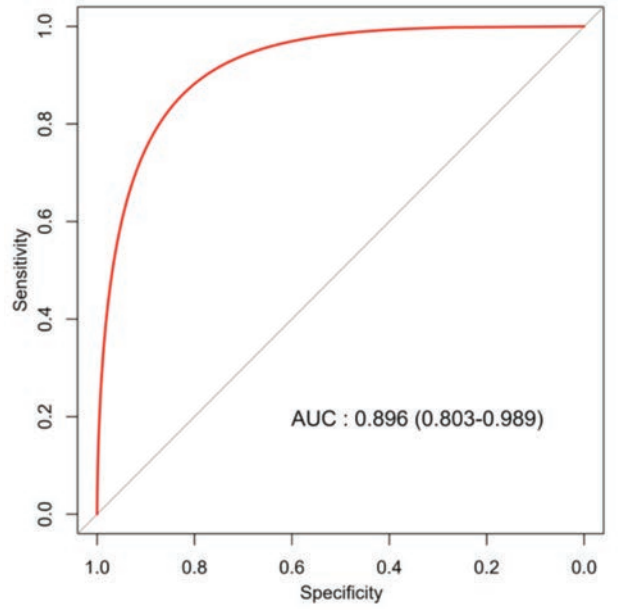
Intraperitoneal hemorrhage; postoperative intraperitoneal hemorrhage; Bold indicated statistical significance.

Figure 2. Example of Nomogram Prediction



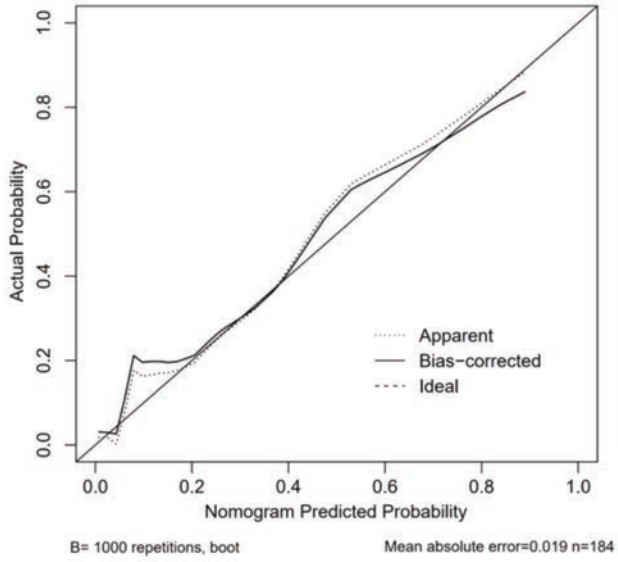
Abbreviations: TE, tracheal extubation

Figure 3. Receiver Operating Characteristic Curve for the Nomogram



Abbreviations: AUC, area under the curve

Figure 4. Calibration Curve of the Nomogram



Discussion

Mortality after LT is the consequence of multiple interacting factors and cannot be explained by a single etiological factor.[21] According to our results, we found hospital length of stay, ICU length of stay, BMI, MELD score, TE time, postoperative intraperitoneal hemorrhage, and postoperative infection to be risk factors for mortality after LT. Among them, postoperative infection, postoperative intraperitoneal hemorrhage, TE time, and BMI were the most independent prognostic factors for mortality after LT. Although our study showed that BMI was an independent factor for mortality after LT, whether BMI is a risk factor for LT remains controversial.[22,23] Several studies have shown that post-LT outcomes of morbidly obese (BMI ≥40) patients are similar in terms of patient and graft survival outcomes compared with outcomes of nonobese patients.[22,24-26] Therefore, BMI was excluded as a risk factor for death after LT in our study. Thus, we developed a nomogram model based on 3 factors to accurately predict mortality risk after LT.

Postoperative infection remains the most common complication and mortality factor after LT.[22,23] We also found that postoperative infection was a major cause of mortality after LT. We observed that 55% of patients in the nonsurvivor group had postoperative infections compared with 8.5% of patients in the survivor group ($P < .001$). The cause of post-LT infections may be related to the patient's usual immunocompromised status secondary to immunosuppressive therapy, poor liver function, and clinical conditions. Patients whose liver function does not recover after LT are more likely to be infected.[27,28]

Postoperative intraperitoneal hemorrhage is another potentially fatal complication after LT that usually requires emergency surgical treatment.[19,29] Several studies have demonstrated low short-term survival in patients with postoperative intraperitoneal hemorrhage.[19,29,30] We observed that postoperative intraperitoneal hemorrhage was an independent risk factor for mortality after LT. The following factors may increase the incidence of postoperative intraperitoneal hemorrhage. During LT, the graft may develop an ischemia-reperfusion injury, which may lead to graft loss or primary failure.[31] In addition, the recipient may develop a hypocoagulable state after LT. Early thrombocytopenia after LT is common due

to platelet activation and depletion after graft reperfusion.[32] Moreover, the coagulation changes in LT patients are extremely complex, and patients may develop coagulopathies postoperatively.[33]

After LT surgery, patients are returned to the ICU with endotracheal intubation. Longer intubation times are associated with higher complications, including ventilator-associated pneumonia and increased mortality.[34] Glanemann and colleagues[35] concluded that LT patients did not benefit from mechanical ventilation. In the setting of mechanical ventilation, increased pulmonary vascular resistance due to lung inflation may increase right ventricular afterload, and the inferior vena cava and hepatic vein reflux caused by related tricuspid regurgitation may produce venous congestion.[36] Conversely, decreased intrapleural pressure in spontaneously breathing patients can improve venous return, including hepatic blood flow. Therefore, spontaneous ventilation can improve hepatic venous return and liver graft circulation, thereby enhancing liver graft recovery.[37] This is consistent with our study that TE time was a risk factor in patients after LT surgery.

Although Child-Pugh classification is commonly used for LT, there are some problems. First, the extent of ascites and encephalopathy are both subjective assessments by physical examination alone. Second, both ascites and encephalopathy may be affected by treatments such as diuretics, albumin infusions, and lactulose, and it is unclear whether ascites and encephalopathy are scored best or worst or independent of specific treatments.[38,39] Several studies have demonstrated that Child-Pugh score was not a predictor of mortality in patients after LT.[40] To reduce subjective assessments, we did not use Child-Pugh in this study but instead used MELD score.

The study has several limitations. First, the cohort of patients was from a single center with potential selection bias, including ethnicity, graft size, living donor age, surgical techniques, and surgical indications. Second, more than 30% of data for cold ischemia time (CIT) were missing for this study, so we excluded this variable. However, all LT procedures were performed according to national guidelines for donation after cardiac death in China,[41] for which CIT is less than 12 hours. Also, it is still controversial whether CIT of within 12 hours has a significant effect on LT survival.[42-44] Moreover, the preservation method plays a key role in the consequences of CIT[43]; older donor age and CIT have a cumulative effect on LT survival.[45,46] Therefore, the absence of CIT did not affect the results of our study. Finally, the relatively small sample size may also limit the generality of our conclusions. Therefore, the nomogram is likely to be influenced by single-center perioperative care and clinical management, and multicenter validation is required in future studies.

Our results suggest that reducing TE time, postoperative infection, and intraperitoneal hemorrhage during the perioperative period of LT can significantly improve patient outcomes. In addition, the nomogram model that we developed to predict mortality risk in LT shows good distinguishing power in predicting the prognosis after LT. Although it cannot replace the clinician's judgment of the prognosis, it can provide a convenient tool for individualized assessment of mortality in patients after LT.

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Induction Versus Maintenance Immunosuppression After Intestinal Transplant: Determining Which Treatment Most Impacts Long-Term Patient And Graft Survival

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Abstract

Objectives: **Immunosuppressive strategies for intestinal transplant have changed over time. However, specific intestinal transplant-oriented protocols and reports on long-term maintenance regimens are scarce. Our objective was to evaluate the impact of 2 different initial immunosuppressive protocols based on thymoglobulin (group A) and basiliximab (anti-interleukin 2 antibody) (group B) and of changes to maintenance immunosuppression over long-term follow-up in intestinal transplant recipients.**
Materials and Methods: **We performed a retrospective analysis of a prospectively established protocol for intestinal transplant immunosuppression, conducted between May 2006 and December 2020. We analyzed 51 intestinal transplant recipients, with 6 patients excluded because of early death or graft loss. Acute cellular rejection frequency and grade, number of acute cellular rejection episodes, time to the first acute cellular rejection episode, response to treatment, number of patients who progressed to chronic allograft rejection, kidney function, infections, incidence of posttransplant lymphoproliferative disorder and graft-versus-host disease, and patient and graft survival were analyzed.**
Results: **In the study groups, there were 87 acute cellular rejection episodes in 45 patients (33 in group A and 54 in group B). We found degree of acute cellular rejection to be mild in 45 patients, moderate in 18, and severe in 24 (not significant between groups). Our comparison of induction therapy (thymoglobulin [group A] vs interleukin 2 antibody [group B]) did not show any statistical difference during clinical follow-up. Long-term review showed that all patients were on tacrolimus. Five-year patient and graft survival rates were 62% and 45% for group A and 54% and 46% for group B, respectively (not significant).**
Conclusions: **Long-term patient and graft outcomes reflected the use of an individualized follow-up with adjustments and changes in immunosuppressive medications according to the patient's clinical course and complications rather than based on the induction immunosuppressive protocol used.**

Key words: *Immunological risk, Immunosuppressive protocol, Intestinal transplant*

Introduction

Despite the improvements in surgical techniques and clinical management, the intestine is still one of the most challenging organs to be transplanted. The main reason is the immunological characteristics of the intestine, which are unique and not yet fully understood.[1,2]

Back in 1989, the use of tacrolimus and steroids became the gold standard for maintenance immunosuppression in intestinal transplant (ITx), allowing long-term survival and a reduction in the number of rejection episodes.[3-5] Because of a greater immunoreactivity of the intestine compared with other organs, required levels of tacrolimus were higher in order to reduce acute cellular rejection (ACR) rates.[6,7] Renal failure, higher infection rates, and post-transplant lymphoproliferative disorders (PTLD) were some of the negative consequences of this

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

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Experimental and Clinical Transplantation (2022) 12: 1105-1113

DOI: 10.6002/ect.2022.0359

approach; therefore, the use of induction as primer immunosuppressive therapy in an effort for lower maintenance in the long-term was implemented.[4] Since then, most of the large-volume centers and the Intestinal Transplant Registry[4,6,8] have analyzed early and long-term results on ITx based on the use of an induction therapy.[9-11] These analyses opened several questions: can we justify a 10-year survival based on a given induction therapy, and are we minimizing the importance of the changes made as part of the individual clinically required adjustments in the long-term maintenance immunosuppression during a patient's clinical course?

Thus far, these questions have remained unanswered, and no immunosuppressive protocol or specific drug has been developed solely for ITx recipients.[12-18] Thus, the aim of this study was to evaluate the effects of the initial immunosuppressive protocol and the effects of the clinically indicated changes instrumented in the immunosuppressive therapy during the long-term follow up in a cohort of patients seen at a single center from 2006 to 2020.

Materials and Methods

A retrospective analysis of a prospectively established protocol and database was conducted between May 2006 and December 2020. The study was approved by our Institutional Review Board (approval number 1477-1119).

Immunological risk, definitions, and group assignments

At the time of transplant, the induction therapy for each patient was selected according to the following criteria: ABO compatible mismatch, high panel reactive antibody titer (>30%) requiring the use of a desensitization protocol, retransplant,[19] multiorgan graft (combined or multivisceral), or B-cell-positive crossmatch at the time of the transplant (group A). The other criteria were ABO compatible match or low panel reactive antibody titer (<30%) and single-organ transplant (isolated ITx) (group B).

Immunosuppression by group

The primary immunosuppressive protocol assigned for group A was thymoglobulin (4.5-10.5 mg/kg), tacrolimus, sirolimus, and steroids.[7] Five patients in group A were highly sensitized and received intravenous immunoglobulin pre-ITx, achieving a successful desensitization. For group B, the induction was interleukin 2 (IL-2) antibody (given intravenously at 10 mg in patients <30 kg or at 20 mg in patients >30 kg and administered on post-ITx days 0 and 4), tacrolimus, mycophenolate mofetil (MMF), and steroids.

Within the first month after ITx and during the rejection episodes, the target tacrolimus level was 12 to 15 ng/mL (during years 2006-2008). Starting in 2009, the protocol was modified, with reduction of target tacrolimus levels to 10 to 12 ng/mL (during years 2009-2020) and tacrolimus levels gradually decreased to the 4- to 6-ng/mL range after the third month post-ITx and in the long term follow-up. Sirolimus target levels were initially 10 to 12 ng/mL and then reduced to 6 to 10 ng/mL at month 3 post-ITx (during years 2006-2008). From 2009 to 2020, the sirolimus target levels were reduced to 8 to 10 ng/mL for the first 3 months and to 4 to 6 ng/mL thereafter. All recipients had a prospective crossmatch done before the engraftment; ITx was not carried out in patients with positive T-cell crossmatch.[20]

Follow-up

Surveillance endoscopies and protocol biopsies were performed twice per week during the first 6 weeks post-ITx, weekly for the next 6 weeks, monthly up to the end of the first year, then 3 times per year up to year 3, and then every 6 months up to year 5 and yearly thereafter.

Immunosuppression and acute cellular rejection

All rejection episodes were identified through protocols or clinically indicated biopsies according to the accepted pathology criteria.[21,22] In case of a confirmed rejection episode, patients were treated based on the rejection severity. Patients with mild episodes received 2 intravenous boluses of methylprednisolone (20 mg/kg for pediatric patients or 1 g for adult patients), given as a single daily dose. Patients with moderate rejection were treated with similar steroid boluses followed by standard steroid tapering. Patients with severe rejection were treated with thymoglobulin as a daily 1 mg/kg dose over 7 days together with a steroid bolus on the first day and tapering, given simultaneously with sirolimus (it was either initiated or the doses were increased if the patient was already receiving it).

Variables analyzed

We analyzed the following variables: biopsy-proven ACR frequency and severity of episodes, number of ACR episodes, time from ITx to the first ACR episode, response to treatment, number of patients who progressed to chronic allograft rejection, tacrolimus target levels, creatinine levels as marker of kidney function, number of infection episodes, incidence of PTLD and graft-versus-host disease (GVHD), and patient and graft survival at 30 days and 6 months as well as at 1, 3, 5 and 10 years posttransplant. We used variables to compare risk between groups.

Statistical analyses

Variables are presented as mean ± SD. We used chi-square test and Z score to compare proportions and Kaplan-Meier with log-rank to analyze and compare survival rates. $P < .05$ was considered significant. We used SPSS version 25.0 for statistical analysis. Timelines were plotted using the "ggplot[2] package" in the R environment.

Results

Between May 2006 and December 2020, 51 ITx procedures were performed at our center. Six patients were excluded due to death or graft loss during the first week post-ITx. There were 21 patients in group A and 24 patients in group B. Patient characteristics are shown in Table 1.

Transplant description

Of 45 patients, 32 received isolated ITx, 4 patients received combined liver-intestine transplant, 1 patients received combined liver-intestine-kidney transplant, 3 patients had multivisceral transplant, 1 patient had multivisceral with kidney, and 1 patient had modified multivisceral. Three patients were retransplanted, with 1 the recipient of the multivisceral with the kidney graft and 1 the recipient of a combined liver-intestine-kidney graft.

Donor features

Intestinal transplant was performed with organs from deceased donors. Mean donor age was 5.6 ± 5.4 years for pediatric patients (<18 years) and 24.9 ± 7.7 years for adult patients (>18 years). Thirty-six ITx procedures were done with ABO compatible donors and 6 patients had ITx procedures with ABO compatible mismatched donors. Total ischemia time was 7:28 ± 2:09 hours. Warm ischemia time was 39.43 ± 11.83 minutes (39.50 ± 13.59 min in group A and 39.36 ± 10.31 min in group B; P = not significant [NS]). Forty-three donors were cytomegalovirus (CMV) IgG positive (19/21 in group A and 18/24 in group B).

Table 1. Characteristics of Intestinal Transplant Recipients

Characteristic	Group A (n = 21)	Group B (n = 24)	*P*
Mean age ± SD, y	17 ± 14	17 ± 18	
Median age, y	12	8	
Male patients, No. (%)	14 (67%)	19 (79%)	.54
Adult patients, No.	7	9	1.00
Pediatric patients, No.	14	15	
Diagnosis at evaluation, No. (%)			.74
Intestinal atresia	1 (5%)	2 (8%)	
Gardner syndrome	1 (5%)	0 (0%)	
Gastroschisis	0 (0%)	2 (8%)	
Hirschsprung disease	3 (14%)	5 (21%)	
Necrotizing enterocolitis	1 (5%)	1 (4%)	
CIPO	1 (5%)	2 (8%)	
Thrombosis	0 (0%)	0 (0%)	
Trauma	0 (0%)	2 (8%)	
Volvulus	6 (29%)	6 (25%)	
Other	1 (5%)	0 (0%)	
Ischemia	2 (10%)	1 (4%)	
Microvillus inclusions disease	1 (5%)	1 (4%)	
Postsurgical complications	0 (0%)	1 (4%)	
ACR-resistant enterectomy	3 (24%)	0 (0%)	
Cystic lymphangioma	1 (5%)	1 (4%)	
Indication for transplant, No. (%)			.84
Lack of vascular access	6 (29%)	12 (50%)	
Catheter-related sepsis	2 (10%)	3 (13%)	
IFPNALD	7 (33%)	7 (21%)	
Unresectable tumor	1 (5%)	0 (0%)	
No reconstructible GIT	1 (5%)	1 (4%)	
Hypersensitized	1 (5%)	0 (0%)	
Exfoliative ACR/enterectomy	2 (10%)	0 (0%)	
Familial adenomatous polyposis	1 (5%)	0 (0%)	
Microvillus inclusion disease	0 (0%)	1 (4%)	
Mean time on wait list ± SD, days	247 ± 251	183 ± 201	.34

Abbreviations: ACR, acute cellular rejection; CIPO, chronic intestinal pseudo-obstruction; GIT, gastrointestinal tract; IFPNALD, intestinal failure parenteral nutrition-associated liver disease

Group A had immunosuppression based on thymoglobulin, and group B had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

Immunosuppression

Induction therapy was given as established in our protocol, with 21 patients in group A receiving thymoglobulin and 24 patients in group B receiving IL-2 antibody.

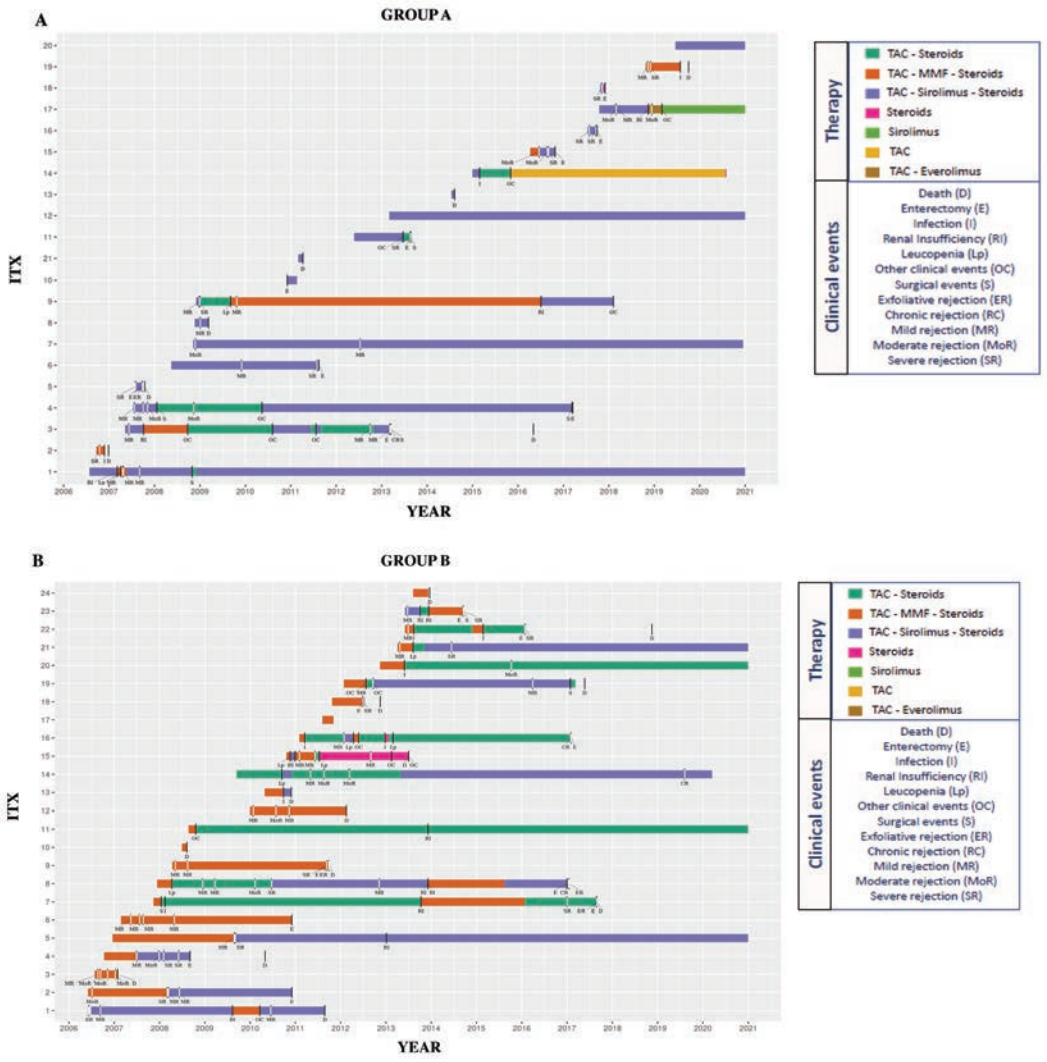
Long-term immunosuppression and adjustments due to clinical requirements are shown in Figure 1 (changes during the first 30 days were graphed as different initial therapy). In all cases, immediate post-ITx maintenance immunosuppression was started as planned by the predetermined protocol.

At day 30 post-ITx, 20/21 patients in group A continued under follow-up: 16 patients (80%) remained on sirolimus/tacrolimus/steroids, 3 patients (15%) were switched from sirolimus to MMF, and 1 patient

(5%) was switched to other therapy. At day 90 post-ITx, 11 patients (52%) remained on sirolimus/tacrolimus/steroids, 1 patient (5%) was switched to MMF, and 2 patients (10%) were left on double therapy. At the end of year 1, 11/21 group A patients continued under follow-up: 7 patients (33%) remained on sirolimus/tacrolimus/steroids, 2 patients (10%) were on MMF/tacrolimus/steroids, 1 (5%) was on double therapy, and 1 patient (5%) was switched to another drug. At the end of year 5, there were 7/21 patients in group A on follow-up: 4 (19%) were on sirolimus/tacrolimus/steroids, 1 (5%) was on MMF/tacrolimus/steroids, 1 (5%) was on double therapy, and 1 (5%) was receiving other therapy (Figure 1A).

In group B, at 30 days post-ITx, 23/24 patients were under follow-up, with 20 patients (87%) remaining on MMF/tacrolimus/steroids, 2 patients (9%) switched from MMF to sirolimus, and 1 patient (4%) left on tacrolimus/steroids. At 90 days post-ITx, 22/24 group B patients were under follow-up: 16 (66%) remained on the initial protocol, 3 (13%) were switched to sirolimus, and 3 (13%) were left on double therapy. At the end of 1 year, 17/24 group B patients were under follow-up: 6 (25%) remained on MMF/tacrolimus/steroids, 5 (21%) were on sirolimus/tacrolimus/steroids, 5 (21%) were on double therapy, and 1 (4%) was switched to other therapy. At year 5, there were 8 group B patients under follow-up: 1 (4%) remained on

Figure 1. Long-Term Immunosuppression Changes and Clinical Events During Patient Follow-Up



Abbreviations: TAC, tacrolimus

(A) Group A follow-up. **(B)** Group B follow-up. Group A had immunosup-pression based on thymoglobulin, and group B had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

MMF/tacrolimus/steroids, 5 (21%) were on sirolimus/tacrolimus/steroids, and 2 (8%) were on double therapy (Figure 1B).

The main reason for changing from MMF to sirolimus was the occurrence of ACR, and the main reason for changing from sirolimus to MMF was progressive renal insufficiency. All patients were kept on tacrolimus for the entire follow-up, except for 1 patient in group A, who received only sirolimus. No differences were found in tacrolimus levels over time between group A and group B, and mean target levels were within the expected level at 30 days (15.1 ± 4.7 ng/mL), 1 year (8.6 ± 2.9 ng/mL), and 5 years posttransplant (8.0 ± 2.5 ng/mL) in all patients.

Acute cellular rejection

The total number of ACR episodes was 87 (33 patients in group A vs 54 patients in group B): 45 with mild episodes (16 in group A vs 29 in group B), 18 with moderate episodes (7 in group A vs 11 in group B), and 24 with severe episodes (10 in group A vs 14 in group B) but with no significant difference between ACR number of episodes and severity when comparing both groups (Table 2 and Figure 2A). The global mean time from ITx to the first ACR episode was 148 ± 501 days (66 ± 150 days in group A vs 219 ± 672 days in group B; *P* = NS).

During the first 30 days post-ITx, 10 patients (48%) in group A and 8 patients (33%) in group B experienced ACR, and, from day 30 to day 90, 3 patients (14%) in group A and 2 patients (8%) in group B experienced ACR. By the end of the first year, 30 of 45 patients (66%) developed new ACR episodes, with 13 (44%) in group A and 17 (56%) in group B (*P* = NS between groups and periods; Table 3). The long-term rejection-free survival rate was 38.5% in group A and 26.9% in group B (*P* = NS). Figure 2B shows the rejection-free survival at the end of the first year.

Table 2. Number of Rejection Episodes by Group

Group	No. of Rejection Episodes			
	Mild	Moderate	Severe	Total
A (n= 21)	16	7	10	33
B (n= 24)	29	11	14	54
Total	45	18	24	87

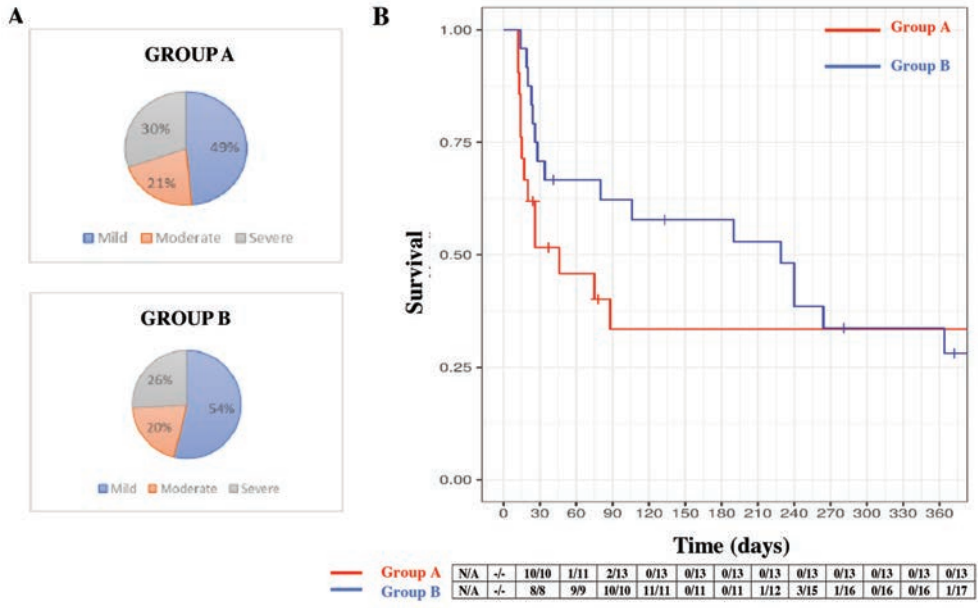
Group A had immunosuppression based on thymoglobulin, and group B had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

Table 3. Time to First Acute Cellular Rejection Episode

Time to First Rejection, days	No. of Episodes		
	Total	Group A	Group B
<30	17	10	8
30-90	5	3	2
90-365	7	0	7
>365	3	2	1
No rejection >365	12	6	6

Group A had immunosuppression based on thymoglobulin, and group B had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

Figure 2. Total Number, Severity of Acute Cellular Rejection, and Association With Immunosuppression Therapy



(A) Number and percentages of rejection episodes by degree and by group. **(B)** Years free of acute cellular rejection (survival) by risk group (*P* = not significant). Group A had immunosuppression based on thymoglobulin, and group B had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

The total number of graft losses related to rejection episodes was 7/87 (8%) in group A and 8/87 (9%) in group B (*P* = NS). Two graft losses due to chronic rejection were identified in group A and 2 in group B (*P* = NS).

Infectious episodes

Figure 3 shows the distribution of the infections during the immediate posttransplant, induction, and maintenance periods. There were a total of 38 infectious episodes. During the first 30 days post-ITx, 5 episodes (13%) were diagnosed in group A (3 bacterial and 2 viral episodes) and 3 episodes (7%) were diagnosed in group B (2 bacterial and 1 viral episodes) (*P* = NS). From day 31 to the end of the first year, 6 episodes (16%) were recorded in group A (3 bacterial, 2 fungal, and 1 viral) and 5 episodes (13%) in group B (1 viral, 2 bacterial, and 2 fungal) (*P* = NS). During the maintenance period, 4 infectious episodes (11%) were observed in group A (3 bacterial and 1 parasitic) and 13 episodes (34%) were observed in group B (4 bacterial, 1 fungus, and 8 viral) (*P* = .03 between groups).

From a total of 15 viral infections (3 in group A and 12 in group B; *P* = .03), 13 occurred in pediatric patients and 2 occurred in adults patients. The etiologies were CMV (n = 4), Epstein-Barr virus (EBV; n = 8), coinfection of CMV and EBV (n = 2), or another virus (n = 2). After year 1 post-ITx, a diagnosis of EBV-related PTLD was shown in 8 children and in 1 adult.

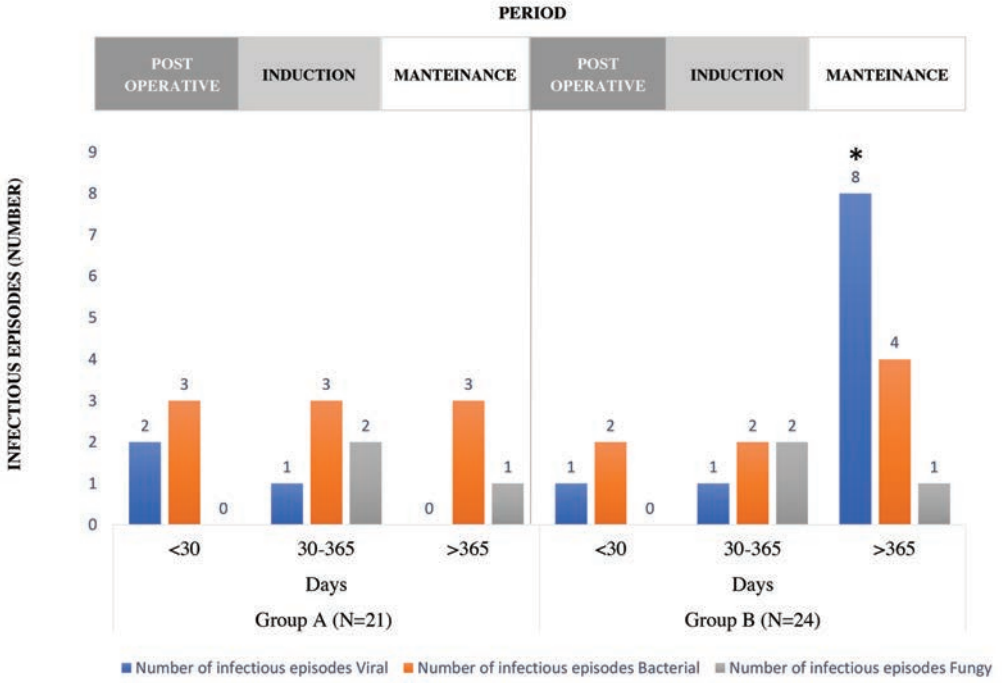
Kidney function

The mean estimated creatinine clearances for group A and group B were 147 and 130 mL/min at 30 days, 124 and 100 mL/min at 60 days, 108 and 106 mL/min at 90 days, and 107 and 90 mL/min at the end of year 1 (*P* = NS at each time point and by groups).

Other variables

There were 9 patients with PTLD (median day of appearance at 1525 ± 703 days), including 8 pediatric patients and 1 adult patient, with 7 patients in group B (1 adult and 6 pediatric patients) and 2 pediatric patients in group A (*P* = 0.1). Furthermore, 8 of 9 patients (89%) developed EBV-related PTLD. We also observed 1 patient with GVHD in group A who was a recipient of a retransplant with multivisceral graft, including a kidney and spleen.

Figure 3. Total Number of Infectious Episodes



Group **A** had immunosuppression based on thymoglobulin, and group **B** had immunosuppression based on basiliximab (anti-interleukin 2 antibody). **P* = .03 viral infections group A versus group B. From 15 viral infections, 13 occurred in pediatric patients and 2 occurred in adult patients.

Mortality and long-term survival

Overall, at 30 days, 6 months, and 1, 3, 5, and 10 years posttransplant, patient survival rates were 95%, 73%, 68%, 68%, 62%, and 53%, respectively, in group A and 100%, 91%, 82%, 68%, 54%, and 37%, respectively, in group B respectively (Figure 4A). Graft survival rates at 30 days, 6 months, and 1, 3, 5, and 10 years posttransplant were 95%, 67%, 62%, 51%, 45%, and 29% in group A, respectively, and 100%, 92%, 79%, 58%, 46%, and 21%, respectively, in group B (Figure 4B). There were no significant differences in patient or graft survival between groups (P = NS).

In our patient cohort, mortality occurred in 21 of 45 patients (47%). At 180 days, 7 patients had died due to infectious episodes (5 in group A and 1 in group B) and rejections (1 in group A). At 1 year, 3 patients had died from infections (2 in group B) and GVHD (1 in group A). Seven patients died between 1 and 5 years post-ITx. The causes of death were PTLD (1 in group A and 1 in group B), infectious episodes (3 in group B), suicide (1 in group B), and unknown (1 in group B). Four deaths were related to PTLD (1 in group B), rejection (1 in group 1), and infectious diseases (1 in group A and 1 in group B).

Discussion

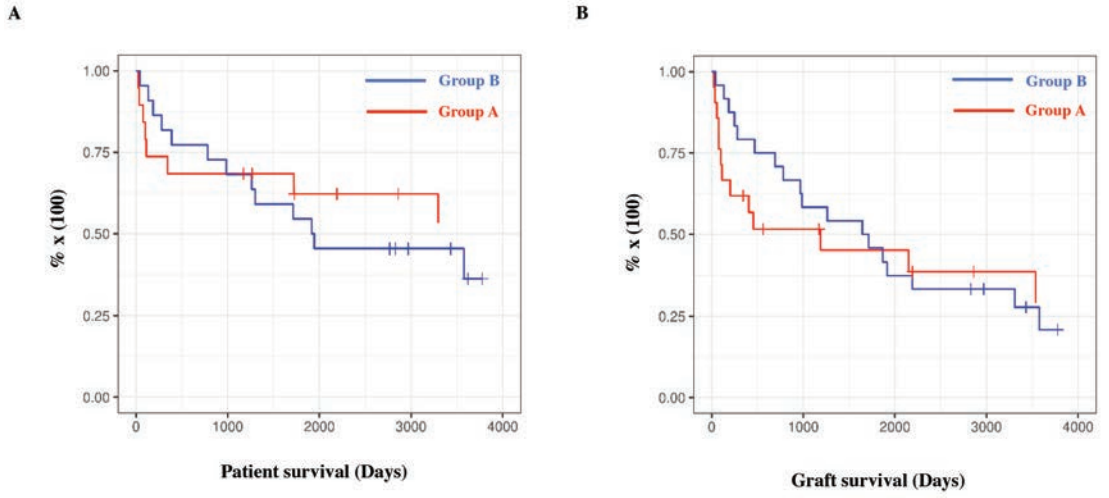
Despite improvements made in the field of ITx over the past decades, immunological management remains the major challenge and continues to be the main cause of graft loss and death.

In 2006, protocols were established with an aim for a close follow-up to evaluate the long-term results of immunosuppression. The induction therapy of each transplant candidate was defined ahead of the procedure; the protocols were designed based on the protocols used for ITx recipients, kidney transplant, and the unpublished experience at the Intestinal Transplant Program at Mount Sinai Hospital.[23-29] Under the premise that inclusion of the liver in ITx increases the receptor lymphoid cells load with a higher risk of ACR and GVHD, thymoglobulin was proposed for induction therapy[3,30,31] in multivisceral grafts. The protective effects of the liver, when transplanted along with other organs, has been proposed, mainly based on its immunoglobulin absorptive capacity, which can reduce the chances of ACR. However, a direct effect on cellular-mediated rejection has not been proven.[32-34] Results from single centers and the International ITx Registry have shown a positive effect on long-term graft survival, but not on early graft survival, supporting our hypothesis.[4,35-37]

Despite single-center reports on the use of thymoglobulin or IL-2 antibody,[6,10,38] our study represents, to our knowledge, the first and the largest prospective single-center comparison of thymoglobulin and IL-2 antibody induction in ITx recipients and to consider changes in the immunosuppressive regimen during follow-up.

In this study, we observed a higher incidence of graft and patient survival in favor of the regimen

Figure 4. Patient **(A)** and Graft **(B)** Survival



Group **A** had immunosuppression based on thymoglobulin, and group **B** had immunosuppression based on basiliximab (anti-interleukin 2 antibody).

used for group B within the first year posttransplant but also with comparable results in the long-term. The early mortality rate observed in patients receiving thymoglobulin may be related to the fact that most multiorgan recipients receive thymoglobulin, implying the inherited complications associated with this regimen as a main reason for graft and patient loss but not to rejection. Although the number of patients in our study was limited, when nonrejection-related early deaths were removed from the analysis, we observed no significant difference in the early incidence of rejection between the groups, supporting that thymoglobulin administration reduces the risk of more severe types of rejection and graft loss early posttransplant.

With regard to maintenance therapy and modifications over time, the introduction of sirolimus as a third drug resulted in a significantly lower incidence of early rejection and has improved graft survival in kidney transplant recipients.[39-41] Although equivalent results were reported in ITx, the major benefit observed was tolerability of the drug for periods longer than 1 year.[41] In our experience, 20% of the patients in group A discontinued sirolimus due to intolerance (15%), worsening renal function (5%), or other side effects (5%). These patients were switched to MMF (15%) or double therapy (5%).

If we analyze the development of infectious complications by group, group A had higher mortality associated with bacterial infections during the first year posttransplant. In the long-term, the infectious episodes then became mainly viral. The first observation might be related to the need for a more aggressive therapy in patients with higher immunological risk, and, as a consequence of the accumulative immunosuppressive effect, the second observation might be associated with a higher proportion of pediatric patients surviving the ITx procedure in the long-term once immunosuppressive doses and levels are reduced.[15]

Our study confirmed the null hypothesis that long-term results after ITx are the result of long-term individualized care, close follow-up, and personalized immunosuppressive management. When we analyzed the drugs used for maintenance, crossover between regimens could be observed and switches occurring because of clinically necessary reasons (rejection episodes, infectious complications, renal disfunction, or other clinical observations such as leukopenia, hypertension, diabetes, or diarrhea). The multiple confounders make it extremely difficult to interpret differences between groups. Therefore, the pretransplant stratification proposed is adequate to classify groups in the early after ITx period. In the long-term, clinical care can equalize immunological risks and rejection results, supporting the statement that long-term results cannot be justified as result of the elected induction therapy, as previously proposed.

Conclusions

Induction therapies chosen based on immunological risk impact early results and may have an effect on infectious outcomes[6,10]; however, the long-term outcomes are the consequence of the long-term maintenance immunosuppressive regimens, according to the clinical events that each patient has during the course after ITx.

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Safety and Efficacy of Co-transplantation of Hematopoietic Stem Cells Combined With Human Umbilical Cord-Derived Mesenchymal Stem Cells in Children With Severe Aplastic Anemia: A Single-Center Experience

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Abstract

Objectives: **The most important problems that limit the effectiveness of allogeneic hematopoietic stem cell transplantation in patients with severe aplastic anemia are graft failure and graft-versus-host disease. Mesenchymal stem cells can support normal hematopoiesis and prevent graft-versus-host disease. We aimed to analyze the effects of combined transplant of human umbilical cord-derived mesenchymal stem cells and matched donor allogeneic hematopoietic stem cells in children with severe aplastic anemia.**

Materials and Methods: **We retrospectively examined 15 pediatric patients with severe aplastic anemia who received fludarabine-based reduced intensity conditioning regimen and intravenously infused human umbilical cord-derived mesenchymal stem cells at a dose of 1×10^6/kg recipient body weight within 12 to 18 hours before hematopoietic stem cells infusion. We evaluated the engraftment rate, the frequency and severity of graft-versus-host disease, and the overall survival rate.**

Results: **No patients had adverse events related to intravenously human umbilical cord-derived mesenchymal stem cells infusion. All patients achieved successful engraftment and sustained donor chimerism. The median time for neutrophil and platelet engraftment was 14 and 25 days, respectively. The frequency was 20% for grade III/IV acute graft-versus-host disease and 15.3% for chronic graft-versus-host disease. Patients were followed-up for a median of 33 months (range, 2-89 months). The 5-year overall survival rate was 80%.**

Conclusions: **Combined transplant of matched donor hematopoietic stem cells with human umbilical cord-derived mesenchymal stem cells is safe in pediatric patients with severe aplastic anemia. The achievement of engraftment in all of our patients and the acceptable frequency of acute and chronic graft-versus-host disease and survival rate are encouraging.**

Key words: *Acquired aplastic anemia, Allogeneic hematopoietic stem cell transplantation, Mesenchymal stem cells*

Introduction

Acquired aplastic anemia is a hematopoietic disorder caused by destruction of hematopoietic stem cells (HSCs) and progenitor cells, due to hematotoxic agents such as drugs, chemical agents, radiation, and virus. The severity of the disease is evaluated by the criteria published in 1976.[1] Severe aplastic anemia (SAA) is a life-threatening disease, and there are 2 treatment options currently: immunosuppressive therapy and allogeneic hematopoietic stem cell transplantation (HSCT). In children and young adults with HLA-matched sibling donors (MSD) or matched family donors (MFD), a related HSCT is the initial treatment of choice. For patients with an insufficient hematological response with immunosuppressive therapy, matched unrelated donor (MUD) HSCT and haploidentical donor HSCT options should be considered.[2] The most important problems that limit

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Acknowledgements: *The authors thank all of the Labcell laboratory team members who contributed to the production of MSCs, especially Muhammet Yılancı, PhD, and Merve Kongur, PhD. The authors have not received any funding or grants in support of the presented research or for the preparation of this work and have no declarations of potential conflicts of interest.*

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Experimental and Clinical Transplantation (2022) 12: 1114-1121

DOI: 10.6002/ect.2021.0027

the effectiveness of allogeneic HSCT are graft failure and graft-versus-host disease (GVHD).[3]

Mesenchymal stem cells (MSCs) are characterized by self-renewal, low immunogenicity, and multi-directional differentiation into several mesenchymal lineages.[4] Mesenchymal stem cells are also primary cells that support the proliferation of HSCs by providing scaffold and secreting cytokines and adhesion molecules.[5] Furthermore, MSCs have immunomodulatory and immunosuppressive effects by inhibition of T-cell proliferation to alloantigens. It has been demonstrated that coinfusion of MSCs can support hematopoiesis, enhance donor engraftment of HSCs, and reduce the incidence of GVHD following HSCT.[5,6] Bone marrow (BM) stroma is damaged by hematotoxic agents, such as drugs, chemical agents, radiation, and viruses; this could poorly affect hematopoietic engraftment in patients with SAA who are undergoing HSCT. Therefore, combined transplant of MSCs plus HSCTs in patients with SAA is quite rational.[7]

In this study, we retrospectively analyzed the efficacy and safety of human umbilical cord-derived (hUC)-MSC infusion in 15 pediatric patients with SAA who received matched (related and unrelated) donor HSCT. The primary endpoint of our study was to evaluate the engraftment rate and the frequency and severity of acute GVHD (aGVHD) and chronic GVHD (cGVHD). The secondary endpoint was to determine the overall survival rate.

Materials and Methods

Patients

From June 2014, we performed combined transplant with hUC-MSCs and HSCs in 15 pediatric patients with SAA who underwent allogeneic HSCT. The use of MSCs was approved by the National Ministry of Health for each patient. Informed consent was obtained from parents or legal guardians before treatment. Patients met the following inclusion criteria: (1) were in line with criteria of the International Aplastic Anemia Study Group, aplastic anemia diagnostic criteria for SAA1; (2) were age <18 years old; and (3) had received MUD HSCT and did not respond to at least 1 immunosuppressive therapy cycle. Exclusion criteria included patients who did not fulfill all of the inclusion criteria.

Of 15 SAA pediatric patients enrolled in the study, 10 were males and 5 were females with a median age of 11 years (range, 2-18 years). Twelve patients (80%) had not responded to previous immunosuppressive therapy, including 1 or more courses of steroids ± cyclosporine ± anti-human antithymocyte globulin (ATG), and all were transfusion dependent. The median time from diagnosis to HSCT was 17 months (range, 2-172 months) (Table 1).

Donors and HLA matching

Seven patients had matched related donors (6 were MSD, 1 was MFD), and 8 patients had MUD (HLA 10/10: 5 patients, HLA 9/10: 3 patients). ABO

Table 1. Characteristics of Pediatric Patients With Severe Aplastic Anemia Who Received Hematopoietic Stem Cell Transplantation (N = 15)

Patient No.	Age, y/Sex	Time From Diagnosis to HSCT, mo	Previous Therapy	Type of Donor	Stem Cell Source	ABO Matching	Conditioning Regimen	Total ATG Dose	GVHD Prophylaxis
1	18/F	172	CsA+CS	MSD	BM	Matched	Flu+CTX+ATG	10 mg/kg	CsA+MTX
2	9.5/M	90	CS	MSD	BM	Matched	Flu+CTX+ATG	15 mg/kg	CsA+MTX
3	5/M	12		MSD	BM	Major	Flu+CTX+ATG	10 mg/kg	CsA+MTX
4	9/F	12	CsA+ATG+CS	MSD	BM	Matched	Flu+CTX+ATG	30 mg/kg	CsA+MTX
5	19/M	41	CsA+ATG+CS	MSD	BM	Minor	Flu+CTX+ATG	20 mg/kg	CsA+MTX
6	12/F	2	CS	MSD	BM	Mix	Flu+CTX+ATG	20 mg/kg	CsA+MMF
7	12.5/M	12	CS	MFD	BM+PBSCs	Minor	Flu+CTX+ATG	30 mg/kg	CsA+MTX+MMF
8	2/M	17	CS	MUD (10/10)	PBSCs	Matched	Flu+CTX+ATG	10 mg/kg	CsA+MTX
9	16/M	3		MUD (9/10)	PBSCs	Matched	Flu+CTX+ATG	10 mg/kg	CsA+MTX
10	3/M	22	CsA+ATG+CS	MUD (10/10)	BM	Minor	Flu+CTX+ATG	15 mg/kg	CsA+MTX+MMF
11	17/M	16	CsA+ATG+CS	MUD (10/10)	PBSCs	Major	Flu+CTX+ATG	30 mg/kg	CsA+MM
12	11/F	21	CsA+ATG+CS	MUD (9/10)	PBSCs	Minor	Flu+CTX+ATG	10 mg/kg	CsA+MMF
13	16/F	58	CsA+CS	MUD (9/10)	BM	Minor	Flu+CTX+ATG	10 mg/kg	Tac+MMF
14	9/F	4		MUD (10/10)	BM	Matched	Flu+CTX+ATG	20 mg/kg	CsA+MMF
15	10/M	47	CsA+ATG+CS	MUD (10/10)	PBSCs	Matched	Flu+CTX+ATG	20 mg/kg	Tac+MMF

Abbreviations: ATG, antithymocyte globulin; BM, bone marrow; CsA, cyclosporine; CS, corticosteroid; CTX, cyclophosphamide; F, female; Flu, fludarabine; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; M, male; MFD, matched family donor; MMF, mycophenolate mofetil; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor; PBSC, peripheral blood stem cell; SAA, severe aplastic anemia; Tac, tacrolimus

blood group mismatching was detected in 8 patients (Table 1).

Conditioning regimen

All patients received pretransplant reduced intensity conditioning regimens with cyclophosphamide, fludarabine, and ATG (rabbit ATG), with 50 mg/kg of cyclophosphamide on days -7, -6, -5, and -4; 30 mg/m^2 of fludarabine on days -5, -4, -3, and -2; and 2.5 to 7.5 mg/kg ATG on days -5, -4, -3, and -2 (Table 1).

Prophylaxis and diagnosis of graft-versus-host disease

The prophylactic therapy for GVHD was adjusted based on the allogeneic HSCT donor, stem cell source, and pretransplant history of patients. Recipients of MSD HSCT procedures usually received cyclosporine and short-term methotrexate, whereas recipients of MUD HSCT usually received cyclosporine and mycophenolate mofetil (MMF). Details are shown in Table 1. Diagnosis and clinical grading of GVHD were performed according to Glucksberg-Seattle criteria.

Isolation and preparation of human umbilical cord-derived mesenchymal stem cells

Human umbilical cord tissue was donated by third-party donors with their consent. Production of hUC-MSCs was performed at the Acibadem Labcell Cell Laboratory, which meets Good Manufacturing Practice standards. Preparation of hUC-MSCs was as previously reported.[8] The final product was prepared for application after being packaged in a 20-mL vial containing 2 × 10^6 cells/mL or in a 5-mL vial containing 5 × 10^6 cells/mL.

Infusion of human umbilical cord-derived mesenchymal stem cells

Ten patients were administered a single dose of hUC-MSCs on day -1, and 5 patients were administered 2 doses of hUC-MSCs on day -1 and on day +5. Patients were monitored for vital signs and symptoms of allergy during transfusion. A suspension of hUC-MSCs was intravenously infused within 10 minutes. The MSC target dose for infusion was 1 × 10^6/kg recipient body weight. The first dose of MSCs was infused intravenously within 12 to 18 hours before HSC infusion. Median first dose of hUC-MSCs was 1.0 × 10^6 cells/kg (range, 1-1.47 × 10^6 cells/kg), and median second dose was 1.0 × 10^6 cells/kg (range, 1-1.15 × 10^6 cells/kg) (Table 2).

Source and count of hematopoietic stem cells

Nine patients received unmanipulated BM, 5 patients received unmanipulated peripheral blood stem cells (PBSCs), and 1 patient received BM + PBSCs. The median total nucleated cell (TNC) and CD34+ cell counts were 4.29 × 10^8/kg and 3.8×10^6/kg, respectively. In patients who received BM transplant,

Table 2. Clinical Outcomes of Hematopoietic Stem Cell Transplantation in 15 Pediatric Patients With Severe Aplastic Anemia

Patient No.	CD34+ Cells/BW	TNCs/BW	Infusion Day of hUC-MSC	Engraftment	aGVHD	cGVHD	Chimerism	Follow-Up, mo	Outcome/Cause of Death
1	3.35 × 10^6	9.01 × 10^8	-1	Yes	No	Yes, limited	Full donor	48	Alive
2	2.3 × 10^6	4.32 × 10^8	-1	Yes	No	No	Full donor	57	Alive
3	3.36 × 10^6	4.27 × 10^8	-1	Yes	No	No	Mixt	34	Alive
4	9.57 × 10^6	3.91 × 10^8	-1	Yes	No	No	Full donor	70	Alive
5	2.16 × 10^6	2.42 × 10^8	-1, +5	Yes	No	No	Full donor	13	Alive
6	1.64 × 10^6	2.37 × 10^8	-1, +5	Yes	No	No	Full donor	25	Alive
7	2.70 × 10^6	4.22 × 10^8	-1	Yes	No	No	Full donor	7	Dead (CMV pneumonia + TA-TMA + renal failure)
8	8.19 × 10^6	4.59 × 10^8	-1	Yes	No	No	Full donor	59	Alive
9	4.36 × 10^6	6.23 × 10^8	-1	Yes	No	No	Full donor	34	Alive
10	4.49 × 10^6	3.93 × 10^8	-1	Yes	Yes (grade I/II)	No	Full donor	89	Alive
11	5.68 × 10^6	8.74 × 10^8	-1, +5	Yes	Yes (grade I/II)	No	Full donor	24	Alive
12	6.84 × 10^6	9.72 × 10^8	-1	Yes	Yes (grade III/IV)	Yes-limited	Full donor	33	Alive
13	3.8 × 10^6	1.91 × 10^8	-1	Yes	Yes (grade III/IV)		Full donor	2	Dead (PRESS + TA-TMA, Klebsiella septicemia)
14	3.1 × 10^6	2.92 × 10^8	-1, +5	Yes	Yes (grade III/IV)	No	Full donor	13	Alive
15	4.0 × 10^6	4.78 × 10^8	-1, +5	Yes	No		Full donor	2	Dead (achromobacter xylosoxidans septicemia

Abbreviations: aGVHD, acute graft-versus-host disease; BW, body weight; cGVHD, chronic graft-versus-host disease; CMV, cytomegalovirus; hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; PRESS, posterior reversible leukoencephalopathy syndrome; TA-TMA, transplant-associated thrombotic microangiopathy; TNCs, total nucleated cells

the median TNC count was 3.91×10^8/kg and that of CD34+ cells was 3.35×10^6/kg. In patients who received PBSC transplant, the median TNC count was 6.23×10^8/kg and that of CD34+ cells was 5.02×10^6/kg (Table 2).

Definitions and assessment of engraftment and chimerism

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count above 0.5×10^9/L, and platelet engraftment was defined as the first day of a week with the platelet count exceeding 20×10^9/L in the absence of transfusion. Hematopoietic chimerism was assessed using peripheral blood samples of the patient and donor via short-tandem repeated sequence-polymerase chain reaction DNA fingerprinting for all pairs. A BM sample from each patient was analyzed for hematopoietic chimerism every 30 days until 90 days after HSCT.

Statistical analyses

The Statistical Package for Social Sciences statistical package program (SPSS version 16.0) was used for analysis of the data. Data were evaluated using descriptive statistical methods (median, mean, minimum, maximum). Continuous data are presented as median (range) due to the overall small sample size. Categorical data are presented as count and percentage. The cumulative incidence of aGVHD and cGVHD was also evaluated by the Kaplan-Meier estimate. Overall survival rate was evaluated with Kaplan-Meier survival analysis.

Results

Engraftment and chimerism

All 15 patients (100%) achieved hematopoietic reconstitution after HSCT. The median time for neutrophil and platelet engraftment was 14 days (range, 9-25 days) and 25 days (range, 15-95 days), respectively. Although 14 patients achieved full donor chimerism within 30 days after HSC plus hUC-MSC transplant, 1 patient (patient 3) achieved and sustained stable mixed donor chimerism (Table 2). Another patient (patient 5) who was full chimeric on day 30 after HSCs plus hUC-MSCs transplant and had mixed chimerism on day 90 was administered donor lymphocyte infusion, with full chimerism then obtained. None of the patients developed poor graft function.

Graft-versus-host disease

Of the 15 patients, 5 (33.3%) experienced aGVHD, including 2 with grade I/II and 3 (20%) with grade III/IV aGVHD, which occurred at a median time after HSCT of 22 days (range, 14-39 days). Acute GVHD involved the skin in 7.1% of patients (1/14), gastrointestinal system and skin in 14.3% of patients (2/14), skin and liver in 7.1% of patients (1/14), and gastrointestinal system and liver in 7.1% of patients (1/14). The frequency of cGVHD was 15.3% (2/13 patients) (Table 2).

Infectious complications in the 100-day period after hematopoietic stem cell transplant

Severe bacterial sepsis occurred in 3 patients (20%), with 1 patient recovering after antimicrobial treatment and 2 patients who died (Table 2). Invasive fungal infection was seen in 2 patients. Hepatosplenic candidiasis occurred in 1 patient (patient 14). Another patient with a history of invasive pulmonary aspergillosis (patient 11) before HSCT developed an intracerebral fungal lesion, which required surgery on day 19. Both patients were successfully treated. Cytomegalovirus (CMV) reactivation was detected by DNA testing in 8 patients, with 7 patients treated successfully with ganciclovir. However, 1 patient (patient 7), who had transplant-associated thrombotic microangiopathy and renal failure, died during month 7 from CMV pneumonia. BK viruria was detected in 5 patients. None of the patients had positivity for Epstein-Barr virus, parvovirus, adenovirus, or human herpesvirus 6.

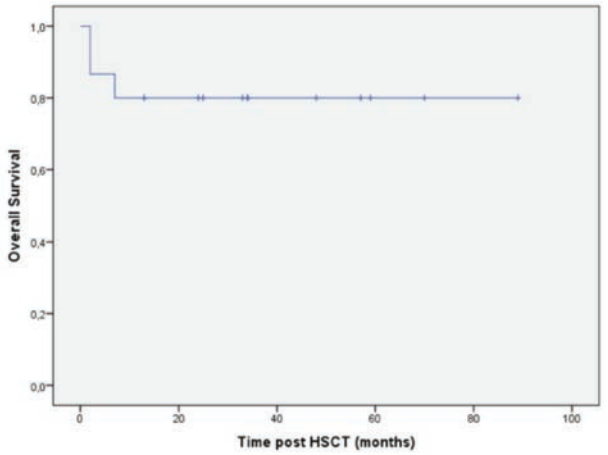
Infusion-related adverse event

No adverse events related to intravenous hUC-MSCs infusion were detected in any patient.

Survival and causes of death

Patients were followed-up for a median of 33 months (range, 2-89 months). The 5-year overall survival rate was 80% (Figure 1). A total of 3 patients died (Table 2). Two patients (patients 13 and 15) died of severe bacterial sepsis 54 and 62 days after HSCT. The other patient (patient 7) died of CMV pneumonia. In patients who died, median time from diagnosis to HSCT was longer than in surviving patients at, respectively, 47 and 16.5 months.

Figure 1. Overall Survival of Children With Severe Aplastic Anemia After Hematopoietic Stem Cell Transplantation (Kaplan-Meier)



Abbreviations: HSCT, hematopoietic stem cell transplantation

Discussion

Because of major improvements in the HSCT procedure in recent years, superiority of allogeneic HSCT over immunosuppressive therapy in SAA patients has been demonstrated, with allogeneic HSCT considered the primary therapy for the effective cure of this disease. Although fludarabine-based reduced intensity conditioning regimens including fludarabine + cyclophosphamide + ATG ± TBI have been shown to reduce rejection and achieve better outcomes in HLA-matched donor HSCT,[9-11] major causes of poor outcomes of HSCT including death are still graft rejection and GVHD.[10] Therefore, studies have focused on the prevention of graft rejection and prevention/minimization of GVHD, including the use of MSCs as a cell-based therapeutic approach.[12]

Mesenchymal stem cells are widely used in cellular therapy in allogeneic HSCT. They may create a more favorable BM microenvironment. In addition, MSCs could prevent and treat GVHD.[13] The number of studies on the use and efficacy of MSCs in adults and children with SAA, especially in haploidentical transplants, has increased over time.[14-17] However, studies in the literature have shown heterogeneity in terms of source (BM-MSCs versus hUC-MSCs) and dose of MSCs, frequency of MSC infusions (once vs twice), administration time (before vs after HSCs infusion), and study population (adults vs children). Most of the studies are not randomized controlled studies but are case series. In addition, results of HSCT procedures with different donor types have been presented in the same study. Therefore, it is difficult to compare the results of the studies with each other and to make correct inferences. There are also studies in the literature in which MSCs infusion alone was used in patients with SAA resistant to traditional treatments.[18,19] In these studies, MSCs treatment was found to be safe, although not enough by itself to recover BM. There is little experience in pediatric patients.

Allogeneic HSCT provides a rapid hematopoietic reconstruction in patients with SAA. Lower engraftment rates have been reported with allogeneic HSCT alone.[20,21] In a study that compared 48 children with SAA transplanted from MSD versus 38 children transplanted from MUD, engraftment was achieved in 93.75% of patients after MSD HSCT and in 86.8% of patients after MUD HSCT.22 In addition, Bacigalupo and colleagues reported 17% graft failure in a retrospective study of 100 patients with SAA who underwent HSCT alone from an alternative donor.[10] However, rapid and sustained hematopoietic reconstitution has been reported in nearly all HSCTs in which MSCs combined transplant was performed. Wang and colleagues reported that engraftment was achieved in all 19 children with SAA who received MUD HSCT with UC-MSCs 1 hour before HSCs infusion.[23] In our study, median time for neutrophil and platelet engraftment was 12 (range, 9-21 days) and 14 (range, 8-24 days) days, respectively. However, Wang and colleagues noted that delayed rejection occurred in 1 case at 4 months after HSCT. In our study, allogeneic HSCT combined with infusion of hUC-MSCs resulted in successful engraftment of HSCs in all 15 patients (100%). Although the number of cases in our study is small, it is encouraging that no patient had graft failure, especially in those with MUD. The longer engraftment times in our study compared with the study from Wang and colleagues may be related to our use of BM as a stem cell source in 9 of 14 patients.

Because myeloablative conditioning regimens are not used in patients with SAA, aGVHD is not a serious problem, especially with MSD HSCT. However, high aGVHD rates have been reported in MUD (43%)[24] and haploidentical HSCT (39.2% and 42.1%)[25,26] in patients with SAA who had HSCT alone. In the literature, different aGVHD rates have been reported in studies where MSCs coinfusion was performed with HSCT. Fu and colleagues reported that no pediatric patients who received MSCs

infusion for MUD HSCT developed severe GVHD.[27] Wang and colleagues reported, in 19 MUD (10/10 matched = 9 patients, 9/10 matched = 6 patients, 8/10 matched = 4 patients) who had HSCT plus hUC-MSCs procedures, grade I aGVHD in 9 patients (47.3%) and grade III aGVHD in 1 patient (5.2%).[23] They infused PBSCs, and patients received cyclosporine + methotrexate+ MMF for GVHD prophylaxis. Our study showed that the frequency of grade I to IV aGVHD and grade III/IV was 33.3% and 20%, respectively. All but 1 of the patients without aGVHD were patients who received hUC-MSCs plus HSCT from a 10/10 matched donor (MSD= 6 patients, MFD= 1 patient, MUD = 2 patients). All of our patients who developed aGVHD had MUD HSCT, and we administered MMF instead of methotrexate with cyclosporine/tacrolimus in all but 1 patient. Patients who developed grade III/IV aGVHD were also patients who did not receive methotrexate. This may be related to the use of cyclosporine/tacrolimus + MMF, without use of methotrexate for GVHD prophylaxis. In 1988, Bacigalupo and colleagues reported that the combined use of cyclosporine and methotrexate is a predictor for favorable HSCT outcomes.[28]

In a recently published meta-analysis of patients who received haploidentical HSCT and hUC-MSCs, the rates of aGVHD, grade II-IV aGVHD, cGVHD, 2-year survival, and engraftment were similar to those who only received HSCT.[29] Thus, an important question is whether combined transplant of MSCs has a beneficial effect for another subgroup of patients, especially for those who have matched donors. In our study, aGVHD was not detected in any of our patients who received MSD HSCT. Another question is whether combined transplant for patients with MSD is really necessary. In our opinion, well-designed controlled clinical trials and a meta-analysis seem necessary to find the real effects of combined transplant of hUC-MSCs and HSCs on GVHD rates in patients with SAA who had transplant with matched donors.

Chronic GVHD is a major cause of morbidity in long-term survivors. Chen and colleagues[30] and Perez-Albuerne and colleagues,[24] in studies of HSCT alone in patients with SAA, reported frequencies of cGVHD of 28.3% and 35%, respectively. In our study, the frequency of cGVHD was 15.3%. which is markedly lower than most pediatric studies of patients who received matched donor HSCT alone. Wang and colleagues reported a rate of cGVHD of 5.2%.[23] This low rate can be explained by the low rate of severe aGVHD in that study.

Cytomegalovirus viral load and the risk of CMV disease increase with MSC infusions.[31] In our study, the reactivation rate of CMV in patients was 46.6%, with CMV-related death occurring in 1 patient. Wang and colleagues reported a higher CMV reactivation rate (78.9%).[23] Interestingly, Si and colleagues reported a very low CMV reactivation rate (5.4%) in children with SAA who received hUC-MSCs at 7 to 10 days after allogeneic HSCT from matched and haploidentical donors.[32] The effect of time of infusion of hUC-MSCs during the procedure on CMV reactivation may be debatable.

In our study, no adverse events related to intravenous hUC-MSCs infusion was detected in any patient. However, mild and self-limited side effects such as fever, chills, headaches, hypoxemia, mild dyspnea and diarrhea have been reported after MSCs infusion.[19,33]

Our study reported a mortality rate of 20% and a 5-year overall survival rate of 80%. Median follow-up time was 33 months, and the longest survival was up to 7 years. Three patients died due to infection. None of our study patients had severe GVHD resulting in death.

As a result of the observations in this study, we planned to omit MMF but add methotrexate to the GVHD prophylaxis in patients with MUD. On the other hand, we planned to make an individualized decision for each patient until certain results were reported with controlled studies and meta-analyses regarding the necessity of MSCs coinfusion in patients with MSD.

This pediatric single-center study has several limitations, including a small sample size and lack of a control group. Most of the similar studies in the literature do not have a control group, although some have used a historical control group. This problem may be overcome by performing meta-analysis of suitable case series. Despite these limitations, the achievement of engraftment in all of our patients and the acceptable frequency of aGVHD and cGVHD and survival rate are encouraging.

Conclusions

Our study supported that combined transplant of hUC-MSCs plus HSCs can be used safely and may increase the efficiency of the transplant procedure in

pediatric patients with SAA who undergo matched donor HSCT.

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Case Report

Coadministration of Immunosuppressant Treatments With Pegloticase in the Context of Solid-Organ Transplantation and Gout: A Case Report

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Abstract

Refractory gout can be treated with infusions of pegloticase, which metabolizes uric acid into a product readily excreted in urine. Antidrug antibodies often develop, leading to reduced efficacy and potential infusion reactions. The concomitant administration of immunosuppressive agents has been suggested as a means of mitigating the effects of drug-related immunogenicity, rendering treatment more tolerable, and resulting in better outcomes. This report presents cases of 2 patients with tophaceous gout, each having previously undergone a solid-organ transplant, each taking immunosuppressants to prevent organ rejection, and each successfully treated with pegloticase. Although data from randomized controlled studies are needed, these cases suggest that it may be beneficial to coadminister an immunosuppressive medication to extend drug persistence with pegloticase in the management of refractory gout. This approach could allow patients to receive long-term treatment, resulting in improved patient outcomes.

Key words: *Immunogenicity, Monosodium urate crystals, Uric acid*

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Acknowledgements: *The authors thank Dr. Cynthia Schmidt with the Leon S. McGoogan Health Sciences Library for her assistance with citations and references. Funding for this work was provided by the National Institutes of Health/National Institute on Alcohol Abuse and Alcoholism grant R25AA020818 through the Summer Undergraduate Alcohol Research Program at the University of Nebraska Medical Center. The funders had no role in the design or conduct of this study and no input on the report. Dr. Mikuls has received research funding from and has served as an advisor to Horizon Therapeutics.*

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Experimental and Clinical Transplantation (2022) 12: 1122-1125

DOI: 10.6002/ect.2022.0046

Introduction

Gout results from the deposition of monosodium urate crystals (MSU) due to persistently high serum urate (SU) levels. When SU levels consistently exceed 6.8 mg/dL, MSU deposition may result in arthritis flares and tophaceous deposits characteristic of gout. In non-primate mammals, uricase metabolizes uric acid into allantoin, a product more easily excreted in urine than uric acid. However, humans lack the genetic coding for uricase. Thus, uric acid represents the human end product of purine metabolism.[1]

Pegloticase, approved for management of treatment-refractory gout, incorporates mammalian recombinant uricase conjugated to polyethylene glycol.[2] This allows uric acid to be excreted as allantoin, leading to robust declines in SU. Polyethylene glycol conjugation increases solubility and half-life and decreases drug immunogenicity.[2] In phase 3 trials, the vast majority of patients initially experienced significant decreases in plasma uric acid levels. However, only 42% of patients were considered responders and achieved levels consistently below 6.0 mg/dL throughout months 3 and 6 of treatment.[1] Although both the responders and nonresponders in the study who received pegloticase (8 mg intra-venously [IV] biweekly) initially achieved low plasma uric acid levels, after week 10 the mean levels of eventual nonresponders were consistently >6.0 mg/dL.[1]

Concurrent administration of immunosuppressants to limit formation of antidrug antibodies may limit the reduction of pegloticase's immunogenicity, as previously demonstrated for other biologic medications. For example, infliximab use has been limited by the formation of antidrug antibodies,[3] and, as with pegloticase, resultant antibodies have been associated with both efficacy loss and infusion reactions. Coadministration of an immunosup-

pressant with infliximab, most often methotrexate or azathioprine, improves treatment persistence.[3] Similarly, coadministration of immunosuppressants with pegloticase may preserve its urate-lowering ability, as observed in the patients reported in this study.

The addition of immunosuppressants to pegloticase in gout treatment is a strategy that has been deployed effectively in limited reports. In a proof-of-concept case series, 10 patients with refractory gout were treated with pegloticase biweekly for at least 5 months.[4] Patients were concurrently administered folic acid and methotrexate. All patients tolerated complete courses, maintained target SU levels, and had no infusion-related reactions.[4] Similarly, at the time of data extraction in a retrospective chart review, of 10 patients with gout who were treated with pegloticase and concomitant methotrexate, 5 were complete responders, 3 were "still succeeding" on treatment, and only 2 ended treatment.[5]

When azathioprine was used concomitantly with pegloticase, a patient's gout was successfully treated with 98 weeks of pegloticase infusions.[6] This patient experienced decreased tophi size, SU levels, and flares in addition to no infusion-related reactions. During 2 periods of noncompliance with azathioprine, the patient experienced temporary SU increases that declined once treatment was reestablished,[6] illustrating the possibility that immunosuppression could prevent formation of anti-pegloticase antibodies and possibly even "recapture" treatment response after their formation.

Limited research is available on the relationship between pegloticase and immunosuppressants in the context of solid-organ transplantation. A recent case report presented a heart transplant patient using immunosuppressants while undergoing pegloticase treatment for tophaceous gout.[7] After the first infusion, the patient's SU levels decreased to 1.5 mg/dL and remained steady throughout her 38-week treatment. She experienced tophi resolution, no gout flares, and improvements in her mobility, pain, and overall quality of life.[7] In another study, 9 of 20 (45%) gout patients treated with pegloticase without concomitant immunosuppression developed antidrug antibodies. In contrast, only 1 of 7 (14%) organ transplant patients on immunosuppressants treated with pegloticase developed antidrug antibodies.[8]

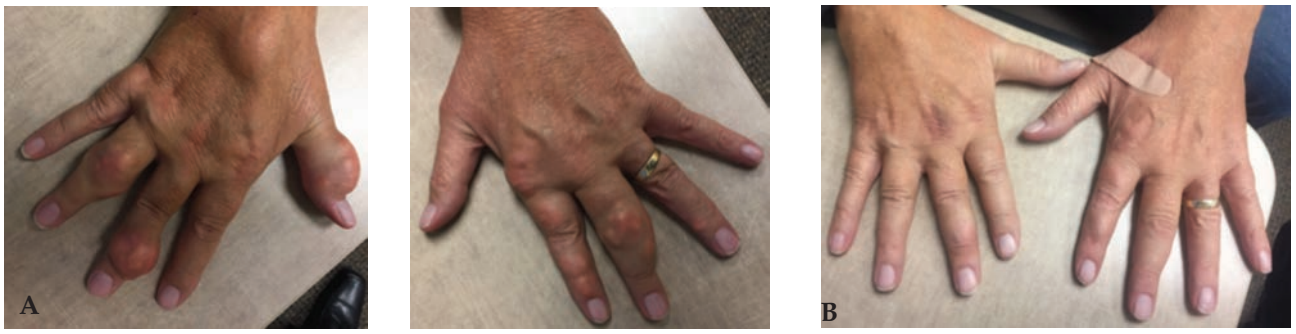
We present cases of 2 patients with treatment-refractory gout who experienced marked and sustained reductions in SU and favorable outcomes with pegloticase. Notably, each had a history of kidney transplant and received immunosuppressants throughout their gout treatment. Coupled with emerging data, the outcomes of these cases suggest that coadministration of immunosuppressant therapy with pegloticase may hinder the formation of antidrug antibodies, allowing for improved treatment persistence and optimization of patient outcomes.

Case Report

Case 1

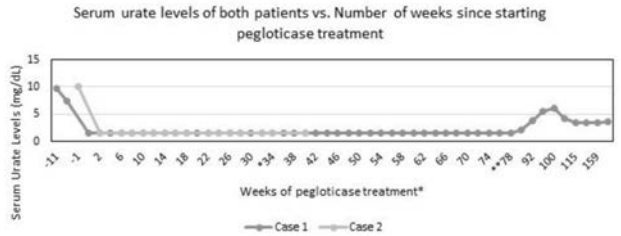
A 55-year-old man presented for evaluation and management of severe gout. He developed immunoglobulin A nephropathy in his twenties and experienced gout flares as his kidney function worsened but was not treated with urate-lowering therapy. The patient had received a kidney transplant from a living related donor (brother) at age 36 years. Immunosuppressive medications posttransplant included mycophenolate mofetil 1 g and tacrolimus 0.5 mg twice daily and prednisone 5 mg daily. He experienced no further flares, but several tophi gradually formed over his knees, elbows, and hands (Figure 1A). At the time of evaluation, 19 years posttransplant, he had an SU level of >10 mg/dL and was treated unsuccessfully with increasing doses of daily allopurinol (gradually titrated from 100 to 300 mg daily). Given the tophaceous burden posed, the decision was made to switch to pegloticase 8 mg IV biweekly rather than to initiate or add another oral urate-lowering agent. The patient experienced a severe flare immediately after the first infusion, which was treated with a prednisone burst and taper. He had no further flares, his SU level remained <1.5 mg/dL throughout therapy (Figure 2), and he reported improved pain and well-being (Figure 3A). Tophi were considerably reduced in size after 6 months of treatment and resolved after 12 to 14 months (Figure 1B). After 40 infusions across 18 months, pegloticase was discontinued and the patient transitioned back to allopurinol (150 mg/day). After an SU concentration increase, his allopurinol dose was increased (300 mg/day) (Figure 2). Nearly 2 years after treatment, the patient has not experienced recurrences of tophi or gout flares, and his SU level has consistently remained below target threshold of 6.0 mg/dL.

Figure 1. Patient 1 Before and After Administration of Pegloticase



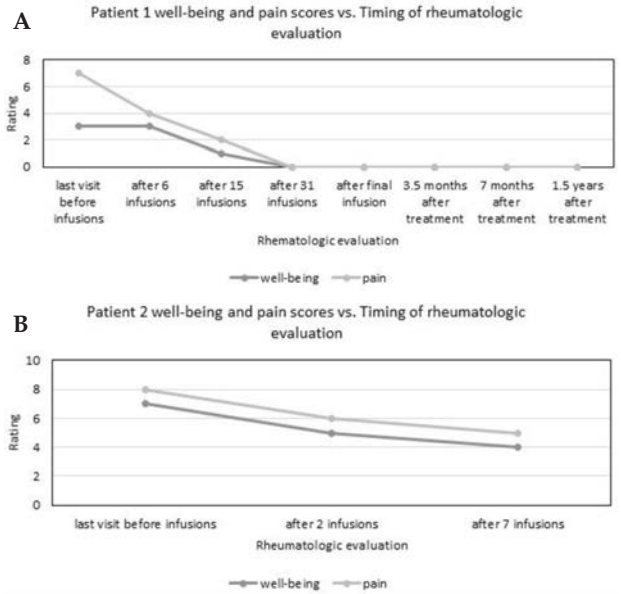
(A) Patient 1 before initiation of pegloticase; tophi were seen on both of the patient's hands, most notably on the thumb, middle finger, ring finger, and wrist of the right hand and on the index and middle fingers of the left hand. **(B)** After 18 months of pegloticase therapy, all of the tophi had decreased in size or resolved.

Figure 2. Rapid Decline in Serum Urate Levels After Pegloticase Treatment in Both Patients



After switching from pegloticase to allopurinol at week 78 (**), patient 1 experienced an increase in his serum urate concentration after transitioning to allopurinol 150 mg daily, which then declined after his daily allopurinol dose was increased to 300 mg daily and has remained <6.0 mg/dL through follow-up.

Figure 3. Well-Being and Pain Scores Over Time in Patients 1 and 2



(A) Patient 1 reported sustained improvements in his pain and well-being throughout pegloticase treatment. **(B)** Patient 2 also reported improvements in her pain and well-being scores. Note: measures not available after infusion 7.

Case 2

A 51-year-old woman sought rheumatologic evaluation for polyarthralgias, myalgias, and intermittent joint swelling. She had a history of "suspected gout," although this was unconfirmed via synovial fluid analysis. The patient had a history of polycystic kidney disease with progressive renal failure and underwent a deceased donor kidney transplant at age 46 years. Initial immunosuppressive medications included sirolimus and tacrolimus, but azathioprine replaced sirolimus after the patient had adverse effects. After the patient developed acute arthritis of the knee, arthrocentesis revealed MSU crystals. She was treated with intra-articular corticosteroids but declined urate-lowering therapy, partly because of the perceived risk of drug interaction with azathioprine and her reluctance to switch her immunosuppressive regimen. The patient later presented with mid and forefoot swelling suggestive of arthritis and newly developed tophi on several toes. Given her tophaceous burden and hesitancy to change immunosuppressive agents, she was treated with pegloticase 8 mg IV biweekly while continuing tacrolimus and azathioprine. She experienced a severe flare after the first infusion but otherwise tolerated treatment well and reported improved pain and well-being (Figure 3B). Tophi resolved after 4 to 5 months, and pegloticase was discontinued after 15 infusions across 8 months, with SU levels remaining <1.5 mg/dL throughout treatment (Figure 2). She transitioned to allopurinol (100 mg/day), and azathioprine was replaced with everolimus to avoid possible drug-drug interaction. Although this patient has not had a follow-up appointment to assess her SU level posttreatment, she has not indicated experiencing any flares since discontinuing pegloticase and instead postponed appointments, suggesting that her disease activity remains low.

Discussion

The long-term effectiveness of pegloticase is limited by the drug's clearance by antidrug antibodies.[1,2] Although 89% of participants in phase 3 trials have developed anti-pegloticase antibodies, those with the highest antibody titers were least responsive to treatment. Only 2% of participants with an antibody titer >1:2430 maintained their urate-lowering response, whereas 63% of participants with an antibody titer <1:2430 maintained the response.[1] These results suggest that antidrug antibody titers discriminate responders from nonresponders, and a lower antibody titer may be necessary to maintain lower urate levels.

There are at least 2 recent studies that examined the efficacy of coadministering immunosuppressants with pegloticase: The REduCing Immunogenicity to PegloticasE (RECIPE) Study[9] and the Study of Pegloticase Plus Methotrexate in Patients with Uncontrolled Gout (MIRROR).[10] The phase 2 RECIPE study was a double-blind, placebo-controlled study of 32 adults with chronic refractory gout who were initiated on pegloticase.[9] In the 12-week study, patients randomized to receive concomitant mycophenolate mofetil (1000 mg twice daily) were more than twice as likely as those randomized to placebo to achieve a SU level of <6.0 mg/dL (86% vs 40%; $P = .01$). The open-label MIRROR Study included 14 participants with gout who were initiated on pegloticase. Eleven of 14 patients (78.6%) satisfied the responder definition, which was defined as maintaining SU levels <6 mg/dL for ≥80% of the time during month 6 of treatment with pegloticase and concomitant methotrexate.[10] Although these studies did not include solid-organ transplant recipients, the results of these studies are informative.

The potential efficacy and durability of pegloticase with concomitant use of immunosup-pressants are illustrated in the 2 cases that we reported here. Although this strategy appears promising, rigorously designed randomized controlled studies are needed to determine whether immunosuppressants should be routinely prescribed with pegloticase in refractory gout treatment, particularly in high-risk populations such as those with a history of solid-organ transplant. Future studies are necessary to identify the comparative efficacy and safety of different immunosuppressive agents in this context. This point may be particularly relevant as comorbidities that are overrepresented in gout, such as chronic kidney disease or diabetes, could place patients at higher risk of treatment-related adverse events related to immunosup-pressive treatments.

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Rapidly Reversible Leukoencephalopathy After Acute Kidney Graft Rejection in a Patient With Systemic Lupus Erythematosus

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Abstract

The finding of white matter damage on brain magnetic resonance imaging may correspond to a wide variety of etiologies. The differential diagnosis may be particularly difficult in immunocompromised patients with a specific autoimmune disease or who are receiving medications after a solid-organ transplant. Herein, we describe the case of a 22-year-old woman who developed serious neurological complications after an acute rejection of a kidney graft that she had received a few months previous to treat a systemic lupus erythematosus-related nephritis. We discuss the possible hypotheses underlying the development of acute leukoencephalopathy in this setting.

Key words: *Acute kidney injury, Autoimmune disease, Central nervous system impairment, Encephalopathy, Renal transplantation, Solid-organ transplantation*

Introduction

The diagnosis of acute central nervous system (CNS) disorders may be difficult in patients who present with multiple diseases, such as immunocompromised patients receiving medications for an autoimmune disease or for organ transplant. Brain magnetic resonance imaging (MRI) is the most appropriate imaging modality to identify the specific types of brain tissue damage. Leukoencephalopathy is a generic term to describe disease processes of various etiologies. Herein, we describe a case of acute toxic leukoencephalopathy (ATL) in a young patient with systemic lupus erythematosus (SLE) who had recently received a renal transplant for rapid worsening of SLE-related nephritis.

Case Report

A 22-year-old African woman was admitted in August 2022 in the emergency department after a long flight back from Africa to Europe. She started to feel unwell during the flight, with abdominal pain, sleepiness, and rapid decrease of consciousness that required emergency admission. Shortly before this, while visiting Uganda for holidays, she had complained of asthenia, abdominal discomfort, and some episodes of diarrhea together with oliguria.

Her medical history showed that she had been diagnosed with SLE at the age of 14 years. Lupus nephritis developed as the main complication 4 years later. In July 2021, she had benefited from a kidney transplant from a living related donor (her mother). An initial episode of acute graft rejection occurred in November 2021 and was controlled by corticosteroids pulse therapy, plasma exchanges, and anti-T-lymphocyte globulins.

The patient was suspected of low adherence to the prescribed immunosuppressive therapy, which included prednisone 10 mg/day, tacrolimus 40 mg/day, and azathioprine 100 mg/day. Tacrolimus was not undetected at admission, and the accompanying relatives suspected that the patient had repeatedly omitted to take at least tacrolimus.

At admission to the emergency department, she had a temperature of 35.5 °C, heart rate of 82 beats/min, blood pressure of 132/85 mm Hg,

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

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Experimental and Clinical Transplantation (2022) 12: 1126-1130

DOI: 10.6002/ect.2022.0297

respiratory rate of 17 breaths/min, and pulse oximetry oxygen saturation of 100%. Her Glasgow coma scale score decreased from 14/15 to 8/15 within 1 hour and she presented with left-sided hemiparesia. She underwent orotracheal intubation. A brain computed tomography scan failed to reveal any lesion at that time (not shown). A lumbar puncture was then performed, and cerebrospinal fluid analysis only revealed 2 elements; multiplex polymerase chain reaction analysis remained negative for neurotropic viruses including JC/BK polyomavirus. Laboratory results showed arterial pH 7.11, bicarbonate level of 8 mmol/L, lactate of 0.5 mmol/L, potassium of 9.7 mmol/L, serum urea of 334 mg/dL, creatinine of 29.5 mg/dL, osmolality of 363 mmol/kg, hemoglobin of 6.2 g/dL, platelet count of 229 × 10^3 cells/μL, lactate dehydrogenase of 329 IU/L, and schistocytes of 1.8%. Specific biomarkers for SLE revealed C3 complement, 0.78 g/L (reference range, 0.90-1.80 g/L); C4 complement, 0.17 g/L (reference range, 0.10-0.40 g/L); antibody against double-stranded DNA immunoglobulin G, 15.0 IU/mL (reference range, <10 IU/mL); and C-reactive protein, 133.4 mg/L (reference range, <5.0 mg/L).

The patient was transferred to the intensive care unit for continuous venovenous hemofiltration. Acute kidney rejection was suspected, so tacrolimus was reintroduced at a daily dose of 40 mg for 4 days and then tapered down to 16 mg/d together with methylprednisolone 4 mg/d, while azathioprine was stopped. The peak serum concentration reached 40.7 ng/mL for tacrolimus. A brain MRI was performed 3 days after admission, and diffusion-weighted imaging (DWI) revealed severe white matter (WM) cytotoxic edema involving diffusely the supratentorial compartment without any other focal parenchymal injury. Only a faint concomitant vasogenic edema was observed on fluid-attenuated inversion recovery (FLAIR) T2-weighted images (that is, FLAIR with longer TE and TR times, for which T2 is the transverse relaxation time, TR is the repetition time, and TE is time to echo) (Figure 1, A-C).

A continuous electroencephalogram recording showed focal ictal discharges within right temporo-occipital area, which were treated with valproic acid. Immunophenotyping excluded a circulating B-cell lymphoma or an immune reconstitution inflammatory syndrome (IRIS). The neurological status rapidly improved, which allowed extubation shortly after the cerebral MRI, and complete recovery without neurological deficit occurred within the next 48 hours. A renal biopsy was performed on day 6 and confirmed acute cellular and humoral rejection. A follow-up brain MRI was performed 5 days after the initial MRI and revealed complete subsidence of cytotoxic edema (Figure 1, D-F). After evidence of the irreversible renal damage, a transplantectomy was initiated.

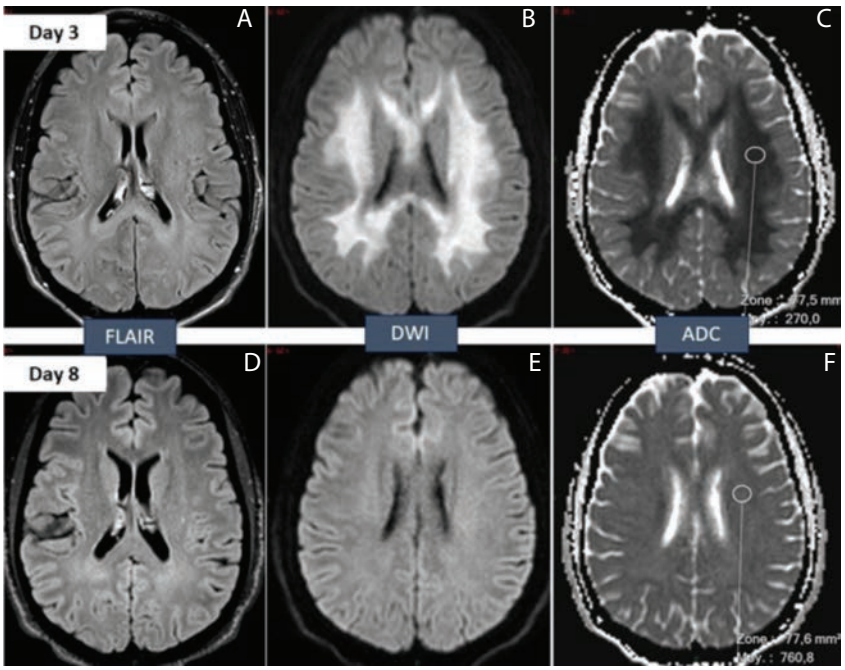
Discussion

Our patient, a young African woman who had previously received a kidney transplant to treat SLE-related nephritis, presented with a combination of severe neurological dysfunctions including loss of consciousness, seizure, and hemiparesis together with high suspicion of acute kidney graft rejection due to low compliance to the immunosuppressive treatment. Brain MRI work-up demonstrated rapidly reversible leukoencephalopathy with a strong prominence of cytotoxic edema of the WM.

In SLE, a wide range of neurological symptoms may be present, including psychiatric disorders, stroke, seizures, myelopathy, chorea, and headaches. Also, a wide range of abnormalities may be observed on cerebral MRI views. The finding of "leukoencephalopathy" (ie, WM damage on brain MRI) may encompass different conditions in SLE.[1] Diffuse leukoencephalopathy on T2-FLAIR sequences with hyperintensity on DWI reflecting cytotoxic edema with free-water diffusivity reduction featured by a decrease in apparent diffusion coefficient (ADC) values is more frequently observed in young women.[2] It may be the first manifestation of juvenile-onset neuropsychiatric SLE. Contrary to the ischemic stroke-related cytotoxic edema, the edema observed in SLE may be fully reversible as illustrated by our patient (Figure 1, E to F). In addition, some patients with diffuse leukoencephalopathy may also present with signs of intracranial hypertension as the consequence of concomitant vasogenic edema, which is best seen on T2-FLAIR views.[3]

In our patient, a combination of vasogenic and cytotoxic edema was observed but with a strong prominence of the cytotoxic edema. Treatment of SLE-related diffuse leukoencephalopathy requires intravenous steroid pulse therapy alone in most cases, but some refractory patients may require an additional treatment regimen with rituximab or cyclophosphamide.

Cgure 1. Magnetic Resonance Imaging Monitoring



Abbreviations: ADC, apparent diffusion coefficient; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; WM, white matter

Images in top row **(A, B, C)** show results from initial examination at day 3, with all 3 views in similar slice location through the supratentorial space. Images in bottom row **(D, E, F)** show results of follow-up examination 5 days later using similar sequences in similar slice location. **(A)** axial transverse FLAIR view shows faint hypersignal intensity of the WM prominently involving the parietal lobes (solid arrow) and the splenium of corpus callosum (dotted arrow) featuring only slight vasogenic edema. **(B)** DWI view shows diffuse abnormal hypersignal intensity within WM (asterisks) featuring strong cytotoxic **(C)** DWI-derived ADC map shows severe hypointensity of the WM (asterisks) with decreased ADC values at 270 × 10-6 mm^2/s (reference range, 700-800 × 10-6 mm^2/s), which confirms strong restriction of free-water diffusivity. **(D)** axial transverse FLAIR view shows moderate subsidence of the vasogenic edema. **(E)** DWI view shows complete normalization, which indicates complete recovery of the cytotoxic edema. **(F)** DWI-derived ADC map confirms normalization of ADC value at 760 × 10-6 mm^2/s.

Progressive multifocal encephalopathy (PML) is a rare and devastating demyelinating disease of the CNS caused by the so-called John Cunningham virus, of which immunological traces are observed in 70% of the general population. Progressive multifocal encephalopathy most often arises in patients with severe underlying immunosuppression, and SLE is associated with the highest risk for PML in patients with rheumatological conditions.[4] The PML complication of John Cunningham virus infection has also been described in kidney transplant recipients.[5] The prognosis of PML is poor, with a mortality rate reaching 60% to 75%.[6] Analysis by MRI plays a key role in the diagnosis of PML. In patients with severe immune deficiency, WM lesions do not enhance after intravenous perfusion of gadolinium-based contrast agent, and no restriction in water diffusion is observed. Conversely, the edges of the lesions can show contrast enhancement together with diffusion restriction in inflammatory PML. Treatment of PML usually relies on reduction of immunosuppression, which could be difficult in patients with underlying autoimmune diseases.[7]

There is also an overlap between PML and the IRIS. This syndrome was initially described in patients with HIV who had received highly active antiretroviral therapy. Very rarely, PML and IRIS have been observed in kidney transplant recipients[8] or in patients with SLE.[9] The mechanism is linked to a reduction or removal of immunosuppression with a paradoxical worsening of neurological symptoms due to recovery of inflammatory capabilities well assessed on contrast-enhanced MRI scans. There is no firm recommendation for treatment of IRIS because pulse steroid therapy and immunosuppression withdrawal carry their own intrinsic risks.

Posterior reversible encephalopathy syndrome (PRES) is a clinical radiological condition characterized by headache, sight disturbances, seizure, and altered mental status together with "pure" vasogenic

edema mainly affecting the subcortical WM of the occipital and parietal lobes. The pathognomonic MRI pattern of the condition combines strongly positive T2-FLAIR images for vasogenic edema and negative corresponding DWI views for cytotoxic edema. A central-variant PRES has been described that involves the basal ganglia and brainstem, with sparing of the posterior lobes.[10] This subtype has been reported on the setting of both SLE and renal transplant, and differences in the radiological presentation at MRI examination allows differentiation between both PRES subtypes, IRIS, PML, and diffuse leukoencephalopathy illustrated by our patient.[10]

Immunosuppressive drugs (eg, mycophenolate, tacrolimus, cyclosporine) for treatment of patients with SLE or for solid-organ recipients can also induce some forms of ATL, which could occur in up to 12.6% of patients.[11] The MRI findings in the condition are described as confluent and symmetric hyperintense areas on DWI prominently involving the cerebral WM associated with restricted water diffusivity (low ADC values appear dark on ADC-mapped views) featuring cytotoxic edema, as observed in our patient (Figure 1, B and C). One putative mechanism for the pattern could be the drug-induced disruption of the cerebral vasculature, which may result in intramyelinic (and not interstitial) edema.

The influence of blood urea nitrogen levels on the pathophysiology of ATL is not precisely known. In the series of 101 patients reported by Ozutemiz and colleagues, serum blood urea nitrogen was increased in 26% of the patients.[11] Coexistence of PRES and ATL has also been discussed.[12] Uremic encephalopathy has been associated with symmetric superficial and deep T2 hyperintensities, but some observations have reported on selective periventricular WM involvement sparing the basal ganglia on DWI.[13]

Finally, there are also cases of obvious association between SLE and B-cell lymphoma and between lymphoproliferative disorders, including B-cell lymphoma from Epstein-Barr virus-driven polyclonal proliferations, with solid-organ transplantation.[14] In immunocompromised patients such as patients with SLE, primary CNS lymphoma can be seen as homogenous, heterogeneous, or sometimes peripheral enhancement of lesions with mass effect and variable water diffusivity restriction.[1,15]

In our observation, clinical and biologic signs indicated that the activity of the SLE disease was weak. Therefore, diffuse demyelinating lesions due to the progression of SLE were deemed unlikely. Progressive multifocal encephalopathy, IRIS, PRES, and primary CNS lymphoma were reasonably excluded by the MRI work-up at admission and by the 5-day follow-up examination, which demonstrated complete subsidence of both types of cytotoxic edema. We concluded that the most likely cause was ATL due to toxic alterations of the blood-brain barrier synergistically caused by renal failure/hyperazotemia and by immunosuppressive drugs. As mentioned, azathioprine had been stopped, but a high dose of tacrolimus had been given immediately after hospital admission. A rapid clinical and radiological recovery together with improvement of the renal function after renal replacement therapy was observed, thereby rendering steroid pulse therapy unnecessary.

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Tacrolimus-Associated Pure Red Cell Aplasia in a Patient With Renal Transplant

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Abstract

Pure red cell aplasia is a relatively rare disease characterized by suppression or absence of erythroid precursors while other cell lineages are normal in the bone marrow. The disease could be secondary to other diseases or an adverse side effect of certain drugs. Tacrolimus is widely used as an immunosuppressive agent in solid-organ transplant without significant myelosuppressive effects. However, several tacrolimus-related pure red cell aplasia cases have been reported to date. Here, we report a case of a renal transplant recipient who developed tacrolimus-associated pure red cell aplasia in the posttransplant period and recovered dramatically after switching from tacrolimus to cyclosporine. Early diagnosis of pure red cell aplasia, which generally requires multiple blood transfusions, is very important because an increased number of blood transfusions can cause immunogenic effects and increased risk for allograft survival. Tacrolimus is a prominent drug for immunosuppression and is suspected to cause pure red cell aplasia during the posttransplant period; therefore, clinicians should consider a switch from tacrolimus to another immunosuppressive agent.

Key words: *Drug-induced pure red cell aplasia, Post-transplant anemia, Renal transplantation, Reticulocytopenia*

Introduction

Renal transplant is the most important option of renal replacement therapy; it reverses the negative effects of end-stage renal disease such as erythropoietin-dependent anemia. Posttransplant anemia may have various causes, including infections, medications, and poor graft function.[1] Various studies have reported drug-related mild-to-moderate anemia in different ratios caused by calcineurin inhibitors, mycophenolate mofetil, and others, after solid-organ transplant.[2] Pure red cell aplasia (PRCA) is a rare cause of posttransplant anemia, and it is characterized by severe normocytic anemia, reticulocytopenia, and absence of precursor erythroid cells in the bone marrow. Tacrolimus is an immunosuppressive agent that is often used during the posttransplant period, without a significant potential for myelosuppression. There are a small number of case reports in the literature of tacrolimus-associated PRCA. Here, we report a renal transplant recipient who developed PRCA while under treatment with tacrolimus, with a complete recovery after the switch from tacrolimus to cyclosporine.

Case Report

A 38-year-old man with end-stage renal disease due to immunoglobulin A nephropathy underwent an uncomplicated living-related renal transplant (from his mother) in August 2019. Four months after transplant (in December 2019), he was admitted to our outpatient hematology clinic and presented with fatigue, palpitation, and a history of erythroid transfusion (5 units) during the previous 1 month. He was under treatment with tacrolimus and prednisolone for immunosuppression; other medications were pantoprazole, metoprolol, and allopurinol. The physical examination was normal except for pallor, tachycardia, and a grade 2/6 systolic murmur. The hemoglobin level was 6.3 g/dL, and leukocyte and thrombocyte counts were normal. Serum creatinine

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

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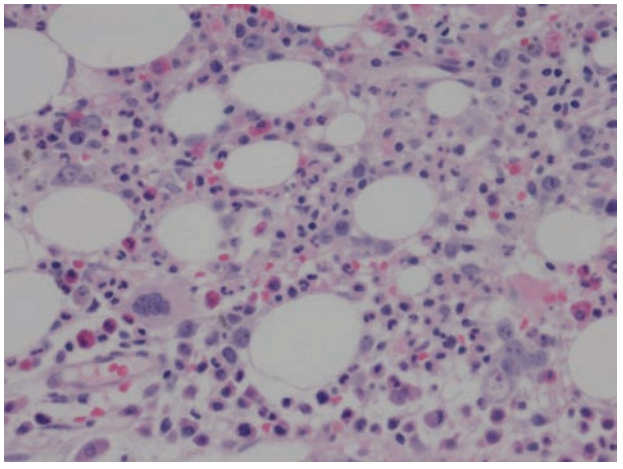
Experimental and Clinical Transplantation (2022) 12: 1131-1133

DOI: 10.6002/ect.2020.0290

was 1.5 mg/dL. A peripheral blood smear showed normocytic normochromic anemia, and the reticulocyte percentage was low (0.11%). There was no blood loss or hemolysis. Direct and indirect Coombs tests were negative. Biochemical results, serum iron studies, and vitamin B12 and folate levels were normal. The results of an enzyme–linked immunosorbent assay for viral infections (hepatitis B and C, cytomegalovirus, Epstein-Barr virus, and parvovirus B19) were also negative; furthermore, DNA for cytomegalovirus and BK virus was not found by polymerase chain reaction. To rule out autoimmune etiologies, we performed antinuclear antibody tests, serum complement levels, and serum and urine immunofixation electrophoresis, but no abnormality was detected. Imaging studies did not show any malignant disease, such as thymoma or lymphoma.

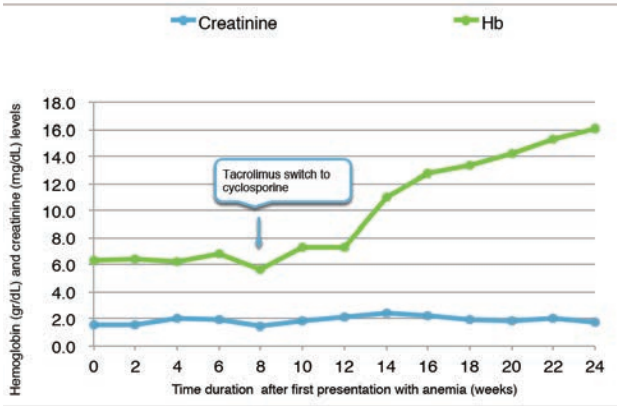
Bone marrow aspiration and biopsy revealed severe hypoplasia of erythroid series with normal maturation of other cell lines that is suggestive of PRCA (Figure 1). While we were investigating anemia, the patient required 5 units of erythroid transfusion. After we excluded other causes, we considered the possibility of tacrolimus as the causative agent because a few tacrolimus-associated PRCA cases have been reported in the literature. After tacrolimus was switched to cyclosporine, the hemoglobin level started to improve at 1 month and transfusion dependency disappeared. Four months after starting cyclosporine, his hemoglobin level was 16.1 g/dL and serum creatinine was 1.7 mg/dL (Figure 2).

Figure 1. Bone Marrow Biopsy With Hematoxylin and Eosin Stain Shows Severe Reduction in Red Blood Cell Precursors



Magnification, ×100.

Figure 2. Line Diagram Showing Serum Creatinine and Hemoglobin Levels of the Patient From Date of First Admission to the Hematology Department to the Present Date



Abbreviations: Hb, hemoglobin

Discussion

Pure red cell aplasia is a syndrome defined by a normocytic normochromic anemia with severe reticulocytopenia and, while other cell lines are in normal maturation, a marked reduction or absence of erythroid precursors exists in the bone marrow. Acquired PRCA is generally secondary to various disorders including systemic lupus erythematosus, chronic lymphocytic leukemia or large granular lymphocyte leukemia, parvovirus B19, thymoma or other solid tumors, drugs, or toxic agents.[3]

Multiple drugs have been reported to date (as case reports) as possible causes of PRCA, such as diphenylhydantoin, fludarabine, recombinant erythropoietin, and azathioprine.[3] In addition, immunosuppressive agents, particularly mycophenolate mofetil, may cause PRCA; mycophenolate mofetil-associated PRCA has been seen in the early period after transplant, and hematologic improvement has occurred rapidly after cessation of mycophenolate mofetil.[4,5] In our patient, mycophenolate mofetil had been already stopped in response to neutropenia and thrombocytopenia approximately 1 month before anemia developed.

Tacrolimus, which inhibits T-cell development and proliferation by decreasing production of interleukin 2, is widely used as an immunosuppressive agent in solid-organ transplant procedures without significant myelosuppression effect. However, several tacrolimus-related PRCA cases have been reported in the literature.[6-9] Although the mechanism by which tacrolimus may cause PRCA is still unknown, some reports have stated that

tacrolimus stimulates transforming growth factor β1 hyperexpression in mammalian cells; in addition, increased transforming growth factor β1 inhibits hematopoiesis and controls erythroid serial differentiation.[10,11] Conversely, tacrolimus has also been reported to be an alternative treatment option for PRCA.[12] However, there are other possible treatment options for PRCA, such as corticosteroids, cyclosporine, alkylating agents, antithymocyte globulin, alemtuzumab, and intravenous immunoglobulin; for example, the response rate in patients treated with cyclosporine (12 mg/kg/day) is 65% to 87%.[13]

Patil and colleagues reported results for 3 renal transplant cases in which tacrolimus was replaced with cyclosporine. For each case, patients improved completely within 4 months, and none of the patients required blood transfusions after stopping tacrolimus.[8]

In our patient, after we excluded other causes of PRCA such as infections (parvovirus B19), lymphoproliferative diseases, autoimmune disorders, and solid-organ tumors (thymoma), we considered a drug-associated cause for PRCA. Although the blood level of tacrolimus was generally in the normal range, we considered this as a possible causative agent for PRCA. After the switch from tacrolimus to cyclosporine, the patient responded to therapy dramatically, and with the cyclosporine treatment his hemoglobin remained in the normal range.

The improvement of anemia after withdrawal of tacrolimus supports the possible etiological role of tacrolimus in PRCA. Alternatively, cyclosporine is also a treatment option for PRCA. Therefore, it is difficult to discern whether the clinical improvement was related to the cessation of tacrolimus or, rather, to a therapeutic effect of cyclosporine.

Conclusions

The evaluation of anemia in renal transplant patients can be a challenging issue that requires careful consideration on multiple etiologies. Mild-to-moderate anemia may occur as a result of infections, drugs, or impaired graft function in patients after solid-organ transplant; however, if transfusion-dependent severe anemia with reticulocytopenia is evident during the posttransplant period, then PRCA should be considered (especially drug-associated PRCA). Furthermore, early diagnosis of PRCA, which generally requires multiple blood transfusions for the patient, is important because an increased number of blood transfusions can cause immunogenic effects and increased risk for allograft survival. Tacrolimus is a prominent drug that is suspected to cause PRCA during the posttransplant period (as in our patient). Clinicians should consider a switch from tacrolimus to another immunosuppressant, as we did.

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CASE REPORT

Severe Atherosclerosis in Donor Liver Vasculature: An Illustrative Case Report and Review of the Literature

Ayokunle S. Olowofela, Oscar K. Serrano, Raja Kandaswamy

Abstract

As the scarcity of transplantable organs continues to increase, juxtaposed with an aging donor population, transplant surgeons are increasingly confronted with marginal organ offers. The presence of atherosclerosis in the donor allograft has been shown to compromise the vascular integrity and predispose to vascular complications in the transplanted liver. Here, we present a case of 54-year-old brain-dead donor who was discovered to have a severely diseased aorta during organ recovery. Pathologic evaluation revealed severe atherosclerosis with calcifications. Because there was no evidence of donor graft dysfunction, we elected to proceed with implantation, although thoughtful consideration was given to aborting the procedure. The donor hepatic artery was resected from the bifurcation of the splenic artery and the common hepatic artery until no further gross atheromas were evident; this segment was then anastomosed with the recipient proper hepatic artery. The recipient is doing well 6 months after transplant without any significant adverse postoperative events. The presence of severe atherosclerosis should not discourage the use of an otherwise adequate graft. Novel newer preservation techniques, such as normothermic perfusion, may enable functional graft evaluation and can increase the utilization of marginal grafts.

Key words: *Deceased donor organs, Extended-criteria donor, Liver transplant, Older donor*

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Acknowledgements: *The authors have no sources of funding for this study and have no conflicts of interest to declare.*

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Experimental and Clinical Transplantation (2022) 12: 1134-1136

DOI: 10.6002/ect.2017.0229

Introduction

Since liver transplantation (LT) became the standard of care for treating end-stage liver disease, there has been an increase in the demand for deceased donor organs. In 2016, 7841 adult LTs were performed in the United States.[1] Unfortunately, the demand for donor organs far outnumbers the available grafts. Each year, over 2600 patients either die waiting for a donor liver or have to be removed from the transplant wait list because they became too sick to undergo LT.[1] This shortage has led surgeons to expand their criteria for acceptable grafts, which may not always be optimal but nonetheless can remove a candidate off the wait list.[2,3]

Vascular complications remain the Achilles heel of LT. These complications are associated with high morbidity and mortality and often lead to graft loss.[4] Considering that cerebrovascular accidents remain one of the primary causes of death in deceased liver donors,[1] it can be inferred that donors with significant atherosclerotic risk are common. Albeit rare and not a contraindication to LT, atherosclerosis of the hepatic artery or celiac trunk[5,6] may complicate the liver engraftment by exposing the vascular anastomosis to potential complications like hepatic artery thrombosis and dissection. Some groups have advised extreme caution when significant calcific plaques extend into the common hepatic artery, with some even suggesting that atheromatosis at the bifurcation of the common hepatic artery or the gastroduodenal artery is a relative contraindication to using the liver graft.[5,7,8]

The presence of atherosclerosis to this extent often results in cancellation of the transplant if the surgeon deems the graft's arterial supply compromised.[5] It has also been suggested that extensive atherosclerosis may result in low hepatocyte viability by preventing effective cooling and adequate solution perfusion of the graft at the recovery.[5] Despite the availability of

diagnostic techniques like abdominal duplex scanning and mesenteric angiography, evaluation of atherosclerosis and patency of the arterial supply of the donor liver is often not performed before organ recovery.[5,9]

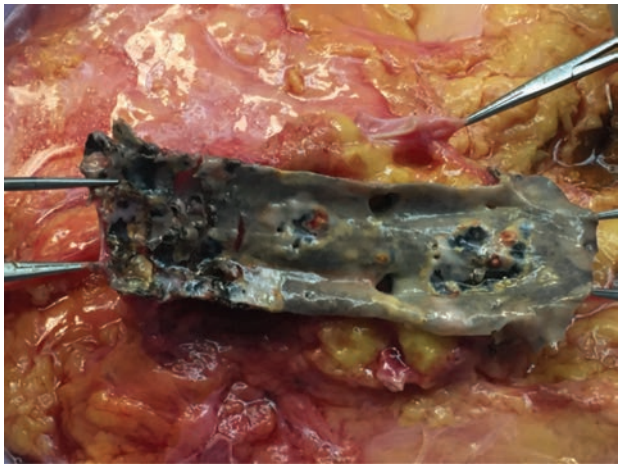
Herein, we present a case of extreme atherosclerosis of the aorta discovered in a brain-dead donor during liver recovery and review the pertinent literature. This successful LT with a marginal liver graft illustrates the potential utility of these organs.

Case Report

The donor was a 54-year-old white male with a history of hypertension, type 2 diabetes mellitus, hypercholesterolemia, and coronary artery disease and who required angioplasty 8 years before presentation. He was found unresponsive at home after cardiopulmonary arrest. After cardiopulmonary resuscitation, he was transferred to the hospital where cardiac catheterization was unsuccessfully performed. He progressed to brain death about 19 hours after admission, and his family decided to pursue organ donation.

During liver recovery, the donor's aorta was found to be black-colored with multiple ulcerated yellow-orange plaques. A piece of the aorta (Figure 1) was retrieved and sent to the pathology laboratory for examination given its unusual appearance. Pathologic analysis revealed severe atherosclerosis with calcifications, with no evidence of aortic dissection or a mycotic aneurysm, which were initially suspected. The donor artery was resected to the bifurcation of the splenic artery and common hepatic artery until no further gross atheromas could be appreciated, and this was anastomosed end-to-end to the recipient's proper hepatic artery. The remainder of the operation proceeded uneventfully. The recipient remained in the hospital for 2 weeks due to a contained bile leak, which was treated with an endoscopic retrograde cholangiopancreatography and papillotomy. After discharge, the patient has been followed closely and is doing well without any significant events. At his most recent clinic visit, he demonstrated normal liver function.

Figure 1. Donor's Severely Atherosclerotic Aorta



Discussion

Donors older than 65 years of age have accounted for 6% to 7% of liver donors in the United States over the past 15 years.[1] The proportion is even higher in countries like Spain where donors older than 70 years accounted for 32.3% of annual liver donations in 2015.[8] These numbers are expected to increase due to an aging donor population, proven adequacy of older donor grafts, and an increasing demand for LT. Grafts from older donors are not without their concerns. These grafts are associated with higher incidence of primary nonfunction and poor early graft function.[10] Older donors also pose the risk of graft atherosclerosis, especially among those with predisposing risk factors like obesity, diabetes, and smoking.

Our review of the literature for cases of atherosclerosis involving the aorta, celiac artery, or hepatic artery in a liver donor yielded a single case report of atherosclerosis involving the hepatic artery. In that report, the liver transplant was cancelled due to concerns about the extensive involvement of the arterial conduit, which caused reduced arterial patency and subsequent low hepatocyte viability.[5] Although purely speculative, it is our impression that most severely atherosclerotic grafts are discarded. In our case, the patency of the graft supply and the graft viability were deemed to be adequate, and LT proceeded as planned with no adverse events in the recipient so far.

The hitherto rarity of atherosclerotic involvement of the celiac trunk or hepatic artery, juxtaposed with concerns about possible vascular complications, has traditionally resulted in the abandonment of the procedure and the discard of an otherwise adequate graft. However, with the growing number of patients on transplant wait lists and an aging donor population, there will be increased offers for

surgeons to use marginal organs. Such offers will require additional consideration in the surgical decision-making process.

The mere presence of atherosclerosis should not dissuade the use of a liver graft. Cardiovascular risk factors in donors, such as hypertension, diabetes, hypercholesterolemia, or prior cardiac procedures, should arouse greater scrutiny of the vasculature of the liver. These donor risk factors may justify more extensive imaging studies, such as ultrasonography or computed tomography, which may elucidate perilous vascular anatomy. Atherosclerotic but otherwise adequate grafts may also benefit from reduced ischemic times as seen in grafts from older donors.[11,12] In these cases, coordination between procurement and recipient teams can help minimize ischemic times to less than 8 hours, as this has been shown to improve recipient outcomes.[11,12]

Finally, the older atherosclerotic marginal liver graft provides an excellent opportunity to evaluate novel techniques for graft assessment and reconditioning, such as normothermic liver perfusion.[13-16] This system allows for the evaluation process to proceed beyond recovery and to obtain a physiologic assessment of the graft to better gauge a marginal organ's biochemical behavior after organ recovery. The information gleaned from the normothermic perfusion phase of a marginal allograft may provide insight into that graft's potential function immediately prior to implantation.

Conclusions

Transplant surgeons are increasingly faced with challenging decisions regarding marginal grafts, including those from older donors. The presence of atherosclerotic plaques in a graft should not be an exclusion factor when utilization decisions are made. We present the successful transplant of a liver graft from a donor with extensive and severe atherosclerosis to illustrate the usability of these grafts. Novel preservation techniques, such as normothermic perfusion, can allow potential functional evaluation and additional objective decision-making, in an attempt to increase the rate of marginal graft utilization.

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Graft-Versus-Host Disease in Pediatric Living Donor Liver Transplant

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Abstract

Acute graft-versus-host disease is an extremely rare complication after pediatric liver transplant. Despite a better prognosis in pediatric than in adult recipients, graft-versus-host disease is associated with high mortality. We report 2 cases of pediatric recipients with acute graft-versus-host disease; both had identical clinical presentation. Remission was achieved in both patients, but the first patient developed Epstein-Barr virus-induced posttransplant lymphoproliferative disease and recurrence of graft-versus-host disease after the immunosuppression regimen was altered. The treatment options and clinical considerations for care of such patients are discussed. It is important to maintain a high degree of suspicion for graft-versus-host disease in every posttransplant patient with persistent skin rash, with or without fever and diarrhea, and confirm the diagnosis.

Key words: *Liver transplantation, Posttransplant lymphoproliferative disease*

Introduction

Acute graft-versus-host disease (GVHD) is a T-cell-mediated disease that occurs when a graft contains immunocompetent lymphoid cells that recognize the recipient (the host) as foreign, with subsequent initiation of an immune response against the host. Incidence of GVHD is 20% to 80% after hematopoietic stem cell transplant, but the incidence after liver transplant (LT) is <1% and is associated with higher mortality,[1,2] despite the fact that LT has been proposed to carry lymphocyte numbers comparable to hematopoietic stem cell transplant.[2,3] The total number of pediatric recipients with acute GVHD after LT reported in the literature is <25 individuals overall.[2,3] Here, we report 2 cases of acute GVHD in pediatric recipients of living donor LT (LDLT), as well as the difficulties in the diagnoses, the clinical courses of treatment, and outcomes.

Case Report

Patient 1

A 4-year-old male child underwent LDLT for acute liver failure of indeterminate etiology with grade 3 hepatic encephalopathy and severe coagulopathy. The LDLT donor was the child's father, who donated his left lateral segment to the child. Postoperative recovery was uneventful, and the recipient was discharged with good graft function after 18 days of hospital stay. At post-LT day 36, this patient presented with generalized maculopapular skin rash and high-grade fever. The rash started on the back of his ears and subsequently spread all over his body, including his palms and soles. We considered several different diagnoses, including measles, Kawasaki disease, vasculitis syndromes, drug reaction, toxic epidermal necrolysis, and hemophagocytic lymphohistiocytosis. Evaluation for infective etiology was negative, but the fever persisted, so we proceeded with a regimen of multiple antibiotics and antiviral and antifungal agents. Results were unremarkable for echocardiogram, bone marrow biopsy, and computed tomography of brain, chest, and abdomen. Adequate graft function of the transplanted liver was maintained, and Doppler ultrasonography results showed no abnormalities.

The skin rash of the patient was persistent and progressive, so we proceeded with a skin biopsy 10 days after presentation. Skin biopsy results

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

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Experimental and Clinical Transplantation (2022) 12: 1137-1140

DOI: 10.6002/ect.2021.0481

suggested acute GVHD (grade 2). We initiated treatment with methylprednisolone at a dose of 2 mg/kg for 3 days followed by oral taper of prednisolone, and his clinical response was characterized by defervescence of fever, improvement in clinical condition, and resolution of rash. He was discharged after a hospital stay of 21 days. Oral steroids were tapered to 0.25 mg/kg/d, and tacrolimus was continued with a target trough level of 8 to 10 ng/mL.

This patient presented with generalized lymphadenopathy at 165 days after LT, and cervical lymph node biopsy results were confirmatory for polymorphic posttransplant lymphoproliferative disease (PTLD). Epstein-Barr virus (EBV) level by polymerase chain reaction was 164 000 copies/mL. Test results for EBV viral load were negative at the time of GVHD diagnosis. Further evaluation with biopsy and positron emission tomography showed involvement of graft liver and bone marrow. Tacrolimus was stopped, and the patient continued with a regimen of oral steroids (0.25 mg/kg/d), as well as weekly treatment with rituximab (375 mg/m^2 body surface area). The patient developed high-grade fever with skin rash 25 days after change of immunosuppression; the rash was typical of GVHD, and skin biopsy results confirmed the recurrence of GVHD. Unfortunately, his clinical condition worsened, and he died from *Klebsiella pneumoniae* septicemia at 208 days after LT.

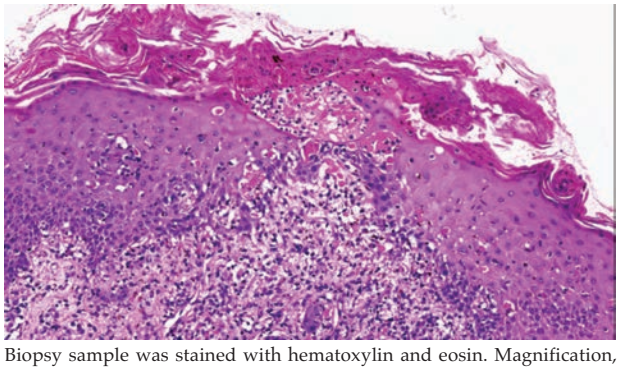
Patient 2

A 22-month-old male child with Alagille syndrome underwent LDLT (father was the donor) for decompensated liver disease. After 16 days of hospital stay, patient 2 was discharged in stable condition with good graft function. He presented 32 days after LT with persistent high-grade fever, diarrhea, and an erythematous maculopapular rash that started behind the ear and extended to the limbs, palms, and soles. After 48 hours, the rash was present throughout the body. Because the rash was typical of acute GVHD, we proceeded with a skin biopsy, the results of which confirmed the diagnosis of acute GVHD (Figure 1). Peripheral blood analysis for donor chimerism showed 8.3% donor lymphocytes (reference level after LT is ≤3%).[2] This patient was started on a regimen of 2 mg/kg of methylprednisolone (for 5 days) with oral and topical tacrolimus. Clinical response was favorable, with a decrease in intensity of skin rash, as well as resolution of diarrhea and fever, so we changed the immunosuppression regimen from methylprednisolone to oral prednisolone (2 mg/kg/d) for 2 weeks followed by taper (0.2 mg/kg every 5 days). However, this patient had recurrence of fever and diarrhea with progressive intensity, and skin rash was extensive at 20 days after initiation of treatment during the period of steroid taper.

Because his symptoms recurred, we obtained a skin biopsy, which showed exacerbation of the GVHD (stage 2 to 3). We initiated a second-line treatment with etanercept (0.4 mg/kg twice weekly, 5 doses), methylprednisolone (4 mg/kg/d for 5 days), and intravenous immunoglobulin (1 g/kg, single dose). Because of extensive skin involvement with severe pruritus, topical tacrolimus was continued and topical mometasone (0.1%) was added. Oral prednisolone was started at a higher dose (5 mg/kg/d) and was tapered slowly. Complete remission of GVHD was evident at 71 days after LT.

The treatment course of this patient was complicated by urinary tract infection with *Proteus mirabilis* and an increase in EBV from 10 000 copies/mL to 592 000 copies/mL after 3 months of treatment. The target trough level of tacrolimus decreased to 6 to 8 ng/mL, and steroids were tapered. There was no clinical evidence or radiological evidence of PTLD. Follow-up in patient 2 has shown good graft function with remission of GVHD at 5 months.

Figure 1. Skin Biopsy Showing Grade 3 Graft-Versus-Host Disease



Biopsy sample was stained with hematoxylin and eosin. Magnification, ×100.

Discussion

Pediatric LT recipients have a lower rate of mortality after GVHD versus adult LT recipients (36% vs 75%, respectively).[2] Children under 5 years of age and

recipients with HLA-mismatched LT donors have increased risk of GVHD development.[3] The diagnosis of GVHD is based on the presence of compatible clinical features, histopathological confirmation (most commonly from skin or gastrointestinal system), and donor chimerism in the recipient's peripheral blood.[2,4] A skin biopsy that shows epithelial cell necrosis in the absence of clinical findings of GVHD is not significant.[2,4] Across the literature, in 33 patients with a clinical suspicion of GVHD, only 7 cases of peripheral blood chimerism GVHD were confirmed; for all of the remaining patients, an alternative diagnosis was reached or they recovered spontaneously.[2,3] In the absence of clinical features, presence of peripheral blood chimerism is nonspecific and may be frequently seen (>70% of recipients) up to 8 weeks after LT.[2,3]

The incidence of acute GVHD in our unit is 0.57% (2 patients of a total of 348 pediatric recipients over 10 years). Diagnosis of GVHD is frequently delayed because of its rarity and its nonspecific initial symptoms. It is important to consider GVHD as a possibility for any patient with fever, diarrhea, or skin rash who is unresponsive to treatment or whose condition deteriorates despite treatment.[5] The most common differential diagnoses considered at the onset of fever/rash due to GVHD include drug-induced complications, viral exanthemas, infectious enteritis (cytomegalovirus and/or *Clostridium difficile* colitis), and organ rejection.[2,4] The time interval between the appearance of first symptom and definitive diagnosis or treatment can vary from 1 to 65 days and has been postulated as a predictor of mortality, which highlights the need for early diagnosis.[4]

Immunosuppression in both of our patients at the time of GVHD diagnosis consisted of tacrolimus (target trough level of 8 to 10 ng/mL in the first 6 months after LT) and oral prednisolone (1 mg/d), and neither patient had a rejection episode after LT. For treatment of GVHD, our first-line treatment consisted of methylprednisolone (2 mg/kg/d) followed by oral taper of 0.2 mg/kg every 5 days and tacrolimus (Table 1). Second-line treatment consisted of addition of etanercept. We have found topical application of tacrolimus and steroid to be an effective adjuvant to the treatment. There is no standardized protocol for management of GVHD after LT. A steroid regimen is the usual first-line treatment, with remission induced in 55% of patients and with relapse in 45%.[3] There is no consensus on the second-line treatment for steroid-resistant GVHD or for GVHD relapse; regimens of infliximab, anti-thymocyte globulin, alemtuzumab, rituximab, and sirolimus have been attempted.[3]

Complete response to treatment in GVHD is defined as resolution of all signs and symptoms of acute GVHD at day 28 after initiation of treatment. Although remission was induced in our first patient, he developed EBV-driven PTLD followed by recurrence of GVHD when the immunosuppression regimen was altered. The EBV counts for our second patient also increased after treatment for GVHD. Pinna and colleagues have described 2 pediatric LT recipients with recurrence of GVHD, but the diagnoses of the first episodes in both patients were not confirmed.[5] Our patients were confirmed to have GVHD in both episodes.

Although reduction or discontinuation of immunosuppression has been attempted in previous studies, in an effort to enable the host immune system to destroy the donor lymphocytes and thereby mediate GVHD, these scenarios were associated with differing clinical responses and rates of remission.[2,4] Survival depends on the response to initial therapy with steroids.[4] It has been postulated that patients with persistent gastrointestinal and bone marrow involvement have higher risk of mortality.[4] Gut involvement due to GVHD could be the explanation for diarrhea in our second patient;

Table 1. Patient Demographics, Treatments, and Outcomes

Patient	Etiology of Liver Disease	Age and Sex	Donor	GVHD Onset After LT	Presenting Symptoms	Diagnosis	Treatment	Complication	Outcome
1	Acute liver failure, indeterminate	49 mo, male	Father	Day 36	Fever, skin rash	Skin biopsy	Steroids, tacrolimus (oral)	PTLD, recurrence of GVHD	Death
2	Alagille syndrome	22 mo, male	Father	Day 32	Fever, skin rash, diarrhea	Skin biopsy, peripheral blood chimerism	Steroids (oral, skin), etanercept, tacrolimus (oral, skin)	Urinary tract infection, cystitis	Survival

Abbreviations: GVHD, graft-versus-host disease; LT, liver transplant; PTLD, posttransplant lymphoproliferative disease

however, we did not perform endoscopy, because (1) the diagnosis of GVHD was confirmed with skin biopsy, (2) screening for infection was negative, and (3) diarrhea subsided with initiation of treatment.

It is important to maintain vigilance for opportunistic infections (bacteria, EBV, adenovirus, human herpesvirus 6) and PTLD in every patient under treatment for GVHD.[4] Prophylaxis with antifungal agents active against molds and low threshold for treatment with ganciclovir or foscarnet should be considered.[4] The risk is further exacerbated by the intense T-cell depression that can occur with second-line agents (antithymocyte globulin, alemtuzumab, etanercept), but this can occur in any patient, as our experience indicates.[4]

In summary, our report describes the treatment protocol and clinical course for 2 pediatric patients who developed acute GVHD after LDLT. It is important to consider GVHD in the differential diagnosis of febrile events after LT, because early diagnosis and treatment is associated with better prognosis.

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First Successful Human Coronary Artery Bypass Surgery Postoperative Heart Transplant: A Case Report

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Abstract

Heart transplant is now the treatment of choice for patients with advanced heart failure who are refractory to medical treatment. With a small number of candidates who meet the traditional criteria of a heart donor, we aimed to alleviate this shortage. In this article, we report a 43-year-old woman with a highly urgent heart requirement, according to acute decompensated heart failure, who received a heart with coronary artery grafts from a 50-year-old woman with the diagnosis of 3-vessel disease. Our review of her 1-year follow-up demonstrated the absence of any cardiac or other problems and survival of the patient. There have been no reports in the relevant literature of transplanting marginal hearts from donors who have previously undergone coronary artery bypass graft before transplant. According to our findings, transplant of a marginal heart with coronary artery grafts can be successful; additional studies with larger samples are warranted to further investigate the results of transplanting marginal hearts from donors who have previously undergone coronary artery bypass graft procedures.

Key words: *Brain death, Coronary artery disease, Heart transplantation*

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Experimental and Clinical Transplantation (2022) 12: 1141-1144

DOI: 10.6002/ect.2022.0312

Introduction

Heart transplant is now the treatment of choice in patients with advanced heart failure who are refractory to medical treatment.[1] Initially, the characteristics of a heart donor were very narrowly defined in order to obtain the best possible results.[2] However, the small number of candidates who meet the traditional criteria of a heart donor has created a dramatic discrepancy between the number of patients who are in need of transplant and those fortunate to receive a suitable donor organ.[3]

Age is one of the criteria that can be successfully expanded to enlarge the pool of potential heart donors. However, it has been observed that older age is a singular risk factor for coronary artery disease (CAD),[4] and therefore older age increases the probability that an aged donor has previously undergone coronary artery bypass graft (CABG). To expand the opportunities to use organs from older donors, transplanting hearts from donors who have previously undergone CABG should be considered. In this case report, we report the 1-year follow-up of a status 1A patient (according to the adult heart allocation policy of the Organ Procurement and Transplantation Network, United Network Organ Sharing) who received a marginal heart transplant along with the 3 coronary artery grafts that had previously been implemented to treat triple coronary artery disease in the donor.

Case Report

Heart donor

A 50-year-old woman, who was referred from another hospital to Shahid-Rajaee Hospital, presented with typical cardiac chest pain and exertional dyspnea (functional class IV) with the diagnosis of 3-vessel disease (confirmed by angiographic results). The patient became a candidate for coronary artery

bypass in Shahid-Rajaee Hospital and received 1 graft from the internal mammary artery to the left anterior descending coronary artery (LAD) and 2 venous grafts to the right coronary and left circumflex arteries. After the operation, the patient did not wake up; with suspicion of brain death through a probable cause of the "sandblast" phenomenon and micro emboli entering the cardiovascular system, brain death assessment was initiated.[5,6] Brain death was confirmed by electrical silence in an electroencephalogram, with positive test results for atropine and apnea. After the consent was obtained from the patient's family for organ donation, she was transferred to the Iran University of Medical Science Transplant Organ Provision Unit (Hazrate Rasoole Akram Hospital).

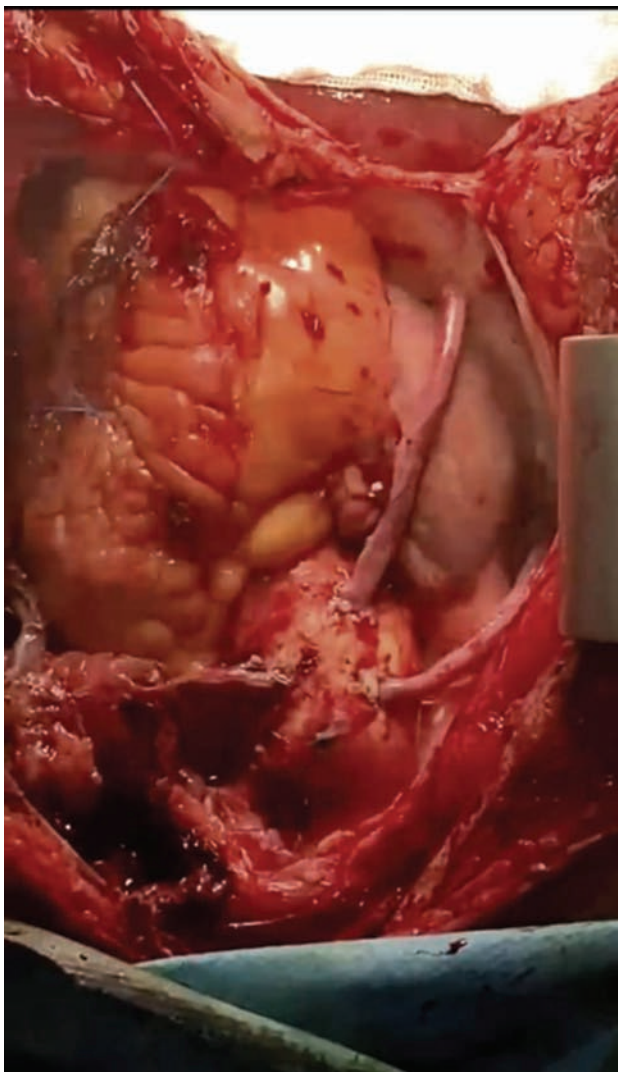
Heart recipient

A 43-year-old woman with a highly urgent heart requirement (status 1A as classified by the Organ Procurement and Transplantation Network, United Network Organ Sharing) according to acute decompensated heart failure. She had been treated by extracorporeal membrane oxygenation, an intra-aortic balloon pump, and daily high doses of epinephrine, norepinephrine, and dopamine. This patient became unstable after hospitalization. Her level of consciousness decreased from 15 to 11 Glasgow coma score, her diuresis reduced to 0 to 5 mL/kg/h, and her mean arterial pressure was under 60 mm Hg with high doses of epinephrine, norepinephrine, and dopamine. According to the decision and permission of the organ allocation unit of the Ministry of Health and Medical Education, the patient was approved as a candidate for an emergency heart transplant, including with a marginal heart.

Heart transplant

The donor operation was performed as a multiorgan procurement procedure. The initiation of heart retrieval was after the completion of other organ preparations. Following the redo sternotomy, the superior vena cava was ligated, making the inferior vena cava free, and the left atrium was vented to prevent left ventricular distension. Then, the ascending aorta was cross clamped, and subsequently, cold cardioplegia solution was applied through the aortic root to induce hypothermic diastolic arrest. The coronary grafts were carefully inspected manually and checked for functionality and availability of the heart circulatory supply. The heart, distal to the aortic end of the grafts with preservation of venal and arterial grafts, was then extracted (Figure 1). Subsequently, the procured heart was supplied with 1.5 L of cardioplegia solution and packed in ice. The heart was preserved at 4 °C, transferred to Shahid-Rajaee Hospital within 90 minutes, and immediately delivered to the operating room.

Figure 1. Donor Heart With Coronary Artery Grafts



For the transplant, the anastomosis of the aortic root was performed distal to the ends of the grafts, and the end of the arterial graft was anastomosed to the recipient's internal mammary artery. Before the operation, the patient had exertional dyspnea (functional class IV that ameliorated to functional class I after transplant) and left ventricular ejection fraction was developed from 10% to 45%. Furthermore,

other assessments produced results within standard reference ranges (25 mm Hg pulmonary artery pressure, 135 mM sodium, 3.6 mM potassium, 1.1 mg/dL creatinine, white blood cell count of 9.0×10^9 cells/L, hemoglobin level of 11, platelet count of 150 000 cells/μL). The patient was out of bed 14 days after the operation and discharged at 8 weeks. She survived for 1 year without any hemodynamic and cardiac deterioration until June 10, 2022.

Discussion

The major aim of transplant is to avoid transplanting an inferior organ, especially in a critically ill recipient.[7] On the other hand, the demand for organ transplants has been rising rapidly due to the higher incidence of end-stage failure of many vital organs, including kidney, liver, and heart, while the supply of organs from ideal donors has remained insufficient to meet the higher demand.[8] One possible solution to this issue is to expand the donor criteria to include organs that would otherwise be considered marginal or high risk. The Institute of Medicine estimates that more than 15 000 patients would potentially benefit from a heart transplant if the acceptance criteria were expanded to include all qualified patients below 55 years of age, and about 40 000 to 70 000 patients would benefit if the acceptance age was extended to 65 years.[9] Donor hearts that are suspected of CAD are generally judged to be unacceptable according to the traditional donor criteria. However, because CAD is a major cardiovascular disease that affects the global human population,[10] is correlated with a higher age,[4] and is commonly treated with CABG, we therefore suggest that the results of transplanting marginal organs from donors who have previously undergone CABG should be studied as a way to increase the number of potential donors.

The possibility of transplanting hearts with CAD has been explored; however, in the known cases where CABG was needed, the procedure was performed either concurrent with the transplant or as a separate posttransplant procedure.

A study by Abid and colleagues reported 4 donor hearts with palpable atherosclerosis on the surface of the LAD. In that study, the organs, which would otherwise have been rejected, were transplanted first, and the atherosclerotic lesion was then bypassed with a left internal mammary artery. Annual surveillance angiography showed patent left internal mammary artery in all patients. Of the 4 patients, 1 patient developed significant diffuse graft accelerated coronary disease involving the LAD at 9 years after cardiac transplant. No other patient has subsequently shown significant progression of proximal CAD.[11]

Marelli and colleagues reported their experiences with the use of donor hearts with mild to moderate CAD transplanted to 22 recipients who were either urgent candidates or who otherwise would not have received a transplant for various reasons. The CABG procedure was undertaken concurrent with the % of the patients. The 2-year survival rate among the patients was 75.6%.[12] Generally, concurrent or postponed CABG is thought to account for the satisfactory results of the studies that have used CAD donor hearts.[13]

Conclusions

There have been no reports in the relevant literature of transplanting marginal hearts from donors who have previously undergone CABG before transplant, and yet this scenario is indeed a rich vein of opportunity that could be tapped. According to our findings, transplant of a marginal heart along with the coronary artery grafts can be successful and hence warrants additional studies with larger samples to further investigate the results of transplanting marginal hearts from donors who have previously undergone CABG.

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Next-Day Discharge after Kidney Transplant During the SARS-CoV-2 Pandemic

Shakir Hussein,[1] Jason Rolls,[2] Madyson Riddell,[1] Jeffrey Wolfe,[1] Rajeev Sharma[1]

Dear Editor:

We present a series of 3 cases of next-day discharge after kidney transplant during the SARS-CoV-2 pandemic; these patients were discharged early to prevent inpatient exposure to the virus at a time when hospitals were overwhelmed with patients infected with SARS-CoV-2 and at a time of significant shortage of nursing staff to take care of these patients. We learned that, if protocols were followed, next-day discharge could be achieved without increasing the readmission rates in carefully selected patients with good family support.

A multidisciplinary approach to decrease length of stay, or Enhanced Recovery After Surgery (ERAS), has been adopted in different forms to shorten the length of stay and to improve outcomes. The elements of this protocol reduce the stress of operation to retain anabolic homeostasis.[1] Early postoperative discharge after major inpatient surgery is associated with lower total surgical episode payments, translating to lower costs for health insurance companies.[2] The ability to reduce cost through length of stay reduction by implementation of a kidney transplant outpatient unit was described by Shapiro and colleagues in 1998.[3] The implementation of a clinical pathway for deceased donor kidney transplant was found to be effective in reducing the length of stay.[4] The feasibility of a modified ERAS protocol that had low morbidity and reasonable readmission rates was demonstrated in kidney transplant recipients.[5] Here, we present 3 patients at a single center who had successful posttransplant day 1 discharge after kidney transplant without a high rate of readmission.

Patient 1 was 33-year-old African American woman with chronic kidney disease stage IV secondary to diabetes mellitus type 2 who had received a preemptive deceased donor kidney transplant from a 19-year-old African American man who died in a motorcycle accident. The recipient's panel reactive antibody (PRA) was 57, and the donor's Kidney Donor Profile Index was 21%. The donor's serum creatinine level on admission was 1.21 mg/dL, which peaked at 1.53 mg/dL and then was 1.0 mg/dL before organ retrieval. Warm ischemia time was 32 minutes, and cold ischemia time was 26 hours and 47 minutes. The recipient's creatinine on admission was 9.01 mg/dL. Her postoperative course was uncomplicated, and patient 1 was discharged home on posttransplant day 1 with a creatinine level of 5.44 mg/dL. Induction immunosuppression included 3 mg/kg thymoglobulin divided into 2 doses along with methylprednisolone. A second dose of thymoglobulin was given on posttransplant day 1 prior to discharge. Maintenance immunosuppression included tacrolimus, mycophenolate mofetil, and prednisone. On posttransplant day 4 in the clinic, the patient's creatinine level was 1.76 mg/dL. She was readmitted to the hospital on posttransplant day 11 with acute kidney injury, hyperglycemia, *Escherichia coli* urinary tract infection, and bacteremia, with creatinine level of 3.56 mg/dL and blood glucose of 314 mg/dL. The patient was treated with antibiotics and intravenous fluids, and her insulin dose was adjusted to achieve euglycemia. After 5 days, the patient was discharged home. At 14 months posttransplant, the patient's creatinine level was 1.09 mg/dL, with no other hospital admissions.

Patient 2 was a 49-year-old African American man with end-stage renal disease due to hypertension who

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

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Experimental and Clinical Transplantation (2022) 12: 1145-1147

DOI: 10.6002/ect.2021.0309

had been on hemodialysis for 2 years; he had received a kidney transplant from a living related (ex-wife) 48-year-old female donor. The recipient's PRA was 0 and creatinine level was 15.01 mg/dL on day of transplant. His postoperative course was uncomplicated, and he was discharged home on posttransplant day 1. Induction immunosuppression included basiliximab and methylprednisolone. A second dose of basiliximab was given on post-transplant day 1 prior to discharge. Maintenance immunosuppression included tacrolimus, mycophenolate mofetil, and prednisone. Creatinine level at the time of discharge was 8.64 mg/dL. Since transplant, patient 2 has not been readmitted to the hospital. At 10 months posttransplant, his creatinine level was 1.58 mg/dL.

Patient 3 was a 64-year-old African American man with end-stage renal disease after a failed first deceased donor kidney transplant. His initial cause of kidney failure was focal segmental glomerulosclerosis. He also had a history of hypertension, dyslipidemia, coronary artery disease, and cerebrovascular accident. Patient 3 was on hemodialysis for 2 years after the first transplant failed and before he received the second kidney transplant. He received a second deceased donor kidney transplant from a 50-year-old female donor with Kidney Donor Profile Index of 53%. At the time of second transplant, the patient's PRA was 7 and his creatinine level was 5.13 mg/dL. Cold ischemia time was 12 hours and 18 minutes. Induction immunosuppression included 3 mg/kg thymoglobulin divided into 2 doses along with methylprednisolone. The second dose of thymoglobulin was given on posttransplant day 1. Maintenance immunosuppression included tacrolimus, mycophenolic acid, and prednisone. Patient 3 was discharged home on posttransplant day 1 with a creatinine level of 4.32 mg/dL. He was never readmitted to the hospital following discharge. At 10 months posttransplant, his creatinine level was 1.9 mg/dL.

According to the Organ Procurement and Transplantation Network, transplant date is determined as "the start of the organ anastomosis during transplant or the start of the islet infusion" (https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf; accessed May 23, 2021). We followed this definition to determine the day of transplant. All patients in our series were African American. Our program serves the metropolitan Detroit area, and 90% of our patients are African American. During early 2020, transplant activities in our hospital were suspended for about 3 months due to the SARS-CoV-2 pandemic. In addition, there was a significant nursing staff shortage. To limit the exposure of transplant patients to the hospital environment and SARS-CoV-2 infection, and because of the nursing staff shortage, we decided to discharge patients early. We did not have an ERAS program in place. Of 21 kidney transplants performed from January through November 2020, 3 patients were discharged the next day after kidney transplant. All patients were discharged with a Foley catheter, which was removed in the clinic around posttransplant day 7. All patients had ureteral stents that were removed 4 to 6 weeks after transplant.

Patient 1, who received preemptive transplant, was readmitted for acute kidney injury, hyperglycemia and *Escherichia coli* urinary tract infection with bacteremia. This could be related to prolonged catheterization and poor catheter care at home in the setting of poorly controlled diabetes mellitus type 2 and immunosuppression. However, this was successfully managed, and patient 1 was discharged home after 5 days without any more readmissions. Patient 2 and patient 3 had uncomplicated postoperative coursed without any hospital readmissions.

The success of these 3 cases was related to strong family support and support by the entire transplant team. We discussed the possibility of early discharge with the patients and their families, nursing staff, social work, pharmacist, and other transplant team members. Strong family support was essential for discharge. Early ambulation and efficient pain control helped achieve this goal. Induction immunosuppression protocols were modified to enable early discharge. Patients were called by the transplant coordinator on the day after discharge, and all patients were seen in clinic within a few days of discharge.

The concept of early discharge, where patients are discharged 2 days after kidney transplant without additional morbidity or higher readmission rates, has been previously demonstrated in large transplant programs in the United States with good resources[6,7]; however, this concept has not been reported with smaller programs. Although our sample size was small, our results underscore the possibility of performing kidney transplant surgeries in appropriately selected candidates with an overnight

stay in smaller transplant programs with limited resources. Given changes in kidney allocation with longer anticipated cold ischemia time, there will be higher rates of delayed graft function, which is associated with longer length of stay.[8] None of the patients in our series had delayed graft function, enabling us to discharge them early. To prevent readmissions after discharge, greater attention is needed on medical comorbidities and on discharge-level factors like electrolyte abnormalities, surgical complications, and delayed graft function.[9]

In conclusion, next-day discharge after kidney transplant is possible in carefully selected transplant recipients, even in smaller programs with limited resources without any additional morbidity or higher readmission rates.

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Index to Volume 20 Issue 12

Author Index

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

Experimental and Clinical Transplantation

Official Journal of the Middle East Society for Organ Transplantation

Volume: 20 Issue: 1 January 2022

Contents

Volume: 20 Issue: 2 February 2022

Contents

Case Report

Letter to Editor

Volume: 20 Issue: 3 March 2022

Contents

Review

Article

Volume: 20 Issue: 4 April 2022

Contents

Volume: 20 Issue: 5 May 2022

Contents

Volume: 20 Issue: 6 June 2022

Contents

Volume: 20 Issue: 7 July 2022

Contents

Volume: 20 Issue: 8 August 2022

Contents

Volume: 20 Issue: 9 September 2022

Contents

Volume: 20 Issue: 10 October 2022

Contents

Volume: 20 Issue: 11 November 2022

Contents

Volume: 20 Issue: 12 December 2022

Contents

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

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

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

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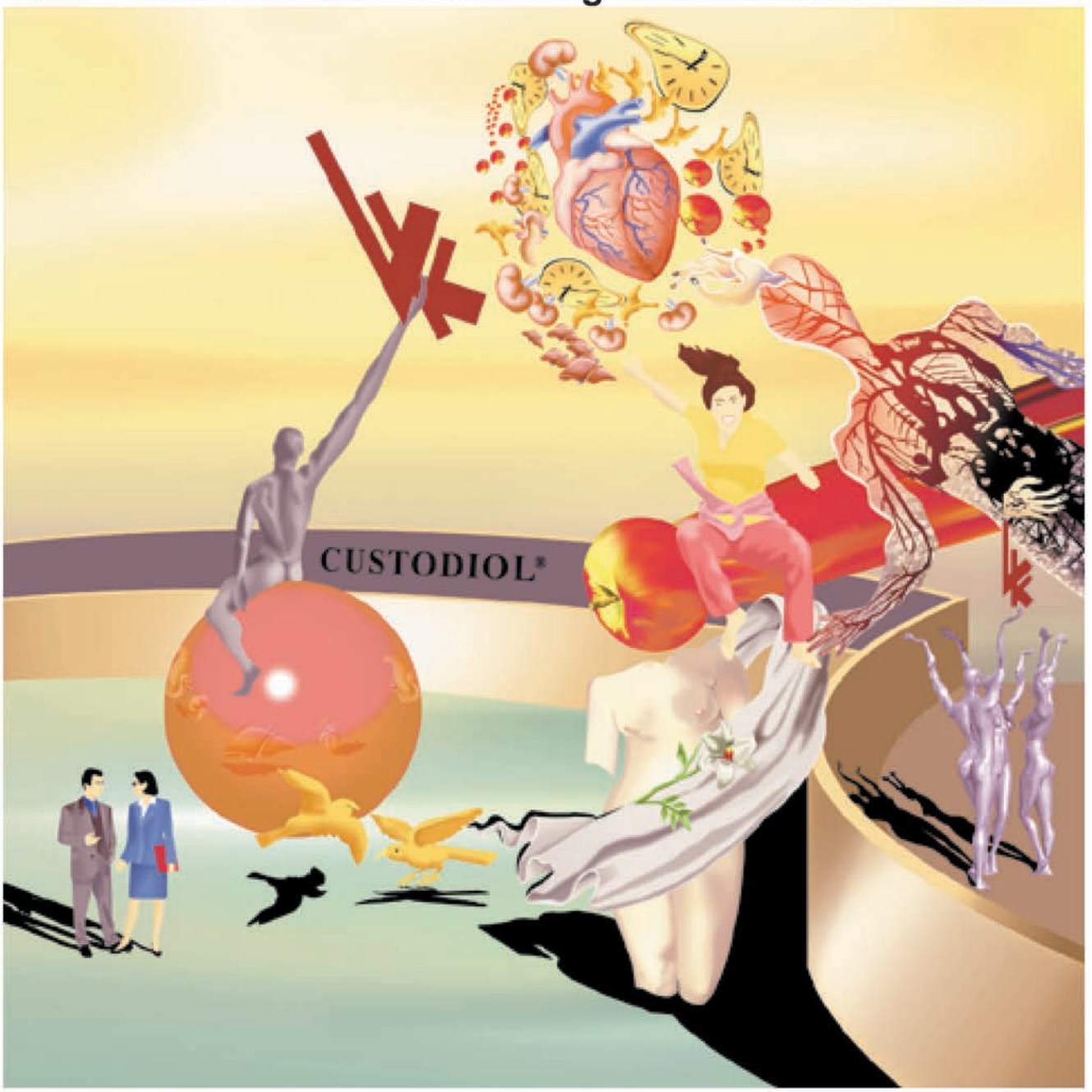

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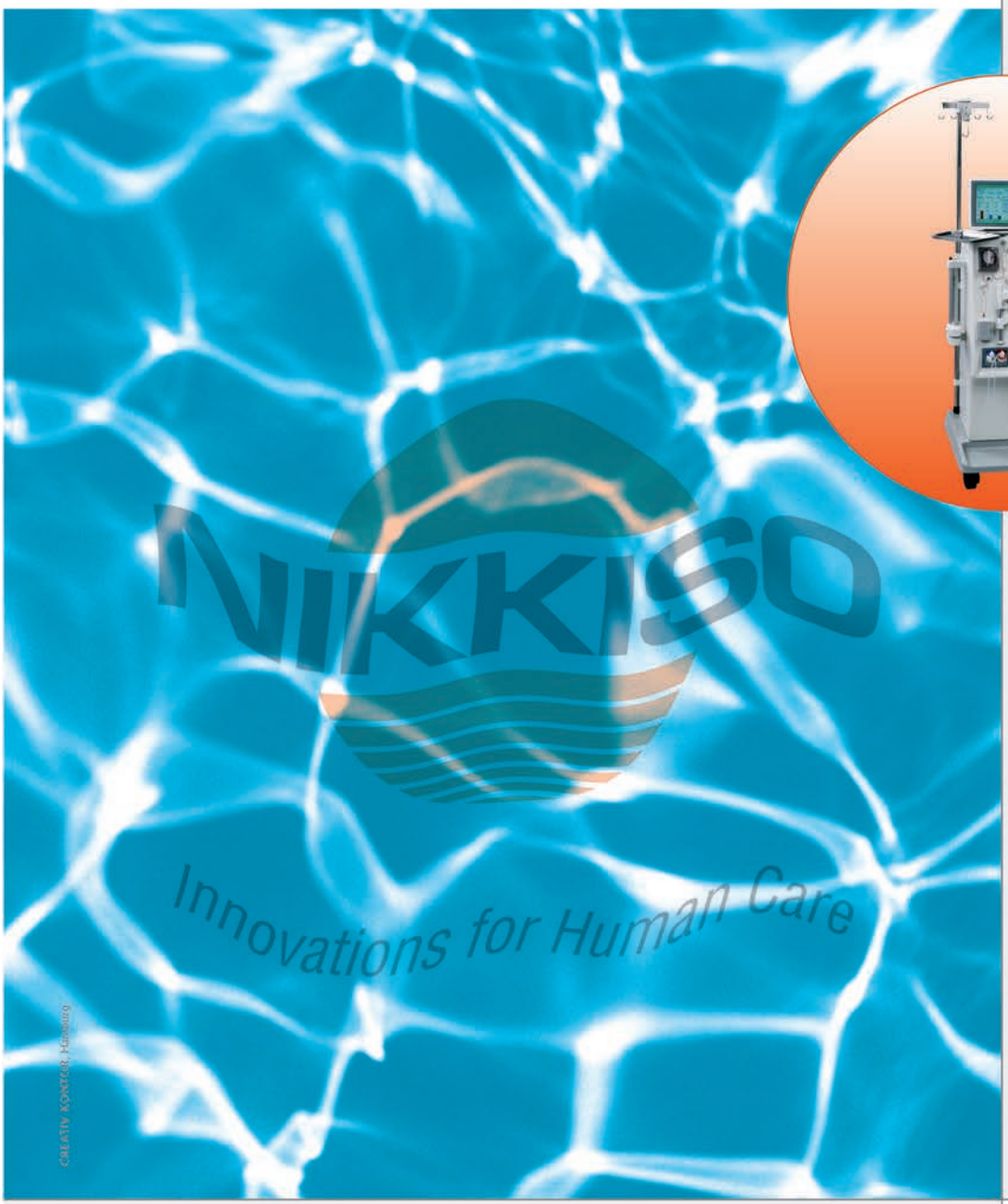

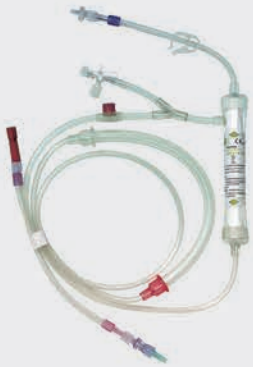

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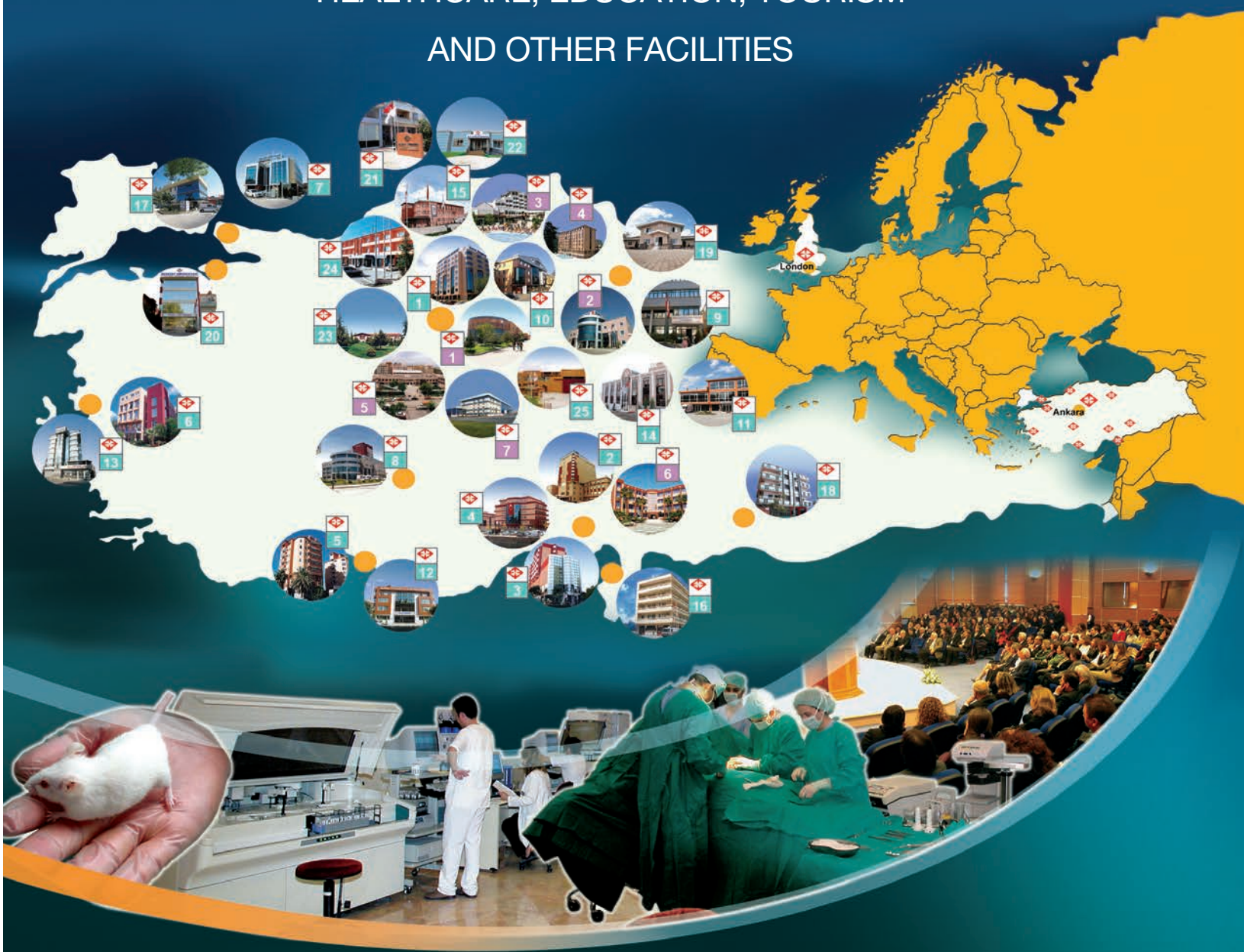

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4. PATALYA THERMAL RESORT HOTEL
5. ANKARA AYŞEABLA SCHOOLS
6. ADANA BAŞKENT PRIVATE SCHOOLS
7. AÇKAR DAIRY PRODUCTS

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2. ADANA DR. TURGUT NOYAN YÜREĞİR TEACHING & MEDICAL RESEARCH CENTER
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12. ALANYA OBA OUTPATIENT CLINICS
13. İZMİR ÇİĞLİ OUTPATIENT CLINICS
14. ANKARA ÜMİTKÖY DIALYSIS CENTER
15. ANKARA YENİKENT DIALYSIS CENTER
16. ANKARA ÇİĞDEM DIALYSIS CENTER
17. İSKENDERUN DIALYSIS CENTER
18. İSTANBUL DIALYSIS CENTER
19. ŞANLIURFA DIALYSIS CENTER
20. TOKAT ZİLE DIALYSIS CENTER
21. YALOVA DIALYSIS CENTER
22. ZONGULDAK DIALYSIS CENTER
23. ZONGULDAK KARADENİZ EREĞLİ DIALYSIS CENTER
24. YAPRACIK PSYCHOSOCIAL REHABILITATION CENTER
25. AYAŞ PHYSICAL THERAPY & REHABILITATION CENTER
26. BASKENT GROUP HEALTH PRODUCTS TRADE and INDUSTRY INC.

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