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MINIREVIEWS

Liver volumetric and anatomic assessment in living donor liver transplantation: The role of modern imaging and artificial intelligence

Mayara Machry, Luis Fernando Ferreira, Angelica Maria Lucchese, Antonio Nocchi Kalil, Flavia Heinz Feier

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Abstract

The shortage of deceased donor organs has prompted the development of alternative liver grafts for transplantation. Living-donor liver transplantation (LDLT) has emerged as a viable option, expanding the donor pool and enabling timely transplantation with favorable graft function and improved long-term outcomes. An accurate evaluation of the donor liver's volumetry (LV) and anatomical study is crucial to ensure adequate future liver remnant, graft volume and precise liver resection. Thus, ensuring donor safety and an appropriate graft-to-recipient weight ratio. Manual LV (MLV) using computed tomography has traditionally been considered the gold standard for assessing liver volume. However, the method has been limited by cost, subjectivity, and variability. Automated LV techniques employing advanced segmentation algorithms offer improved reproducibility, reduced variability, and enhanced efficiency compared to manual measurements. However, the accuracy of automated LV requires further investigation. The study provides a comprehensive review of traditional and emerging LV methods, including semi-automated image processing, automated LV techniques, and machine learning-based approaches. Additionally, the study discusses the respective strengths and weaknesses of each of the aforementioned techniques. The use of artificial intelligence (AI) technologies, including machine learning and deep learning, is expected to become a routine part of surgical planning in the near future. The implementation of AI is expected to enable faster and more accurate image study interpretations, improve workflow efficiency, and enhance the safety, speed, and cost-effectiveness of the procedures. Accurate preoperative assessment of the liver plays a crucial role in

ensuring safe donor selection and improved outcomes in LDLT. MLV has inherent limitations that have led to the adoption of semi-automated and automated software solutions. Moreover, AI has tremendous potential for LV and segmentation; however, its widespread use is hindered by cost and availability. Therefore, the integration of multiple specialties is necessary to embrace technology and explore its possibilities, ranging from patient counseling to intraoperative decision-making through automation and AI.

Key Words: Liver transplantation; Living-donor; Diagnostic imaging; Artificial intelligence; Machine learning; Deep learning

Core Tip: Accurate liver's volumetry (LV) is imperative for successful living-donor liver transplantation to ensure adequate future liver remnant and graft volumes. Manual computed tomography scan delineation conventionally serves as the standard approach; however, it is constrained by factors such as cost, subjectivity, and variability. In contrast, automated LV techniques utilizing advanced segmentation algorithms present superior reproducibility, reduced variability, and enhanced efficiency compared with manual measurements. However, the accuracy of automated LV requires further investigation. The study comprehensively reviewed both traditional and emerging LV methods, including semi-automated image processing, automated LV techniques, and machine learning-based approaches, while analyzing their respective strengths and weaknesses.

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INTRODUCTION

Liver transplantation is the first-line treatment for patients with terminal liver disease. Deceased donor organ shortage and cultural barriers have led to the development of alternative graft types. Living-donor liver transplantation (LDLT) has emerged as an extension of the *ex-situ* graft transection concept, encompassing reduced-size and split-liver techniques. By enabling the expansion of the donor pool, LDLT offers the advantage of timely transplantation and holds the potential for excellent graft function and improved long-term outcomes[1-6]. Moreover, LDLT reduces waiting list mortality.

An adequate preoperative evaluation of the donor is essential for successful LDLT. Sufficient future liver remnant (FLR) and graft volume must be ensured through liver's volumetry (LV) studies[7,8]. An FLR of 30% to 35% of the original liver volume is required for donor safety, whereas at least 4% of the standard liver volume or more than 0.8 and less than 3–3.5 of the graft recipient weight ratio (estimated before the surgery through imaging and confirmed after the graft is weighted) is required to meet the recipient's needs[9,10]. Small grafts are associated with cellular damage due to excessive portal flow, leading to "small-for-size syndrome," whereas large grafts may receive inadequate portal flow, resulting in "large-for-size syndrome"[11-17].

Manual liver volumetry (MLV) conducted on portal venous phase multidetector computed tomography (CT) scans with intravenous contrast is conventionally considered the standard method for measuring LV[7,18,19]. However, it can be costly, time-consuming, subjective, and prone to inter- and intra-observer variabilities. The process entails manual tracing of the liver borders using specialized software, necessitating the expertise of an experienced radiologist, often without the input of the surgeon. The percentage of error (PE) may vary significantly, ranging from 2% to 20%, which can have a dramatic effect on the final graft volume and transplantation outcomes[20-24].

Advancements in medical imaging, computational algorithms, and artificial intelligence (AI) have set the stage for the development and application of automated LV techniques. Automated LV holds significant promise in the evaluation of LDLT, as it utilizes sophisticated segmentation algorithms to delineate liver boundaries from CT or magnetic resonance imaging (MRI) scans. Therefore, enabling volumetric calculations and comprehensive volumetric analysis and allowing for the assessment of lobe-specific volumes, segmental volumes, and overall liver volume. Such automated approaches offer advantages over manual measurements, including enhanced reproducibility, reduced intra- and interobserver variability, and improved efficiency. However, the accuracy of automated LV techniques is yet to be conclusively determined[25-28].

The study aimed to provide a comprehensive review of the literature, presenting both traditional and emerging methods of LV and anatomical liver assessment, while discussing their respective strengths and weaknesses. By examining the current state of LV techniques, the review aimed to contribute to the advancement and optimization of liver transplantation outcomes.

MANUAL LIVER VOLUMETRY

The introduction of multiphasic CT and MRI techniques has led to the widespread adoption of MLV as the standard practice in liver transplant centers to estimate liver volume before accepting a living-donor as a suitable candidate. During the donor evaluation, a complete anatomical analysis of the hepatic veins, portal vein and hepatic arteries is provided by multiphasic CT and MRI. Bile duct anatomy is evaluated in cholangio MRI studies, specially, in left lobe and right lobe donors.

If the donor´s anatomy is suitable for the planned procedure, LV is carried out. The procedure involves manual delineation of the liver borders using sequential image slices to determine the overall liver volume. Subsequently, a transection plane is selected based on the specific type of liver graft and the inclusion of the middle hepatic vein (MHV) [25,29-31] (Figure 1).

Limitations include reliance on operator expertise and medical specialty, leading to discrepancies between the analyses performed by radiologists and surgeons, potentially related to the transection line. Furthermore, the inclusion of blood vessels and bile ducts in the final volume calculation can lead to overestimations[32]. Additionally, the LV procedure itself is time-consuming, typically requiring approximately 20-40 min to complete, which significantly affects the daily workflow of both radiologists and surgeons[19,33]. In terms of accuracy, PE ranges from 5% to 36% when comparing the estimated volume with the actual graft weight (AGW)[34]. It is important to note that errors can occur in both directions, resulting in overestimation and underestimations[8].

It is routinely considered that the density of the liver is equivalent to the density of water; therefore, the AGW is representative of the graft volume[35]. However, studies measuring AGW have identified the necessity of correction factors when estimating graft volume, as highlighted in Table 1. Recently, Lemke *et al*[36], measured the mean physical density of 16 transplanted liver lobes to be 1.1157 g/mL, asserting that the conversion factor was, on average, 12% higher than expected. Tongyoo *et al*[32] demonstrated that the AGW of a right lobe donor liver graft (RLDG) was approximately 91% of the estimated right lobe liver volume. The 9% volume reduction was attributed to intrahepatic blood flushed out of the liver by the preservation solution during back-table preparation[9,31,37]. Other inaccuracies may have been due to the inclusion of the MHV and/or the caudate lobe[38].

SEMI-AUTOMATED IMAGE PROCESSING

Semi-automated methods have been developed to address observer-related issues associated with manual measurements and to enhance the efficiency of LV and hepatic segmentation. An example of such a method is the MeVis Liver Analyzer (MeVis Medical Solutions AG, Bremen, Germany), which is a computer-assisted software that operates on CT images. Moreover, the software employs a modified live-wire algorithm to automatically determine the contours between user-defined boundary points based on the CT values and gradients. The algorithm parameters were tailored to each CT phase, including the venous (V), arterial (HA), and native (N) phases. To ensure accurate liver segmentation, manual correction of automatically delineated contours and manual drawing of the contour parts were performed. Live-wire contours were interactively determined on 3 mm axial two-dimensional (2D) CT slices. The software automatically interpolates and optimizes the contours of intermediate slices, with final adjustments made by the operator through manual corrections, if necessary (Figure 2).

Volumetric calculations, expressed in milliliters (mL), were performed by adding the areas of all segmented regions. Surrounding structures such as major extrahepatic vessels (portal vein, hepatic artery, and inferior vena cava) and the gallbladder fossa were excluded from the volume calculations (Figure 3).

Goja *et al*[39] discovered that semiautomated software tools exhibited the highest correlation (r = 0.82) for measuring right lobe grafts. However, left lobe grafts tend to be overestimated, whereas left lateral segment (LLS) grafts are often underestimated, with an underestimation of approximately 66% of the total LLS grafts. One possible explanation for the underestimation of LLS grafts is that CT scans typically underestimate the volume because the actual surgical plane of transection is approximately 1 cm to the right of the falciform ligament, whereas the radiological plane of transection is exactly at the falciform ligament. Other studies have addressed the accuracy of semi-automated image processing (SAIP), and their results are presented in Table 2.

AUTOMATED LIVER VOLUMETRY TECHNIQUES

Automated LV relies on advanced image-processing techniques and algorithms to accurately segment the liver from CT or MRI scans. The principles and algorithms vary depending on the approach employed. However, some common techniques and concepts are involved.

Image preprocessing

Before liver segmentation, image preprocessing techniques may be applied to enhance the image quality, reduce noise, and improve the contrast between the liver and surrounding structures. These techniques include filtering, intensity normalization, and image enhancement methods.

Table 1 Formulas to estimate liver volumetry by computerized tomography

Ref.	Formula	Research place
Poovathumkadavil *et al*[22], 2010	LV = 12.26 × BW(kg) + 555.65	Saudi Arabia
Noda *et al*[21], 1997	LV = 0.05012 × $BW^{0.78}$	Japan
Johnson *et al*[20], 2005	LV = 0.722 × $BSA^{1.176}$	North America
Yuan *et al*[24], 2008	LV = 949.7 × BSA (m^2) - 48.3 × age - 247.4	China
Yoshizumi *et al*[23], 2003	LV = (0.772 × BSA)/1.08	North America

LV: Liver volume; BW: Body weight; BSA: Body surface area.

Table 2 Results of semi-automated image processing in different analysis

Ref.	Software and comparison	Reports
Pomposelli *et al*[47], 2012	Software MeVis Compared right lobe graft volumes estimated by SAIP with actual graft weights measured during LDLT	A nonsignificant volume difference of approximately 17.5 mL and a low percentage error of approximately 2.8%
Çelik *et al*[34], 2023	CT Liver Analysis, Philips Healthcare-RLDG volumes by manual and SA were compared to AGW	Both manual and SA overestimated the graft weight (manual: 893 ± 155 mL *vs* AGW: 787 ± 128 g, $P < 0.001$, SA: 879 ± 143 mL *vs* AGW, $P < 0.001$). The mean interaction time was 27.3 ± 14.2 min for manual and 6.8 ± 1.4 min for SAIP ($P < 0.001$)
Mohapatra *et al*[31], 2020	Myrian XP Liver 3D software (France)-RLDG, LLDG and LLSDG volumes by manual and SA were compared to AGW	Both manual and SA showed strong correlation with AGW ($r = 0.834$ and 0.856, respectively). The mean percentage error for manual and SA was 14.2 ± 12.5% and 12.2 ± 11.8%, respectively. The overall accuracy improved using SA ($P = 0.015$)
Kalshabay *et al*[25], 2023	Vitrea software, including two different applications for manual segmentation (Volume analysis) and automated segmentation (CT liver analysis) SA software (OsiriX MD) RLDG	The manual method correlated better with AGW ($r = 0.730$) in comparison with the SA ($r = 0.685$) and the automated ($r = 0.699$) methods ($P < 0.001$). The mean error ratio in volume estimation by each application was 12.7 ± 16.6% for manual, 17.1 ± 17.3% for SA, 14.7 ± 16.8% for automated methods
Goja *et al*[39], 2018	AW Volume share 6 (GE Healthcare; Chicago, Illinois, United States) RLDG, LLDG and LLSDG volumes by SA were compared to AGW	RLDGt: There was no statistically significant difference between mean SA and AGW in RL (722 ± 134 *vs* 717 ± 126 gm; $P = 0.06$). LLDG: Correlated strongly ($r = 0.81$, $P < 0.001$), mean SA was significantly high as compared to mean of AGW (460 ± 118 *vs* 433 ± 102 gm; $P = 0.003$). LLSDG: Mean SA was significantly low as compared to mean of AGW (203 ± 48 *vs* 254 ± 49 gm; $P < 0.001$)

CT: Computerized tomography; SA: Semi-automated; AGW: Actual graft weight, RLDG: Right lobe donor graft; LLDG: Left lobe donor graft; LLSDG: Left lateral segment donor graft.

Segmentation algorithms

Segmentation algorithms were used to delineate the liver region of interest from the remaining images. Additionally, such algorithms aim to accurately identify the liver boundaries. Commonly used algorithms include threshold-based methods, region growing, active contours (or snakes), level sets, graph cuts, and machine-learning-based techniques.

Threshold-based methods

Threshold-based methods involve setting intensity thresholds to separate the liver from the background or other organs. The liver is segmented based on predefined intensity ranges or statistical measures such as the mean intensity or intensity distribution.

Region growing

Region-growing algorithms start from a seed point within the liver and iteratively develop the region by including pixels with similar characteristics (*e.g.*, intensity, texture, or gradient) until a stopping criterion is met. The method is particularly useful when the liver has a distinct intensity pattern compared to the surrounding tissues.

Active contours (snakes)

Active contour models, also known as snakes, use an energy-optimization approach to iteratively deform a contour to fit the liver boundary. The contour was attracted to the image edges or intensity gradients, ensuring accurate delineation of the liver boundaries.

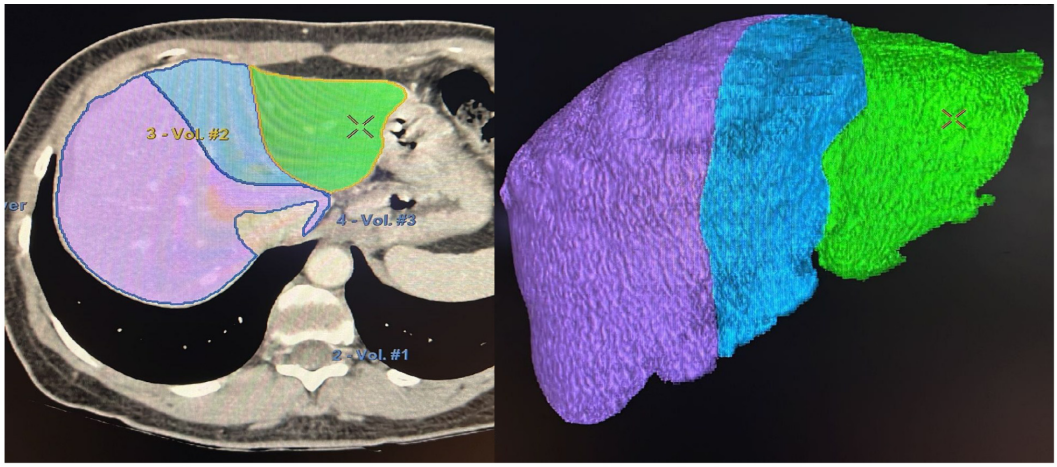

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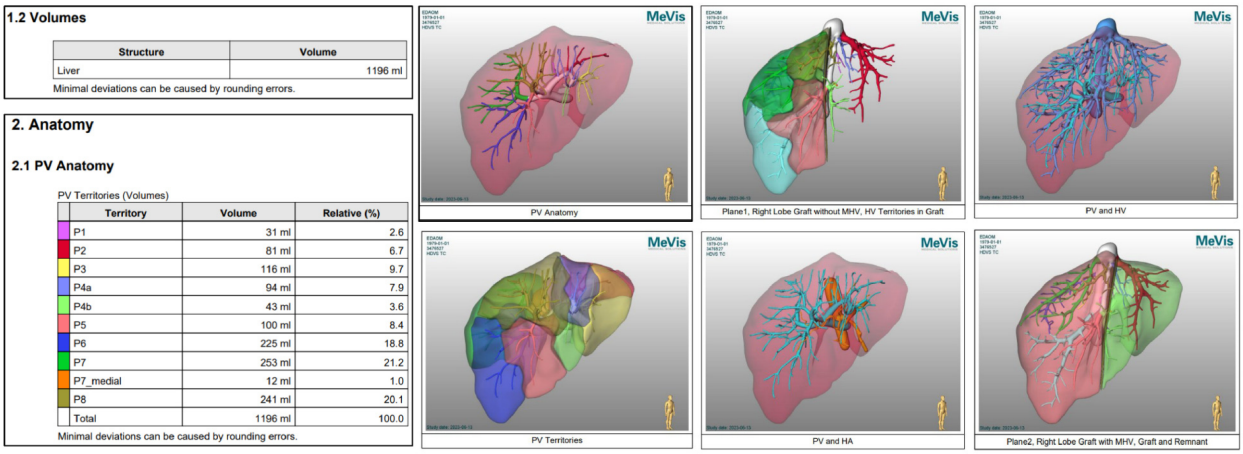
Figure 1 Manual volumetric study performed in our institution for pre-operative living-donor evaluation (Hepatic VCAR-GE Healthcare).



1.2 Volumes

Structure	Volume
Liver	1196 ml

Minimal deviations can be caused by rounding errors.

2. Anatomy

2.1 PV Anatomy

PV Territories (Volumes)

Territory	Volume	Relative (%)
P1	31 ml	2.6
P2	81 ml	6.7
P3	116 ml	9.7
P4a	94 ml	7.9
P4b	43 ml	3.6
P5	100 ml	8.4
P6	225 ml	18.8
P7	253 ml	21.2
P7_medial	12 ml	1.0
P8	241 ml	20.1
Total	1196 ml	100.0

Minimal deviations can be caused by rounding errors.

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Figure 2 MeVis software images and tables output: The software returns multiple images and tables. PV: Peripheral vein; MHV: Middle hepatic vein; HA: Hepatic artery.

Level sets

Level-set methods are mathematical techniques used to evolve a curve or surface over time to delineate the liver boundaries. The methods use the concept of level sets, which represent the evolving contour as a zero-level set of a higher-dimensional function.

Graph cuts

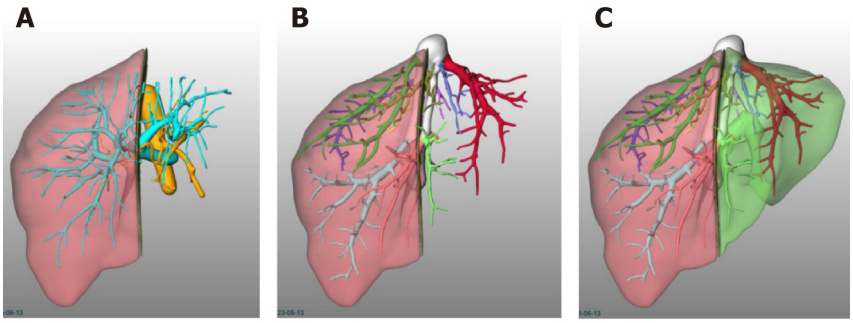
Graph cut algorithms model the liver segmentation problem as an optimization task in a graph framework. The graph is constructed using image features, and the segmentation is achieved by identifying the minimum energy cut that separates the liver from the background.

Machine learning-based techniques and deep learning

Machine learning algorithms, such as random forests, support vector machines, and deep learning models, can be trained on annotated liver images to automatically segment the liver. Such algorithms learn the patterns and features that distinguish the liver from other structures and can provide accurate and robust segmentation results[40].

Most software tools employ a combination of techniques or advanced algorithms that are specific to their methodology. The choice of algorithm depends on factors such as image quality, complexity of liver structures, computational efficiency, and specific requirements of the application. Each algorithm has its advantages, limitations, and parameter settings, which must be carefully considered and optimized for accurate LV. A combination of techniques can be used to improve accuracy and robustness[41].

For example, the initial segmentation can be obtained using thresholding or region growth, followed by refinement using active contours or graph cuts. Hybrid approaches that combine multiple algorithms can leverage the strength of each technique to achieve more accurate LV. Additionally, the validation and evaluation of the automated LV results against the ground truth or manual segmentations are critical for assessing the algorithm's performance and reliability



D

Plane1, Right Lobe Graft without MHV (Volumes)

	Territory	Volume	Relative (%)
	Plane	15 ml	1.3
	Graft	769 ml	64.3
	Remnant	412 ml	34.4
	Total	1196 ml	100.0

Minimal deviations can be caused by rounding errors.

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Figure 3 Resection planes volumetric estimation using MeVis. A: Right Lobe Graft without middle hepatic vein (MHV), peripheral vein and hepatic artery; B: Right Lobe Graft without MHV, HV; C: Right Lobe Graft without MHV, Graft and Remnant; D: Table showing total, plane, graft and remnant liver volumes. MHV: Middle hepatic vein.

[42].

Most computer aided diagnostics used in clinical practice use conventional machine learning approaches, in which the effectiveness depends on the domain expertise of the developers. So, the limitations of conventional learning are linked to the limitations of the human developer. Manual and semi-automated volumetry is dependent on conventional machine learning. Deep learning has emerged as a state-of-the-art machine-learning method for many applications. Deep learning is a type of representation learning method in which a complex multilayer neural network architecture learns representations of data automatically by transforming the input information into multiple levels of abstraction[43].

Deep convolutional neural networks (DCNN) are widely used in image pattern recognition. They can automatically extract relevant features from training samples by adjusting their weights through backpropagation. In contrast to manual feature design, the DCNN learns feature representations during training. When trained with a large and representative dataset, the DCNN features outperformed the hand-engineered features by being highly selective and invariant. The automated deep learning process enables the analysis of numerous cases, surpassing human capabilities. Deep learning proves robust in handling variations across different classes, as long as the training set is diverse and extensive [40-43].

ACCURACY AND RELIABILITY

Automated LV and deep machine learning for LDLT has gained attention in recent years. There has been an increase in the number and quality of AI and machine learning studies in the medical field, mainly those focused on automating the interpretation of 2D image tests (MRI, CT, and radiographs), assembling three-dimensional models of organs and tissues, and volumetric calculations, including virtual segmentation of the liver. In liver resection and liver transplantation, most studies have a small number of cases, focusing on adult liver transplantation and RLDG, with very few studies on left lobe donor graft and left lateral segment donor graft[26-28,42-44]. The higher risk of the small-for-size syndrome in adult liver transplantation justifies the intense volumetric and anatomical studies on RLDG. Usually, for pediatric recipients (< 10 kg), an inaccurate volumetric assessment will rarely lead to insufficient liver volume; in contrast, the risk of the large-for-size syndrome is higher compared to the small-for-size syndrome. In such cases, the surgeon usually reduces the graft on the back table or converts it into a mono-segmental graft before implantation[45].

Automated software allows the surgeon to choose the transection plane; some studies have compared the correlation of these measurements for RLDG when performed by the surgeon using automated software with the manual measurements performed by radiologists. Moreover, both measurements had a good correlation with the AGW (r > 0.80), along with no significant difference between measurements by the surgeon and the radiologist[29].

As it is of paramount importance that the surgeon who is going to perform the procedure to perform the anatomical assessment and to choose the adequate liver segmentation plane, new softwares, focusing on the surgeon's interaction are being developed. A more user-friendly automated platform was developed by a group from the Republic of Korea[46], which they referred to as Dr. Liver. They validated the method in 50 RLDG and compared it to MLV. The correlation with AGW was better for the automated Dr. Liver (r = 0.98) than for the MLV (r = 0.92), although they were both good correlations. However, the percentage of absolute difference (%AD) from AGW of Dr. Liver (3.1% ± 2.8%) was significantly

smaller than that of the MLV (10.2% ± 7.5%). None of the Dr. Liver measurements percentages of %AD was > 10%, while they were 46% for MLV measurements. Evaluation of %AD is very important in clinical practice because an error percentage of more than 10% can result in a small-for-size boundary graft volume. Also, the total time for task completion was shorter for Dr. Liver *vs* MLV (7.3 ± 1.4 min *vs* 37.9 ± 7.0 min).

CONCLUSION

Accurate preoperative assessment of the liver plays a critical role in ensuring the selection of suitable donors and improving recipient outcomes after LDLT. MLV initially emerged as the gold standard for accurate assessment. However, the time-consuming nature of the manual analysis, reliance on operator expertise, and high variability in PE have prompted the adoption of SAIP software tools, and more recently, automated software solutions. AI represents the future of LV and segmentation and offers immense potential in the field, leading to a future fully automated liver segmentation and volumetry based on deep-learning. However, the widespread adoption and daily application of AI are hindered by cost and accessibility limitations. We are responsible for embracing technology and fostering interdisciplinary collaborations in the fields of radiology, engineering, informatics, and surgery. The possibilities afforded by AI are limitless, ranging from patient counseling and education to intraoperative decision-making facilitated by automation and AI assistance.

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FOOTNOTES

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MINIREVIEWS

Anti-thymocyte globulin for treatment of T-cell-mediated allograft rejection

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Abstract

Anti-thymocyte globulin (ATG) is a pivotal immunosuppressive therapy utilized in the management of T-cell-mediated rejection and steroid-resistant rejection among renal transplant recipients. Commercially available as Thymoglobulin (rabbit-derived, Sanofi, United States), ATG-Fresenius S (rabbit-derived), and ATGAM (equine-derived, Pfizer, United States), these formulations share a common mechanism of action centered on their interaction with cell surface markers of immune cells, imparting immunosuppressive effects. Although the prevailing mechanism predominantly involves T-cell depletion *via* the complement-mediated pathway, alternate mechanisms have been elucidated. Optimal dosing and treatment duration of ATG have exhibited variance across randomised trials and clinical reports, rendering the establishment of standardized guidelines a challenge. The spectrum of risks associated with ATG administration spans from transient adverse effects such as fever, chills, and skin rash in the acute phase to long-term concerns related to immunosuppression, including susceptibility to infections and malignancies. This comprehensive review aims to provide a thorough exploration of the current understanding of ATG, encompassing its mechanism of action, clinical utility in the treatment of acute renal graft rejections, specifically steroid-resistant cases, efficacy in rejection episode reversal, and a synthesis of findings from different eras of maintenance immunosuppression. Additionally, it delves into the adverse effects associated with ATG therapy and its impact on long-term graft function. Furthermore, the review underscores the existing gaps in evidence, particularly in the context of the Banff classification of rejections, and highlights the challenges faced by clinicians when navigating the available literature to strike the optimal balance between the risks and benefits of ATG utilization in renal transplantation.

Key Words: Anti-thymocyte globulin; T-cell-mediated rejection; Steroid-resistant

rejection; Biopsy confirmed acute rejection

Core Tip: Anti-thymocyte globulin is a highly efficient induction agent that can prevent acute rejection and delayed graft function. It is widely used for biopsy confirmed acute rejection reversal and steroid-resistant rejection.

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INTRODUCTION

Rejection is one of the common complications after kidney transplantation. About 10%-20% of kidney transplant recipients experience acute rejection (AR) in the first year post-transplant[1,2]. AR can be defined clinically as a rise in serum creatinine in the absence of other pathology, and verified by allograft biopsy using the Banff classification system [3]. AR is associated with an increased risk of long-term graft loss, morbidity, and mortality[4]. Therefore, timely treatment of AR is crucial in improving long-term outcomes in kidney transplantation. A proportion of AR can be resistant to steroids (25%-30% of AR episodes)[2]. Anti-thymocyte globulin (ATG) is a polyclonal antibody used as an induction agent to reduce rejection rates and treat rejections following a kidney transplant. It is available in rabbit-derived (rATG; Thymoglobulin), ATG-Fresenius, and equine-derived forms (eATG; ATGAM). During the early use of ATG, its role in treating steroid-resistant allograft rejection was established[5]. The Kidney Disease Improving Global Outcomes (KDIGO) and British Transplant Society guidelines advise using ATG at induction in high-risk individuals and as an option to manage steroid-resistant acute rejection[6,7]. For this review, we studied peer-reviewed research articles published in PubMed-indexed journals. We reviewed the various clinical trials of ATG, its use in the treatment of acute rejection, steroid-resistant rejection, recurrent rejections, and clinical studies published in similar journals. We excluded reports presented as conference abstracts and those published in languages other than English. We aimed to evaluate the risks and benefits of ATG treatment in rejections and its implications in clinical practice. We envisage that such analysis of the literature will help clinicians and patients evaluate the role of ATG holistically in current transplantation protocols and aid in clinical decision-making at an individual patient level. Lastly, we identify gaps in evidence and outline potential strategies that could help bridge these gaps to improve post-transplant patient and allograft survival.

MECHANISM OF ACTION

ATG predominantly targets T cell antigens (although some of these antigens are present in other cell types) like TCR/CD3, CD2, CD4, CD5, CD6, CD8, CD25, CD28, CD45, and HLA (Human Leukocyte Antigen) class I to induce the immunosuppressive effects. The complement-dependent T cell lysis in the intravascular compartment (*i.e.*, blood) and the phagocytosis of T cells by macrophages in peripheral and secondary lymph nodes are regarded as the primary mechanism of action of ATG. The pre-activated T cells present in blood or peripheral tissues are depleted through antibody-dependent cell-mediated cytotoxicity and Fas-ligand-dependent apoptosis pathways[5,8,9]. The pharmacokinetics of ATG depends on the dose and schedule of administration as well as the number of 'targeted' immune effectors[9]. A lower concentration of thymoglobulin in the 0.1–1 μg/mL range induces lysis of preactivated T cells. A higher concentration (10–100 μg/mL) triggers CD178 (CD95-L) expression by resting T cells and apoptosis of preactivated T cells through pathways mostly involving Fas/Fas-L interactions[10,11]. ATG also modulates cell surface expression of adhesion molecules (ICAM-1, -2, and -3), integrins (LPAM-1 and VLA-4), and chemokine receptors (CXCR4, CCR5, and CCR7), thus interfering with leukocyte-endothelial interactions that play a role in ischemia/reperfusion injury, graft *vs* host disease, and rejection[10,12]. The modulation, particularly in this setting, is the process of internalization of the ATG-antigen complex by endothelial cells. This results in decreased surface antigen which ultimately decreases the interaction of leucocytes with the endothelium and their trans-migration into tissue. ATG has been shown to contain antibodies against a few B-cell antigens, including B-cell-specific and non-specific surface proteins CD19, CD20, CD40, CD80, CD30, CD38, CD95, and HLA-DR. ATG crosslinks with these surface proteins and induces apoptosis (*in vitro*) in naïve and activated B cells at clinically relevant concentrations (1–100 ng/mL). ATG can also bind with Syndecan-1 (CD138), a plasma-cell-specific molecule; however, *in vivo* ATG treatment is not associated with a reduction in either splenic or bone marrow plasma cells[5,9].

ATG interferes with the functional properties of dendritic cells (DCs) including maturation and migration and influences the balance between solid organ rejection and tolerance. Several *in vitro* studies showed the tolerogenic effect of ATG. ATG attaches to Toll-like receptors present on the surface of DCs. The common mechanism is the induction of

complement-mediated DC lysis hampering lymphocyte proliferation[13-15].

Finally, ATG is also known to produce dominant tolerance by the expansion of CD4+CD25high Foxp3+ T-regulatory cells which inhibits the action of CD4+CD25- T cells, CD8+ T cells, B cells, DCs, and natural killer (NK) cells[16-18]. It also associates with the increase of NK-T cells (CD4-/CD8- subset of T cells), which seems to decrease the incidence and severity of acute rejection[19]. Figure 1 summarizes the five documented mechanisms of ATG.

TREATMENT OF T-CELL-MEDIATED REJECTION

T-cell-mediated rejection (TCMR) is a process initiated by the interaction of T-cells with donor antigens predominantly presented by macrophages. The interaction of these biomolecules leads to local inflammation (infiltration of T cells and macrophages) that further leads to recruitment of effector T cells, tubulitis, nephron response to injury including differentiation of the epithelium, and if untreated, nephron loss that will be irreversible. Acute rejection is clinically suspected in patients experiencing an increase in serum creatinine, after the exclusion of other causes of graft dysfunction. Subclinical acute rejection is defined by the presence of histological changes specific for acute rejection on screening or protocol biopsy, in the absence of clinical symptoms or signs. Kidney biopsy remains the gold standard test to diagnose acute rejection, with characteristic infiltration of donor tissue interstitium with host T cells, cells in the monocyte-macrophage lineage, and nephron injury[20]. Treatment of TCMR has changed little over time and sparse data exist comparing one strategy to another.

AR requires a short course of more intensive immunosuppression in addition to baseline immunosuppression therapy. Options include treatments with steroids, antibody preparations, alteration of maintenance immunosuppression, or a combination of these options. Corticosteroid therapy is the most commonly used first-line treatment for acute cellular rejection episodes. Although most patients respond to corticosteroids, the dose and duration of treatment have not been well defined by randomised controlled trials. Table 1 lists the published clinical trials and Table 2 lists published cohort studies, the majority of which are retrospective single centre studies. Treatment of acute cellular rejection with T-cell depleting antibody can be more effective in improving kidney function and preventing graft loss than treatment with corticosteroids alone[21]. However, all these trials were published more than 20 years ago, with the majority between 1970s and 1990s, when Banff classification was yet to be incorporated into clinical practice or clinical trials/studies. In clinical practice, treatment is guided by biopsy features as longer-term graft survival varies with the type of TCMR[22]. The majority of Banff class I lesions respond to methylprednisolone alone; conventionally pulse methylprednisolone at 250–500 mg daily for 3–5 d is recommended by international guidelines[6]. TCMR involving lymphocytic infiltrate of the vasculature (Banff II and III lesions) may necessitate T cell–depleting therapy. Polyclonal antibodies include horse-derived (anti-lymphocyte globulin, ALG) and rabbit-derived (ATG) antibodies against the human lymphocyte or thymocyte, respectively. Most commonly rATG dosed at 1.5 mg/kg for 7-14 doses was used (Tables 1 and 2)[8,21-24]. Reversal of rejections was seen in 50%-90% in clinical trials. Intravenous immunoglobulin (IVIG) and anti-thymocyte serum were also used in the past[25]. Recently, Alemtuzumab had been put forward as a possible treatment option for rejection[26].

STEROID-RESISTANT REJECTIONS

In approximately 25% to 30% of the patients, rejections are not reversed with steroid therapy alone. In these recipients, more intensive immunosuppressive therapy is required to reverse the AR episode. When serum creatinine levels do not recover to within 120% of the pre-rejection baseline value following corticosteroid pulse therapy within 14 d of the steroid medication's initiation, the episode is deemed steroid-resistant[27]. Up until day 5, patients with steroid-responsive and steroid-resistant AR experienced similar changes in their serum creatinine levels. However, at that point, the responders' creatinine levels significantly decreased, while the non-responders' levels stayed high. Therefore, conventionally, physicians typically wait 5 d for classifying a rejection as steroid-resistant[28].

ADVERSE EFFECTS

Infusion of ATG may be complicated by immediate toxicity in the form of fever, chills, or skin rash which are considered self-limiting and managed by symptomatic therapy (paracetamol, antihistamines, and bolus steroids) and reducing rates of infusion. Lymphopenia, neutropenia, and thrombocytopenia can occur, but these are amenable to dosage adjustment. Medium- to longer-term effects include cytopenia, higher rates of infection, and malignancy. Serum sickness is a rare complication caused by the deposition of immune complexes in tissues. Characteristic symptoms include fever, jaw pain, arthralgia, lymphadenopathy, and rash[10,22]. Registry studies have tried to determine whether ATG induction therapy is associated with a greater risk of developing post-transplant lymphoproliferative disease, but results are mixed and remain inconclusive[10]. Tables 1 and 2 outline the frequency of these adverse effects published in the randomised controlled studies and cohort studies, respectively.

Table 1 Summary of randomized clinical trial studies

No.	Ref.	Study design	ATG – dose & duration	Graft outcome	Death	Other adverse events
1	Shield *et al* [50], 1979	Prospective, randomised, single centre, United States; First rejection	eATG 15 mg/kg daily for 14 d (n = 10) *vs* MP 1 g/d for 5 d (n = 10)	Reversal – 8/10 (ATG) *vs* 6/10 (MP); Recurrent rejection 1/10 (ATG) *vs* 5/10 (MP); Graft loss at 12 mo – 1/10 (ATG) *vs* 1/10 (MP)	At 12 mo – 0/10 (ATG) *vs* 1/10 (MP)	Infection – 3/10 (ATG) *vs* 0/10 (MP); AVN – 1/10 (ATG) *vs* 0/10 (MP)
2	Filo *et al*[51], 1980	Prospective, randomised, single centre, United States; First rejection	eATG 10 mg/kg/d for 15 d (n = 35) *vs* MP 30 mg/kg every other day up to 5 doses (n = 43)	Reversal – 32/35 (ATG) *vs* 29/43 (MP); Recurrent rejection – 16/35 (ATG) *vs* 15/43 (MP); Graft survival (91% *vs* 62%); Faster recovery (6.9 d *vs* 8.9 d); Graft loss – 15/35 *vs* 25/43 (MP)	At 12 mo – 1/24 (ATG) *vs* 0/29 (MP)	
3	Hoitsma *et al* [52], 1982	Prospective, randomised, single centre, Netherlands; First rejection	rATG initially 4 mg/kg followed by 2-7 mg/kg for 21 d (n = 20) *vs* prednisolone 200 mg/d, tapered to 25 mg/d in 2 wk (n = 20)	Reversal – 43/50 (ATG) *vs* 35/50 (Prednisolone); Recurrent rejection – 28/50 (ATG) *vs* 35/50 (Prednisolone); Graft loss – 15/50 (ATG) *vs* 28/50 (Prednisolone)	At 12 mo – 0/20 (ATG) *vs* 1/20 (Prednisolone)	Infection – 9/20 (ATG) *vs* 15/20 (Prednisolone)
4	Toledo-Pereyra *et al* [53], 1985	Prospective, randomised, single centre, United States; First rejection	ALG 10 to 20 mg/kg for 10 d (n = 20) *vs* ATG 10 to 20 mg/kg for 10 d (n = 20)	Reversal – 15/20 (ALG) *vs* 16/20 (ATG)		
6	Alamartine *et al*[54], 1994	Prospective randomised, single centre, France; Steroid-resistant rejection	Muromonab-CD3 5 mg/d for 10 d (n = 27) *vs* rATG: 1.5 mg/kg/d for 10 d (n = 32)	Reversal – 25/26 (Muromonab-CD3) *vs* 27/32 (ATG); Recurrent rejection – 25/32 (ATG) *vs* 24/27 (Muromonab-CD3); Graft loss at 12 mo – 11/32 (ATG) *vs* 4/26 (Muromonab-CD3)		CMV infection – 8/27 (Muromonab-CD3) *vs* 18/32 (ATG)
7	Tesi *et al*[55], 1997	Prospective, randomised, multi-centre n = 163 (82 Thymoglobulin, 81 ATGAM); First rejection	rATG 1.5 mg/kg *vs* ATGAM 15 mg/kg (both for 7 to 14 d)	65% treated with THYMO had histology grade improvement (*vs* 50% in ATGAM)	Overall – 3/82 (rATG) *vs* 1/81 (eATG)	CMV infection 20/82 in both groups
8	Mariat *et al* [31], 1998	Prospective, randomised, single centre, France; First rejection	Muromonab-CD3 5 mg/kg for 3 d followed by 2.5 mg/kg for 7 d (n = 29) *vs* rATG 25 mg/d if < 40 kg, 50 mg/d if 40-70 kg & 75 mg/d if > 70 kg; 10 d (n = 31)	Reversal – 25/29 (Muromonab-CD3) *vs* 30/31 (ATG); Recurrent rejection – 11/29 (Muromonab-CD3) *vs* 9/31 (ATG); Graft loss at 12 mo – 6/29 (Muromonab-CD3) *vs* 4/31 (ATG)	At 12 mo – 3/31 (ATG) *vs* 1/29 (Muromonab-CD3)	CMV infection – 12/31 (ATG) *vs* 13/29 (Muromonab-CD3); Malignancy – 0/31 (ATG) *vs* 2/29 (Muromonab-CD3)
9	Gaber *et al* [56], 1998	Prospective, randomised, multi centre, United States; First rejection	Thymoglobulin (rATG) 1.5 mg/kg/d for 7-14 d (n = 82) *vs* ATGam (eATG) 15 mg/kg/d, for 7-14 d (n = 81)	Reversal – 88% (Thymoglobulin) *vs* 76% (ATGAM); Recurrent rejection; 28/82 (rATG) *vs* 50/81 (eATG)	Total 6/82 (rATG) *vs* 3/81 (eATG)	Leukopenia – 57% (rATG) *vs* 30% (eATG); Bacterial infection – 29% (rATG) *vs* 37% e(ATG); Viral infection – 21% (rATG) *vs* 11% (eATG)
10	Theodorakis *et al*[57], 1998	Prospective, randomised, single centre, Germany; First rejection	ATG 4 mg/kg for 7 d (n = 25) *vs* MP 250 mg/d for 3 d (n = 25)	Recurrent rejection – 4/25 (ATG) *vs* 18/25 (MP); Graft loss – 5/25 (ATG) *vs* 3/25 (MP)		
11	Baldi *et al* [58], 2000	Prospective, randomised, single center, Belgium; First rejection	rATG 4 mg/kg day for 10 d (n = 28) *vs* Muromonab-CD3: 5 mg/d for 10 d (n = 28); MP for both groups: 500 mg/d for 3 d	Reversal – 21/28 (rATG) *vs* 14/28 (Muromonab-CD3); Recurrent rejection – 9/28 (ATG) *vs* 10/25 (Muromonab-CD3)	Irreversible rejection in 3/28 OKT3, 2nd rejection in 33% ATG, 39% OKT3	Fever – 21.4% (ATG) *vs* 92.8% (Muromonab-CD3); Headache – 3.5% (ATG) *vs* 46.4% (Muromonab-CD3); Infection – 9/28 (ATG) *vs* 10/28 (Muromonab-CD3); Malignancy 2/28 (ATG) *vs* 0/28 (Muromonab-CD3)
12	Midtvedt *et al*[59], 2003	Prospective, randomised, single centre, Norway; First rejection	ATG 2 mg/kg followed by 1 mg/kg if & when T cells > 50 (n = 27) *vs* muromonab-CD3: 5 mg, then 2.5 mg (n = 28)	Reversal – 26/27 (ATG) *vs* 27/28 (Muromonab-CD3); Recurrent rejection – 12/27 (ATG) *vs* 14/28 (Muromonab-CD3); Grafts loss at 12 mo – 3/27 (ATG)	At 12 mo – 2/27 (ATG) *vs* 1/28 (Muromonab-CD3)	CMV infection – 14/27 (ATG) *vs* 11/28 (Muromonab-CD3); Malignancy – 1/27 (ATG) *vs* 1/28 (muromonab-CD3); Bacterial pneumonia – 3/27

			vs 4/28 (Muromonab-CD3)			(ATG) *vs* 3/28 (Muromonab-CD3)

ATG: Anti-thymocyte globulin; rATG: Rabbit Anti-thymocyte globulin, eATG: Equine Anti-thymocyte globulin; MP: Methylprednisone.

Table 2 Summary of non-randomized clinical studies

No	Ref.	Study design	ATG -dose/duration	Graft outcome	Death	Adverse events
1	Hardy *et al* [60], 1980	Prospective, non-randomised, single centre , United States, *n* = 20 (10 ATG)	eATG – 15 mg/kg (max 750 mg) for 21 d + MP (750, 200 & 150 mg for 3 d) (*n* = 10) *vs* MP (750, 200 & 150 mg for 3 d) (*n* = 10)	Reversal – 9/10 (ATG) vs 8/10 (control); Recurrent rejection 2/10 (ATG) *vs* 4/10 (control); Graft loss at 12 mo – 4/10 (ATG) *vs* 5/10 (control)	0 in both groups	3 serious complications in control group and 1 in ATG
2	Richardson *et al*[30], 1989	Prospective, non-randomised, single centre, United Kingdom	rATG (2-3 mg/kg for 5-10 d) reduced to 1-2 mg/kg if leukopenia or thrombocyt-openia (*n* = 27)	70.3% graft survival with mean follow-up time of 13.3 mo; 8 out of 27 failed (6 due to rejection, 1 death, and 1 renal artery stenosis)	1 death	6 UTIs, 1 pseudomembranous colitis, 8 CMV and 5 HSV, 2 deaths
3	Clark *et al* [45], 1993	Prospective, non-randomised, single centre, United Kingdom	Group 1: rATG, 2.5-5 mg/kg/d) for 10-14 d (*n* = 10); Group 2: As per T cell count for 10-14 d (*n* = 17)	76% graft survival at 1 year group 2 (*vs* 60% in group 1); Group 1 – (4 rejections); Group 2 – (4 rejections)	2 deaths (group 1) *vs* 0 deaths (group 2)	Group 1: 3 serious viral infection, 6 minor infections; Group 2: 11 minor infections
4	Uslu *et al* [61], 1997	Retrospective, non-randomised, single centre, Turkey	rATG 5 mg/kg for 13.7 ± 3.7 d (*n* = 9) OKT3 5 mg/d for 11.4 ± 1.9 d (*n* = 5)	Graft survival: 78% ATG *vs* 20% OKT3 with median f/u 405 d		OKT3 – 1 CMV, Fever > 38 in 80% pts in both groups, Leukopenia (35% ATG *vs* 0 in OKT3)
5	Sharma *et al* [46], 2003	Prospective, non-randomized, single centre, India	ATG 1.5-1.8 mg/kg alternate d, mean duration 5 doses (*n* = 33)	90% graft survival in first year and 73% at 20 mo. Graft loss in 4; Recurrent rejection in 8/33 at 3 mo	1 death	11 pneumonia, 3 UTI, 1 peritonitis, 2 CMV, 5 leukopenia
6	Colak *et al* [62], 2008	Retrospective, non-randomised, single-centre, Turkey	ATG 3-5 mg/kg/d 10-14 d (Dose adjusted with other parameters) (*n* = 23)	Graft function improved in 19 cases (83%)	1 death	9 infections (3 pulmonary aspergillosis, 2 CMV, 4 pulmonary/urinary bacterial infections)
7	Kainz *et al* [33], 2009	Retrospective, non-randomised, multi centre, Austria	N/A *n* = 399 (368 ATG, 31 OKT3)	Median actual graft survival 9.5 yr ATG *vs* 4.5 yr OKT3	N/A	N/A
8	van der Zwan *et al* [38], 2018	Retrospective, non-randomised, single centre, Netherlands	rATG – 4 mg/kg repeated after 4 d if CD3 > 200, for 2 wk (*n* = 103)	Median allograft survival 7.0 yr. At one yr 78.2% had functioning graft; At 5 yr 55.6% functioning graft; 49 lost graft in median f/u 6.8 yr	17 deaths	97 bacterial, 8 fungal, 27 CMV reactivation, 4 EBV reactivation, 6 BK viraemia), 14 malignancy (12 solid, 2 lymphoma)

ATG: Anti-thymocyte globulin; EBV: Epstein-Bar virus, CMV: Cytomegalovirus, rATG: Rabbit anti-thymocyte globulin; OKT3: Muromonab CD3, UTI: Urinary tract infection; N/A: Not applicable.

DISCUSSION

Despite the advancement of immunosuppressant therapy, AR remains one of the major problems in the field of clinical renal transplantation. The current approach in the management of acute kidney rejection in adults and children is based on the 2009 KDIGO guidelines[29]. These guidelines recommend corticosteroids for the initial treatment of acute cellular rejection. They advise adding or restoring maintenance prednisone in patients with rejection episodes who are not on steroids. They also recommend using lymphocyte-depleting agent or muromonab-CD3 (OKT3) for TCMR that does not respond to corticosteroids and for recurrent acute cellular rejections. The lymphocyte-depleting agent ATG has been used extensively for treating and preventing AR in kidney transplant recipients[21]. ATG has also been used as first-line therapy for those with severe acute TCMR including vascular lesions (Banff II or higher categories), and as rescue therapy for steroid-resistant acute TCMR (Tables 1 and 2). It has been shown that steroid-resistant rejection can be a significant problem in patients immunosuppressed with triple therapy (combination of tacrolimus [Tac], mycophenolate mofetil [MMF], and steroids) and 70% of such rejections can be reversed following ATG treatment[30]. A systemic review by Webster *et al*[23] was one of the comprehensive studies describing the advantages of using ATG over steroids for the treatment of steroid-resistant rejection. They studied 21 trials (49 reports, 1394 randomised participants) and concluded that in treating first rejection, ATG was superior to steroids in reversing rejection (relative risk [RR] = 0.57; 95% confidence interval [CI]: 0.38-0.87) and preventing graft loss (death-censored RR = 0.74; 95%CI: 0.58-0.95). However, there

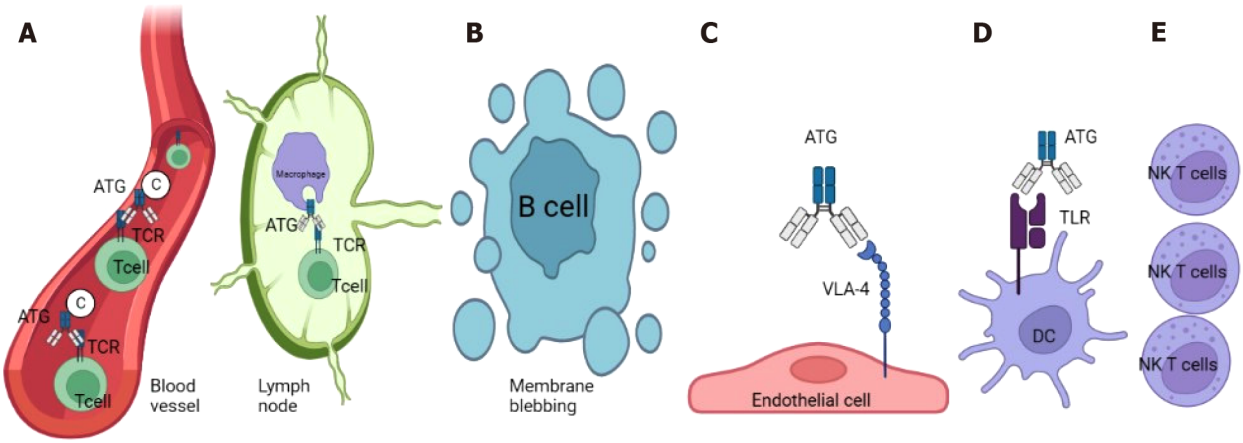

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Figure 1 Mechanisms of action of anti-thymocyte globulin. A: T-cell depletion in blood through complement-mediated lysis and in secondary lymphoid tissue by T cell apoptosis; B: B-cell apoptosis by anti-thymocyte globulin (ATG); C: ATG-VLA-4 complex leading to decreased adhesion proteins in endothelial cells required by leukocyte/endothelium interaction; D: Dendritic cell maturation by HLA1/ATG interaction; E: Increased natural killer T cells.

was no difference in preventing subsequent rejections (RR = 0.67; 95%CI: 0.43-1.04) or death (RR = 1.16; 95%CI: 0.57-2.33) at 1 year between ATG and steroids. Additionally, they also found no benefits with the use of muromonab-CD3 over ATG or ALG in reversing rejection, preventing subsequent rejection, or preventing graft loss or death. A decade later, in 2017[21], Webster *et al*[23] updated the review with 11 new trials (76 reports, 1680 participants). The updated meta-review concluded that antibody therapy was still better than steroid therapy (RR = 0.50; 95%CI: 0.30 to 0.82) for reversing the first acute rejection and preventing subsequent rejections (RR = 0.70; 95%CI: 0.50 to 0.99) and tended to help prevent graft loss (death-censored RR = 0.80; 95%CI: 0.57 to 1.12). There was no benefit of muromonab-CD3 over ATG in reversing rejection, preventing subsequent rejection, or preventing graft loss or death[29].

Muromonab-CD3 (Orthoclone, OKT3) is the first monoclonal antibody used clinically for immunosuppression. It eliminates CD3+ T cells from the peripheral circulation to produce the immunosuppressive effects. A few noticeable studies compared muromonab-CD3 with monoclonal and polyclonal antibodies in the treatment of steroid-resistant rejection[31,32]. Using clinical records stored in the Austrian Dialysis and Transplant Registry, Kainz *et al*[33] conducted a retrospective descriptive analysis of 399 (368 ATG treated *vs* 31 OKT3 treated) patients diagnosed with biopsy-confirmed acute rejection between 1990 to 2005. Their study suggested that ATG treatment for rejecting allograft exhibited longer graft survival over OKT3 treatment (median graft survival 9.5 years in ATG group *vs* 4.6 years in OKT3 group) and increased risk of graft loss in OKT3 group (hazard ratio = 1.73; 95%CI: 1.09-2.74; P = 0.019). ATG was better tolerated compared to OKT3, with a lower frequency of cytokine release syndrome.

Clinicians all around the world have backed studies to find a better alternative or newer, safer but more effective immunosuppressive regimen. Due to cost-effectiveness, adverse infusion reaction, prolonged duration of inpatient stay, and need for central venous access for ATG, Alemtuzumab (CD52-specific monoclonal antibody), which can be given subcutaneously in a single dose, has been put forward with some promising results. A propensity-matched controlled study of 116 patients treated with Alemtuzumab, in comparison to 108 patients treated with ATG, showed similar patient and allograft survival[26] whilst having superior infection-free survival with Alemtuzumab. The authors suggested that Alemtuzumab therapy may therefore be an alternative therapy for glucocorticoid-resistant, recurrent, or severe acute kidney transplant rejection.Registry data show that the incidence of AR has been steadily falling. The rate of AR used to be more than 50% in the 1970s, which has markedly dropped to 10%-20% today as per the United States, Australian, and New Zealand registries[2]. This can be attributed to the improvement of induction and maintenance of the immunosuppressive regimen. During the 1980s, the triple therapy regimen, which was the combination of low-dose cyclosporine, azathioprine, and prednisolone, was prescribed for maintenance immunosuppression[34,35]. Over the years, various combinations have been tried to find the optimal regimes. As of today, the best results overall are achieved with Tac, MMF, and steroids. A randomised trial conducted by Gonwa *et al*[36] demonstrated that this triple therapy regimen showed overall better outcomes in terms of graft and patient survival compared to other drug combinations. The study also showed that this combination provided particular benefits to kidney allograft recipients who develop delayed graft function/acute tubular necrosis. The landmark Symphony trial consolidated evidence for reduced exposure to calcineurin inhibitors in kidney transplantation, in conjunction with induction with daclizumab, MMF, and corticosteroids[37]. van der Zwan *et al*[38] recently showed the long-term outcome of the use of rATG with the combination of Tac + MMF + steroids for the treatment of AR. They concluded that early detection of AR followed by Tac + MMF + steroids with ATG provides better allograft functioning and survival. Survival after rATG was comparable to the overall survival of all kidney transplantation patients (P = 0.10).

However, there is a paucity of studies using ATG in current immunosuppression era and contemporary classification of AR. Only few studies in Tables 1 and 2 used Banff classification in the description of AR and when used, was from earlier classifications[39], at which point the role of antibody mediated component was less well understood.

The ATG dosage and duration varied widely among randomised studies as well as cohort studies described in Tables 1 and 2. The optimal dosing schedule in patients at high or low immunological risk has yet to be determined. Préville *et al* [40] derived data from a non-human primate model (cynomolgus monkey) which suggested that T-cell depletion with rATG is dose-dependent and that the optimal total dose required to achieve lymphocyte depletion in both peripheral blood and secondary lymphoid tissues (spleen and lymph nodes) is approximately 6.4 mg/kg. About 40% of patients treated with Thymoglobulin (mean of 6 doses at 1.5 mg/kg/d) have a recovery of > 50% of the initial lymphocyte count at 3 mo. Yet, time to immune reconstitution is characterized by not only a high intra-individual variability in the immune cell subpopulations (T and B cells, NK cells, DCs) but also an interindividual variability leading to prolonged lymphopenia for some patients up to 5 years[8]. When used as induction agent, a significant difference in infection rates was reported with rATG dose of < 7 mg/kg compared to use of > 7 mg/kg[41,42]. Since then, other studies have attempted to use the lower dose while optimizing the immunosuppressive effects of ATG[43,44]. However, in the context of AR treatment, guidance for use of ATG at 1.5 mg/kg remains broad at 7-14 d. It is difficult to pre-determine precise duration based on published studies. Variation in effects with intermittent dosing and continuous dosing was also reported (Tables 1 and 2). For CD3 count (T cells) < 200, 4 mg/kg bolus dose was used followed by re-dosing after 4 d, and for CD-3 count < 50[45], ATG was limited to 5 doses[46].

There is a need for further studies to unravel implications of ATG in treatment of rejections. These include: (1) Identifying patients most likely to benefit from ATG therapy. Clinical risk factors and kidney biopsy findings will need to be tested as a multivariate prediction model in determining outcomes that would enable choice of right patients; (2) It is possible that some of the intra-graft mRNA expression profiles (immune and non-immune biomarkers) could predict response to pulse glucocorticoid therapy in transplant recipients and likewise additional therapy to ATG[47]; (3) Evaluating benefit of ATG in late rejections compared to its benefit in treating early rejections; (4) Finding the optimal balance of immunosuppression in renal allograft recipients. Suboptimal immunosuppression can lead to rejection while over-immunosuppression can lead to life-threatening post-transplant infections. There remains no precise way to monitor the intensity of immunosuppression to prevent infectious complications[21]. Reports of CMV infection (Tables 1 and 2) were considerably high in published studies and prophylactic treatment with Valganciclovir for 3-6 mo is common practice lately; (5) Role of Torque-Teno Virus measurement (as a biomarker of immunosuppression to predict over/under-immunosuppression) is still in an infantile state[48]; (6) Role of ATG treatment in rejections due to non-compliance with maintenance immunosuppression medications. Currently, outcomes of treatment of such rejections is unclear; and (7) Role of anti-ATG antibodies in negating therapeutic potency of ATG needs to be established[49].

CONCLUSION

In conclusion, ATG emerges as a valuable therapeutic option for managing acute T-cell-mediated rejections, particularly in cases refractory to steroid treatment or characterized by higher grade rejections, such as Banff II or III. While the established standard dosing regimen recommends 1.5 mg/kg for a duration spanning 7 to 14 d, it is imperative to underscore the complexity of tailoring ATG therapy to individual patients, where striking the optimal balance between risks and benefits remains a formidable clinical challenge. To further advance our comprehension of this crucial treatment approach, it is imperative that we embark on comprehensive investigations. Large-scale studies, ideally based on registries, should be conducted with meticulous phenotyping of transplant recipients and thorough analysis of renal transplant biopsy characteristics. Such endeavours are indispensable in augmenting the existing body of scientific knowledge, ultimately enabling us to address the pertinent questions surrounding the precise use of ATG in the management of acute T-cell mediated rejections.

FOOTNOTES

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MINIREVIEWS

BK viral infection: A review of management and treatment

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Abstract

BK viral infection remains to be a challenging post-transplant infection, which can result in kidney dysfunction. The mainstay approach to BK infection is reduction of immunosuppression. Alterations in immunosuppressive regimen with minimization of calcineurin inhibitors, use of mechanistic target of rapamycin inhibitors, and leflunomide have been attempted with variable outcomes. Over the past few years, investigators have explored potential therapeutic options for BK infection. Fluoroquinolone prophylaxis and treatment was found to have no benefit in kidney transplant recipients. The utility of cidofovir is limited by its nephrotoxicity. Intravenous immunoglobulin is becoming a popular option for treatment and prophylaxis for BK infection, as it increases the neutralizing antibody titers against the most common BK virus serotypes. Virus-specific T cell therapy is an emerging treatment option for BK viremia. In this review, we will explore management and therapeutic options for BK infection and recent evidence available in literature.

Key Words: BK infection; Kidney transplant; Treatment; Management

Core Tip: BK viral infection is a significant post-transplant infection, which can result in kidney dysfunction if left unaddressed. The mainstay approach to BK infection is reduction of immunosuppression. Data on specific therapies have remained equivocal. In this article, we will review recent evidence available in literature on treatment approaches to BK viral infection.

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INTRODUCTION

BK virus is a DNA virus that belongs to the human polyomavirus family. It was first isolated in 1971 from the urine of a Sudanese kidney transplant recipient with initials B.K[1]. BK infection is common in the general population, approaching >90% seroprevalence by age 4[2]. It persists following primary infection and may reactivate following immunosuppression[1]. BK virus infection is a common and important post-transplant viral infection that can result in kidney dysfunction if left unaddressed. The evolution of BK infection often involves viruria, that progresses to viremia, and eventually leads to nephropathy. Severe BK virus-associated nephropathy (BKVAN) can result in loss of the kidney allograft. Effective treatment for the eradication of BK infection remains elusive. The most recent guidelines from the American Society of Transplantation Infectious Diseases Community of Practice (AST-IDCOP) recommends a stepwise approach in immunosuppression reduction as the primary intervention for BK viremia and nephropathy. The AST-IDCOP did acknowledge the lack of randomized controlled trials to provide evidence for using tacrolimus or cyclosporine, switching mycophenolate to mechanistic target of rapamycin (mTOR) inhibitor or leflunomide, and using intravenous immunoglobulin (IVIg) and cidofovir[2]. Studies that employed the use of fluoroquinolones in either prophylaxis or treatment have had varying outcomes. Finally, virus-specific T-cell therapy (VST) is a new emerging therapeutic option under current investigation. In this systematic review, we seek to present the most recent evidence surrounding management approaches and therapeutic options for BK infection following organ transplantation.

ALTERATIONS IN IMMUNOSUPPRESSIVE REGIMEN

BK virus infection poses a threat to the survival of kidney transplants, and a considerable proportion of infected patients face irreversible graft failure. The occurrence of this infection appears to be linked to the level of immunosuppression rather than any specific immunosuppressive agent. The optimal approach for treating BK infection is still uncertain, however, reducing immunosuppression is widely recognized as a primary therapy for BK infection. Although systematic studies in this area are lacking, several studies have shown that reduction in immunosuppression results in better viral clearance and preservation of graft function.

A retrospective study done in the Medical College of Wisconsin on 24 kidney transplant recipients with BK viremia (> 7000 copies/mL) showed that a 44% and 41% reduction in mycophenolate mofetil (MMF) and tacrolimus respectively, caused a significant decline in the BK DNA copies per milliliter of plasma ($P < 0.0001$) within a mean period of 5.8 mo. Only three patients (13%) developed acute cellular rejection, successfully treated with intravenous bolus steroids. After 43.5 mo, all except for one patient have a stable functioning graft[3]. In a similar study, post-transplant surveillance for BK DNA polymerase chain reaction and urinary cytology was done in 229 kidney transplant recipients. Patients found to have BK viremia and BKVAN received treatment with a 30%-50% reduction in tacrolimus and/or MMF dosages. After 5 years, overall patient survival and graft survival were 95.6% and 92.1% respectively. Following the reduction of immunosuppression, complete resolution of BK viremia was achieved in all patients and without any increase in acute rejection rates. Among the viremic patients without BKVAN, recurrent BK viremia did not occur. The seven patients diagnosed with BKVAN successfully cleared viremia within an average time of 5.9 mo, while having a stable glomerular filtration rates (GFR) in five years[4]. There have been several studies that compared reduction of immunosuppression *vs* other treatment approach in controlling BK virus infection. In 2010, Johnston *et al*[5] published a systemic review of 40 studies examining the effect of immunosuppression reduction alone or in combination with cidofovir, leflunomide, IVIg, or ciprofloxacin. Results showed a death-censored graft loss rate of 8/100 patient-years for immunosuppression reduction alone and 8 and 13/100 patient-years for the addition of cidofovir or leflunomide respectively, suggesting that there does not seem to be a graft survival benefit of adding cidofovir or leflunomide to immunosuppression reduction for the management of BKVAN. The same finding was seen in the study done by Halim *et al*[6] in 55 kidney transplant recipients where administration of three different anti-BK virus agents (leflunomide, IVIg, ciprofloxacin) added no benefit to long-term outcome in patients with BKVAN ($P = 0.32$). A recent retrospective study compared treatments for BK DNAemia in 43 kidney transplant recipients. The study evaluated immunosuppression reduction *vs* mTOR inhibitors plus IVIg. Results indicated that the immunosuppression reduction group experienced a significantly faster decrease in BK DNAemia compared to the mTORi±IVIg group ($P < 0.001$). Viral clearance was notably higher in the immunosuppression reduction group compared to the mTORi ± IVIg group ($P = 0.033$). There were no significant differences in death-censored graft loss, rejection rates, or graft function at 12 mo. This study further supports that standard BK virus (BKV) DNAemia treatment of reduction in immunosuppression as having superior outcomes compared to any other treatment approach[7].

LEFLUNOMIDE

Leflunomide, an immunosuppressive medication, has been explored as a potential treatment for BKVAN in kidney transplant recipients. The therapeutic benefit of using leflunomide in this context lies in its antiviral activity against various viruses such as herpes simplex (HSV-1) and cytomegalovirus (CMV). In vitro studies have shown that the active metabolite of leflunomide (A77 1726) has some anti-viral properties by a dose-dependent reduction in BK large T antigen expression. This reduction in antigen expression, however, did not translate to a reduction in BK virus DNA replication [8]. This finding was echoed by a retrospective single-center study done by Krisl *et al*[9] where 52 patients with BK viremia (with or without nephropathy) did not show any significant BK viral clearance after treatment with leflunomide compared to the control group. The rate of BK clearance was 30.8% in the leflunomide group *vs* 60.9% in the group that did not receive leflunomide (P = 0.02). Furthermore, graft failure occurred in 15% of patients in the leflunomide group and 7% in the no leflunomide group (P = 0.32).There are some studies that showed partial improvement in BK virus clearance and renal function. A prospective open-label study where 12 kidney transplant recipients diagnosed with BKVAN had MMF changed to leflunomide. Results showed that renal function improved in 50% of patients, remained stable in 16.6%, and deteriorated in 33.4%, with graft loss in 17% of cases. Clearance of BK viremia was observed in 42% of cases[10]. A similar study was done in 12 kidney transplant recipients whose MMF was changed to leflunomide upon diagnosis of BKVAN. Results showed that T-cell proliferation tend to be higher with leflunomide treatment compared to MMF therapy (8.4 ± 7.7% *vs* 12.4 ± 10%, P = 0.2). However, the difference was not statistically significant. BK viral clearance was observed in 41.6% of cases treated with leflunomide within 6 mo. Stable creatinine clearance was also noted in 50% of these patients within 6 mo of treatment. Of note, however, one patient in this study developed end-stage kidney disease because of concurrent acute antibody-mediated rejection and BKVAN[11].

Although these studies have shown dismal results, several case reports and studies have shown encouraging findings with the use of leflunomide in treating BK infection in kidney transplant recipients. One such study was done in 13 patients with biopsy-proven BKVAN treated with leflunomide in combination with a low-dose calcineurin inhibitors and prednisone after cessation of MMF therapy. Findings showed that 12 patients (93%) had undetectable viral load after mean treatment of 109 d. There was noted graft improvement in 13% of cases. However, overall graft function at follow-up was not significantly better than at diagnosis (P = 0.69). Leflunomide was well-tolerated and no serious adverse effects or episodes of graft rejection were reported[12]. Another study involving 26 patients with biopsy proven BKVAN investigated treated with either leflunomide alone or leflunomide plus a course of cidofovir and followed them for six to 40 mo. Results showed that 84% of cases had viral clearance in six months (P < 0.001). Follow-up after 12 mo or more showed creatinine levels not significantly changed compared to baseline in 16 patients. After follow-up of 40 mo, graft loss was at 15%[13].

The utilization of leflunomide in kidney transplant recipients with BK virus infection remains a topic of ongoing debate. A high-powered and robust randomized trial could prove essential in definitively establishing the relationship between this treatment and critical clinical outcomes such as effective viral clearance and the enduring maintenance of long-term graft function.

FLUOROQUINOLONES

Fluoroquinolones are often utilized in kidney transplant recipients due to their broad spectrum of activity. They have been demonstrated to inhibit BK replication in its natural host cells by blocking large T antigen helicase activity in polyomavirus, and possibly by inhibition of host cell proteins like topoisomerase II[14]. This perceived efficacy against the said virus was the impetus for several retrospective studies to investigate its role as prophylaxis for BK virus among kidney transplant recipients. One such study was performed by Gabardi *et al*[15] wherein they compared two groups of kidney transplant recipients with documented BK virus infection, one that used a fluoroquinolone (ciprofloxacin or levofloxacin adjusted according to renal function) for 30 d and another group that did not. In this study, sulfamethoxazole/trimethoprim was the primary antibiotic used for pneumocystis prophylaxis, whereas fluoroquinolone in combination with atovaquone use was used for those with sulfa allergy or G6PD deficiency. The results showed that there was lower BK viremia rate at one year post-transplant among those who received a fluoroquinolone compared to those who did not (4% *vs* 22.5%, respectively; P = 0.03). Another study retrospectively analyzed two groups of kidney transplant recipients, one with no BK virus prophylaxis (group I, n = 106), and another that used ciprofloxacin for 30 d to cover for BK virus prophylaxis (group 2, n = 130). The investigators evaluated the levels of BK viruria and viremia between the two groups over a period of 12 mo. On the third month after transplantation, there was a higher risk of developing BK viruria and viremia in group 1 *vs* group 2 (viremia: 0.161 *vs* 0.065, P = 0.0378; viruria: 0.303 *vs* 0.146, P = 0.0067). In the subsequent six, nine, and 12 mo though, there was no difference in the mean blood and urine BK viral load between the two groups, even after adjusting for corticosteroid regimen. This raised the possible benefit of increasing the duration of prophylactic treatment[16]. These studies were among those that inspired the randomized controlled trials that ensued.

Lee *et al*[17] conducted the first prospective, multicenter, double-blind, placebo-controlled trial that investigated the efficacy of levofloxacin in the treatment of BK viremia among adult kidney transplant recipients. A total of 43 patients were randomized to either receive levofloxacin 500 mg daily (with renal dose adjustment), or placebo for 30 d, with appropriate adjustment of immunosuppression according to the standard of practice at each institution. After three months of treatment, there was no significant difference in the percentage of BK viral load reduction between the levofloxacin-treated group and placebo (70.3% *vs* 69.1%, respectively, P = 0.93). Results were similar at one month (58% *vs*

67.1%; *P* = 0.47) and six months (82.1% *vs* 90.5%; *P* = 0.38). Hence, the use of levofloxacin did not improve BK viral load reduction, BK viral load clearance, or allograft function. Furthermore, those who used levofloxacin had a higher rate of Achilles tendonitis. Knoll *et al*[18] carried out a randomized clinical trial among 154 adult kidney transplant recipients looking into the efficacy of a three-month course of levofloxacin for the prevention of BK viruria within the first year of transplant. Apparently, levofloxacin administration showed no advantage as the rate of BK viruria was not significantly different between the two groups [29% in the levofloxacin group *vs* 33.3% in the placebo group; hazard ratio 0.91; 95% confidenceinterval (CI): 0.51-1.63; *P* = 0.58]. In addition, there was an increased risk of resistant infection among isolates usually sensitive to quinolones in the levofloxacin group *vs* placebo (58.3% *vs* 33.3%, respectively; risk ratio 1.75; 95%CI: 1.01-2.98), and increased risk of suspected tendinitis (7.9% *vs* 1.3%; risk ratio, 6.16; 95%CI: 0.76-49.95), albeit not statistically significant. Another point against the use of fluoroquinolone for the prevention of BK virus infection was noted in a trial that compared BK viremia between a group that received a three-month course of ciprofloxacin *vs* placebo. At six months post-transplant, more patients in the ciprofloxacin group had BK viremia compared to the placebo group (18.8% *vs* 7.5%, respectively, *P* = 0.03). Moreover, prolonged fluoroquinolone use resulted in a significantly higher rate of fluoroquinolone-resistant gram-negative urinary tract and bloodstream infections in the ciprofloxacin arm[19]. A meta-analysis that included two randomized controlled trials and six retrospective cohort studies reinforced that fluoroquinolones are not effective for prevention of BK viremia in kidney transplant recipients, and do not reduce the incidence of BKVAN or graft loss[20]. The latter studies constitute the evidence that fluoroquinolones have no role for the prevention of post-transplantation BK polyomavirus infection.

CIDOFOVIR

Cidofovir is a nucleotide analog of cytosine that is approved for the treatment of CMV in human immunodeficiency virus-positive patients, and has demonstrated *in vitro* activity against murine and simian polyomavirus strains[21,22], as well as a related human polyomavirus (JC virus) *in vivo*[23]. It decreases viral DNA synthesis upon incorporation with the nascent chain. Nephrotoxicity is its major adverse effect because it is taken up rapidly by proximal tubular cells by organic anion transporters at their basolateral membrane but secreted slowly into the lumen, resulting in high intracellular drug concentrations that can cause tubular necrosis. Hydration and co-administration with probenecid, a competitor of cidofovir for the transporter, can have a nephroprotective effect[24]. It is this adverse effect that precludes its recommendation for treatment of BK, such that its use should be weighed against the possible risk of worsening renal function.

In a cohort of 21 kidney transplant recipients with biopsy-proven BKV interstitial nephritis (BKVIN), Kuypers *et al*[25] investigated the effect of adjuvant low-dose cidofovir treatment *vs* no cidofovir, after lowering immunosuppressive drug therapy, on graft function, viral load, and graft outcome. Eight patients received cidofovir at 0.5-1.0 mg/kg at four to ten weekly courses. In the cidofovir-treated group, there was an improvement in creatinine clearance from 29.3 mL/min to 32.0 mL/min (range: 24-46) after a median follow up period of 24.8 mo (range 8-41) upon completion of treatment. Graft function did not acutely deteriorate during treatment except for one patient, but ultimately no graft loss occurred in this group. Blood viral load decreased in all patients treated with cidofovir. Once the BK viremia resolved, graft function improved but did not attain baseline levels. Adverse reactions noted include nausea in three patients, and development of pruritic maculopapular rashes in one patient. In contrast, nine of the 13 patients who did not receive cidofovir lost their graft after a median of eight (4-40) months. They also noted in this study that peak cidofovir concentrations were dose-dependent, and that probenecid treatment appeared to be unnecessary as it did not influence peak serum concentrations. This study was designed to be a preliminary report suggestive of the favorable effect of cidofovir on renal graft survival, function, and preservation, warranting a randomized controlled prospective study to follow suit. Another study by Kuypers done four years later investigated 41 kidney transplant patients with BKVIN, of whom 26 received cidofovir at 1 mg/kg to a maximum of ten weeks, without probenecid, and 15 did not receive cidofovir. Both groups had immunosuppression reduction. Similar to the previously mentioned study, there was a significantly higher occurrence of graft loss in the group that did not receive cidofovir (73.3% *vs* 15.4%, *P* = 0.0002). No renal toxicity was noted in the cidofovir group. The observed adverse effects include anterior uveitis in three patients, and skin rash during infusion with cidofovir[26].

A retrospective review of kidney and kidney-pancreas transplant recipients who received cidofovir combined with reduced immunosuppression for BKVAN or high-level viremia showed that adjunct cidofovir administration resulted in preserved renal function and no graft loss when viral clearance happened within six months of treatment. On the other hand, long term cases of BK infection (more than six months) were associated with a 15% decline in estimated glomerular filtration rate. Factors associated with long term BK infection include older age, delayed graft function, and higher peak BK viral load, suggesting that this subset of patients will not benefit as much from adjunctive cidofovir[27]. The course of cidofovir treatment among BK- infected individuals following bone marrow transplant manifesting as hemorrhagic cystitis have also been useful as the findings suggest applicability to kidney transplant recipients. In an open-label, non-randomized, single-dose pilot study done among hematopoietic stem cell transplant (HSCT) pediatric patients with symptomatic infection of adenovirus, nucleoside-resistant CMV, human polyomavirus (BK or JC virus), and/or nucleoside-resistant HSV, cidofovir was used to investigate virologic response, as well as safety and pharmacokinetics, with a focus on nephrotoxicity. Of the 12 patients in the study, four had BK viruria, and all four had unsuccessful viral clearance. One out of the four developed nephrotoxicity[28]. In a systematic review that compared intravesical *vs* intravenous route of cidofovir administration among stem cell transplant patients with BK polyomavirus hemorrhagic cystitis, there were more patients in the intravesical cidofovir group *vs* the intravenous cidofovir group who achieved a complete treatment response (88.2% *vs* 68%). Furthermore, no nephrotoxicity was observed in those that received the intravesical route,

whereas 9.3% had renal failure in those that received the drug intravenously. This better toxicity profile warrants more investigation due to its potential benefit[29]. All of the above mentioned studies are either preliminary or pilot studies done on a small population, or descriptive, retrospective ones. One randomized, double-blind, placebo-controlled, dose escalation study of cidofovir in kidney transplant patients with BKVAN was initiated in 2006 by the National Institute of Allergy and Infectious Diseases but closed early in 2013 due to failure to enroll in a timely manner.

Brincidofovir, a prodrug of cidofovir, which is less nephrotoxic due to its decreased accumulation in proximal tubules, is approved for the treatment of smallpox in pediatric and adult patients. Its use in BKVAN was described in a HSCT patient who had no reduction in immunosuppression. No drug-related adverse reactions occurred. Stable kidney function was maintained without the need for dialysis[30]. Another case was described in a pediatric kidney transplant recipient with BKVAN who was treated with brincidofovir after treatment failure with decreased immunosuppression, ciprofloxacin, and leflunomide. The treatment resulted in decrease in BK viral load, decrease in serum creatinine to baseline levels, and stabilization of renal function thereafter[31]. A phase 2, open-label, randomized, controlled, multiple ascending dose study on the safety and tolerability of IV brincidofovir in adult kidney transplant recipients with BK infection is currently underway in multiple study sites in Australia and Japan.To date, the role of cidofovir in the treatment of BK infection in kidney transplant recipients remains to be adjunctive at best, until a well-designed and high-grade study can better define its potential benefit.

IVIG

The effectiveness of IVIg against BK infection is still uncertain. IVIg is currently considered an additional treatment choice for patients with refractory BK infection despite aggressive adjustment in immunosuppressive medications. The proof of the effectiveness of IVIg is limited to case series, retrospective studies, and prospective cohort studies.

IVIg is believed to quell BKV-associated kidney disease by acting on various parts of the immune system, including dendritic cells, macrophages, and granulocytes. It is thought to demonstrate its effect by interacting with certain receptors like Fc gamma receptors[32]. Commercially available IVIg preparations contain strong antibodies that can counteract different strains of the BK[33].

In 2006, Sener *et al*[34] suggested that IVIg could be used as a treatment for BKVAN. A case report from 2009 demonstrated that IVIg helped restore kidney function, reduced BK levels, and improved histopathological findings in a pediatric kidney transplant recipient who did not respond adequately to immunosuppression reduction and cidofovir [35].

A study showed that 0.4 g/kg/d (n = 16) or 1 g/kg/d (n = 17) of IVIg administration resulted in increased BKV-neutralizing antibodies (NAbs), which persisted for 22 ± 7 days[36]. In one retrospective study involving 30 patients with BKVAN, 1 g/kg of IVIg was administered to patients who did not respond to eight weeks of the immunosuppression adjustment and leflunomide, with mean BKV loads of 205314 copies/mL. After one year of follow-up, 27 patients (90%) showed a positive response in clearing viremia, with decrease of BK viral loads to 697 copies/mL. It also showed a good graft survival in 12 mo[37].

Another retrospective, single-center cohort study involving 50 patients with BKVAN showed that 1g/kg of IVIg in addition to immunosuppression adjustment led to better clearance of viremia. It showed fewer graft losses with IVIg group (27.3% *vs* 53.6% for control, P = 0.06), although graft and patient survivals were not statistically different[38]. In contrast, a retrospective analysis by Naef *et al*[39] yielded conflicting outcomes. This study involved 860 kidney transplant recipients with BK viremia. A total of 52 out of 131 patients with high-level BK viremia received IVIg. At one year follow-up, the IVIg group exhibited lower estimated GFR compared to patients who did not receive IVIg (44 mL/min *vs* 52 mL/min) and failed to show advantages in shortening the duration of BK viremia or reducing rejections. On the other hand, IVIg might play a role in preventing BKVAN. In one study, 174 kidney transplant recipients were divided into the following three groups retrospectively based on their risk of BKV infection: patients with low NAbs (high-risk) with IVIg, high-risk patients without IVIg, and patients with high NAbs (low-risk) without IVIg. The IVIg group received 0.4 g/kg of IVIg every three weeks for one to three doses, for the first three months following transplant. At 12 mo post-transplant, the incidence of BK viremia in high-risk patients who received IVIg was significantly lower than untreated high-risk group (6.8 % *vs* 36.6%, P < 0.001), and similar to the low-risk group (10.1%)[40].

The AST-IDCOP states that these studies are difficult to evaluate given other concurrent antiviral intervention, widely variable empirical dosing, and initiation of treatment late in the course of the disease[2]. An ongoing randomized controlled trial (NCT 02659891), aims to shed more light on the potential benefits of IVIg in treating BKVAN.

MONOCLONAL ANTIBODIES

Efficacy and safety of first-in-class human IgG1 monoclonal high-affinity neutralizing antibody against BKVAN is currently under investigation (NCT 04294472). This phase 2, randomized, double-blind, placebo-controlled clinical trial evaluated the safety and efficacy of monoclonal antibody (MAU868) in kidney transplant recipients who had BK viremia within one year of enrolment. It involved 28 patients of whom 20 received MAU868 and eight received placebo. Results showed that the MAU868 group had more effective viral load clearance than the placebo group at week 16 through week 36. All patients tolerated MAU868 well. Further investigation regarding its safety and efficacy is warranted.

VST

VST is an emerging therapeutic option for BK infection. Pioneering work towards the development of T-cell therapy started in the early 1990s, mostly geared towards reconstitution of cellular immunity against CMV and isolation of antigen-specific T cells[41]. Over the recent few years, several trials have been conducted to test the clinical utility of VST for BK infection. In a study that included 16 HSCT recipients who developed BK infection, all achieved clinical benefit following VST. Viral load reduction of 85.5% and 96% were noted at week 6 and 12 post-infusion, respectively. Thirteen out of 14 patients who had hemorrhagic cystitis had resolution of hematuria. One of two patients with BKVAN had improvement in renal function[42]. In another study involving 59 HSCT patients with BK hemorrhagic cystitis who received BK-specific cytotoxic T-cell therapy, 67.7% mounted a response and had significant clinical improvement at day 14. Response rate increased to 81.6% at day 45 and was noted to be durable thereafter. Significant decrease in urine BK viral load was also noted among responders[43]. A phase II trial on Posoleucel, a multivirus-specific T-cell therapy derived from healthy, seropositive, third-party donors, was conducted among 59 HSCT recipients who developed CMV, epstein-barr virus (EBV), HHV-6, adenovirus, JC, and BK infection. Of the 27 patients who developed BK infection, all had partial response after 6 wk of treatment with Posoleucel. Of the 23 patients who had BK hemorrhagic cystitis, 74% had resolution of symptoms and macroscopic hematuria. Nine of 24 patients also had documented increase in IFN-γ ELISpot levels[44].

Multivirus-specific T-cell (MVST) lines that target CMV, EBV, Adenovirus, and BK were generated by Roubalová *et al*[45] and they found predominance of CD8+ phenotype in CMV-specific T cells and CD4+ phenotype in BK-specific T cells. The authors suggested modification of the protocol to prevent antigenic competition for MVST to be efficacious treatment of BK infection. Koukoulias *et al*[46] developed a glucocorticoid-resistant, multi-pathogen specific T cell product named Cerberus that targets Adenovirus, CMV, EBV, BK, and Aspergillus. This allows capture of common opportunistic infections among transplant recipients regardless of the intensity of immunosuppression.

In general, most trials conducted on VST claim potential widespread utility of this therapy against multiple post-transplant viral infections while avoiding the nephrotoxic and myelosuppressive effects of certain antivirals. VST is more widely utilized in HSCT recipients. Conceptually, since T-cell reconstitution is central to the management of viral infections, it seems intuitive that VST should have application in the management of BK infection in other solid organ transplant (SOT) recipients. Adenoviral vector-based multivirus-specific T-cell immunotherapy that targets CMV, EBV, Adenovirus, and BK has been developed and demonstrated rapid *in vitro* expansion of multivirus-specific T cells from SOT recipients and *in vivo* priming of antiviral T-cell immunity[47]. Autologous BK-specific T cell lines have been generated from viremic kidney transplant recipients[48]. BK-specific CD8+ T-cells have also been generated *in vitro* from peripheral mononuclear cells derived from healthy donors and pulsed with synthetic peptide pools[49]. These proofs of concept of T-cell therapy paved the way for a promising novel therapy for the prevention of BK infection before kidney and other solid organ transplantation and the treatment of BKVAN after transplantation[48,49]. Jahan *et al*[50] reported a case of a 54-year-old female kidney transplant recipient who developed BKVAN, necessitating reduction in mycophenolate and tacrolimus, administration of IVIg, leflunomide, cidofovir, and ciprofloxacin, but had worsening BKVAN and graft dysfunction. The patient eventually received BK-specific T-cell therapy derived from the patient's daughter and infused over ten sessions. Despite note of significant reduction in BK viral load, the kidney allograft eventually failed due to interstitial fibrosis and tubular atrophy. The authors proposed that early T-cell therapy might be more effective in treating BKVAN. Administration of VST in three SOT recipients, including kidney, heart, and heart-kidney transplants, elicited complete response in one and partial response in two patients[51]. Of the case reports that described the use of VST in kidney transplant recipients who developed BK infection, there were no reports of acute rejection, graft-versus-host disease (GVHD), or death with use of VST[52].

It is worth noting that rare but serious adverse effects of VST, including cytokine release syndrome, diffuse alveolar damage, hepatic sinusoidal obstruction syndrome, multi-organ failure[53], and GVHD[52] have been reported in literature. Other potential logistical limitations of VST include the need for donor immunity to the viral target, as well significant cost, labor, time, and regulatory burden for manufacturing the therapy[52,54]. Some investigators opted to utilize HLA-matched or partially matched T-cell donors, although this did not seem to affect the clinical outcome[43]. Other concerns involve antigenic competition between high and low frequency T-cells and multiple antigens[55] and the efficacy of VST in the setting of lifelong and more intense immunosuppression among SOT recipients[52].

BK VACCINE

An emerging preventative measure for BK infection is the administration of virus-like particle vaccines to induce high levels of neutralizing antibodies against BK even prior to transplantation. Peretti *et al*[56] immunized macaques and mice and were able to demonstrate broad neutralizing response to heterologous BK and JC virus genotypes following the priming dose in macaques and the booster dose in mice. The authors proposed the potential clinical value of BK vaccination among patients awaiting organ transplant to prevent kidney dysfunction and failure from BKVAN or potential transplant rejection following immunosuppression reduction.

DISCUSSION

BK viral infection poses a significant threat to SOT and HSCT recipients and may eventually lead to renal dysfunction and even loss of the renal allograft. Immunosuppression reduction is the mainstay approach to the management BK viral infection. This treatment, however, has a risk of acute rejection that may necessitate use of other anti-rejection therapy that can worsen the current BK virus infection. A cautious and stepwise approach in immunosuppression reduction coupled with close monitoring of renal function, have been found to be an effective approach to find the right balance between treating the BK virus and preserving graft function.

Changes in immunosuppressive regimen do not seem to have significantly different outcomes. Outcomes data on the use of leflunomide, fluoroquinolones, cidofovir, and brincidofovir remain equivocal. Leflunomide and fluoroquinolones are readily available and relatively well-tolerated. However, leflunomide has a potential risk of leukopenia, peripheral neuropathy, gastrointestinal effects, and liver dysfunction or damage[57]. Fluoroquinolones pose a risk of gastrointestinal effects, tendinitis, tendinopathy, tendon rupture, aortic aneurysm and dissection, neuropathy, arrhythmia, and labile blood sugars[58] and potentially higher rates of fluoroquinolone-resistant infections. Cidofovir may be nephrotoxic and myelosuppressive while brincidofovir may cause gastrointestinal effects, predominantly diarrhea[59]. IVIg and monoclonal antibodies are relatively well-tolerated but might carry the risk of headaches, flu-like symptoms, and rarely renal dysfunction, thrombosis, and hemolytic anemia[60]. Viral-specific T-cell therapy and vaccines are some of the emerging management approaches to BK viral infection. Viral-specific T-cell therapy may incur significant time, labor, and cost, while posing rare but potential risks of multi-organ failure and GVHD[52,53]. Certainly, the use of the above agents in addressing BK viral infection should be weighed against their potential adverse effects (Table 1).

Future perspectives

There are definite unmet needs in therapeutic options for BK viral infection. High quality ideally randomized controlled trials, on currently existing medications, as well agents in development, should be conducted. The value of viral-specific T-cell therapy and vaccines should be further investigated.

CONCLUSION

BK viral infection is an important post-transplant infection that can eventually lead to renal dysfunction. Mainstay for management is reduction in immunosuppression. However, this poses a risk for acute rejection. Over the years, alterations in immunosuppressive regimen, use of mTOR inhibitors and leflunomide, fluoroquinolones, cidofovir, and IVIg have been attempted and investigated, and resulted in variable outcomes. BK-specific T-cell therapy and vaccines are emerging options for the management and prevention of BK infection. Nevertheless, effective and durable treatment for BK infection remains elusive. In addition, there is paucity of randomized, controlled trials to provide high-level evidence to support certain management strategies. Indeed, there is a need to pursue studies that will provide evidence to support best management approaches for BK infection post-transplant. These studies might define the future landscape for BK management, while minimizing adverse effects of treatment and optimizing graft and patient survival.

Table 1 Summary table of studies on management of BK infection

Ref.	Study type/period	Subjects	Key findings (include *P* value if available)
Alterations in immunosuppression			
Vela *et al*[7], 2022	Retrospective study; Mar 2013-Oct 2020	43 kidney transplant recipients with BK DNAemia; 26 received mTORi + IVIg; 17 had immunosuppression reduction	BK DNAemia and viral clearance reduced faster and more significantly in subjects with reduced immunosuppression (P < 0.001 and P = 0.033 respectively). Death-censored graft loss, rejection rates, and kidney graft function at 12 mo did not differ significantly
Halim *et al*[6], 2016	Cohort study	55 kidney transplant recipients with BK viremia and/or BKVAN nephropathy; 22 received leflunomide + IVIg + ciprofloxaci; 33 had immunosuppression reduction alone	Administration of leflunomide, IVIg, and ciprofloxacin added no benefit to the long-term outcome of patients with established BKVAN. Treatment of BKVAN by reduction of immunosuppression alone appears to be more effective
Huang *et al*[4], 2015	Prospective study; Mar 2006-Oct 2008	229 kidney transplant recipients with BK viremia and BKVAN 30%-50% reduction in FK and/or MPA	BK viremia resolved in 100% of patients without increased acute rejection. All patients with BKVAN had viral clearance and showed no decline in GFR
Saad *et al*[3], 2008	Retrospective, single center study; Sept 2001-Dec 2003	24 kidney transplant recipients: 16 with BKVAN; 8 with BK viremia	Reduction in immunosuppression alone results in clearance of the BK viremia with good long-term outcome
Leflunomide			
Krisl *et al*[9], 2012	Retrospective, single center study; Jun 2001-Dec 2009	76 kidney transplant recipients with BK viremia with or without BKVAN; 52 received leflunomide; 24 did not receive	No difference in BK viral clearance. Multivariate analysis demonstrated that mycophenolate mofetil discontinuation, BK viremia without nephropathy, and mean BK viral load were

		leflunomide	significantly associated with BK viral clearance. Leflunomide use lacked this association
Canivet *et al* [11], 2009	Prospective study; Jan 2006-May 2008	12 kidney transplant recipients with BKVAN; MMF switched to leflunomide	Not statistically significant T cell markers, BK DNAemia clearance in 41.6%, creatinine clearance stable or improved in 50%, no significant adverse events
Teschner *et al* [12], 2009	Prospective study	13 kidney transplant recipients with BKVAN; MMF switched to leflunomide	12 had viral clearance at a mean of 109 d. Graft function stabilized or improved (mean [median] creatinine concentration at diagnosis, 2.39 [2.5] mg/mL, *vs* 2.27 [2.0] mg/dL at follow-up). 1 graft loss due to refractory rejection. Leflunomide concentration did not correlate with treatment efficiency
Faguer *et al* [10], 2007	Prospective study; Jul 2002-Apr 2006	12 kidney transplant recipients with BKVAN; MMF switched to leflunomide	42% had BK clearance. 66.6% had stable or improved renal allograft function
Josephson *et al* [13], 2006	Prospective study; Apr 2001-Apr 2004	26 kidney transplant recipients with BKVAN; 17 received leflunomide alone; 9 received leflunomide + cidofovir	84% of cases blood and urine viral load levels uniformly decreased over time ($P < 0.001$). Mean serum creatinine levels stabilized over the first 6 months of treatment, and with 12 mo or more of follow-up. 16 patients had fairly unchanged serum creatinine
Fluoroquinolones			
Patel *et al*[19], 2019	Prospective, randomized, placebo-controlled trial; Jan 2013 -Oct 2016	200 adult solitary kidney transplant recipients; 133 received ciprofloxacin as BK prophylaxis; 67 did not receive ciprofloxacin	BK viremia at 6 mo post-transplant occurred in 25 (18.8%) patients in the ciprofloxacin group and 5 (7.5%) in the placebo group ($P = 0.03$). Increased risk of fluoroquinolone-resistant infections in those who received ciprofloxacin
Knoll *et al*[18], 2014	Prospective, double-blind, placebo-controlled randomized trial; Dec 2011 -Jun 2013	154 adult kidney transplant recipients; 76 received a 3-mo course of levofloxacin; 78 received placebo	BK viruria occurred in 22 (29%) in the levofloxacin group *vs* 26 (33.3%) in the placebo group (HR 0.91, 95%CI: $P = 0.58$). Increased risk of resistant infection among isolates usually sensitive to quinolones in the levofloxacin group *vs* placebo (58.3% *vs* 33.3%, respectively); (RR 1.75; 95%CI: 1.01-2.98) as well as a nonsignificant increased risk of suspected tendinitis (7.9% *vs* 1.3%; RR 6.16; 95%CI: 0.76-49.95)
Lee *et al*[17], 2014	Prospective, multicenter, double-blinded, placebo-controlled trial; Jul 2009 -Mar 2012	43 adult kidney transplant recipients with documented BK viremia; 22 received levofloxacin for 30 d; 21 received placebo	At the 3-mo follow up, there was no significant difference in BK viral load reduction between the levofloxacin and placebo group (70.3% *vs* 69.1%, respectively, $P = 0.93$). The percentage reductions in BK viral load were also equivalent at 1 mo (58% *vs* 67.1%, $P = 0.47$), and 6 months (82.1% *vs* 90.5%, $P = 0.38$)
Wojciechowski *et al*[16], 2012	Retrospective study; First cohort (group 1): Jul-Dec 2009 Second cohort (group 2): Jan-Jun 2010	236 adult renal transplant recipients; Group 1: 106 did not receive BK virus prophylaxis; Group 2: 130 received ciprofloxacin as BK virus prophylaxis	At 3 mo post-transplant, the group that did not receive ciprofloxacin (group 1) had a higher risk of developing BK viremia than the group that received ciprofloxacin (group 2) (0.161 *vs* 0.065, $P = 0.0378$) and viruria (0.303 *vs* 0.146, $P = 0.0067$), but this difference progressively narrowed until there was no significant difference anymore at 12 mo for both viremia (0.297 *vs* 0.261, $P = 0.6061$) and viruria (0.437 *vs* 0.389, $P = 0.5363$)
Gabardi *et al* [15], 2010	Retrospective analysis; Jan 2004-Dec 2008	185 adult kidney transplant recipients; 25 received a 30-d course of ciprofloxacin; 160 did not receive a fluoroquinolone	Higher rate of BK viremia in those who did not receive a 1-mo course of levofloxacin 36 (22.5%) *vs* 1 (4%) who received levofloxacin; $P = 0.03$
Cidofovir			
Schneidewind *et al*[29], 2018	Systematic review	189 adult patients with BK virus associated hemorrhagic cystitis after allogenic stem cell transplant; 172 received intravenous cidofovir; 17 patients received intravesical cidofovir (2 patients received both routes of administration)	Complete response: 68% in intravenous cidofovir group, 88.2% in intravesical cidofovir. Kidney toxicity: 9.3% in intravenous cidofovir group, none in intravesical cidofovir group
Papanicolaou *et al*[30], 2015	Case report	58 yr old male post hematopoietic stem cell transplant; developed biopsy-proven polyomavirus associated nephropathy; received brincidofovir 100 mg twice weekly for 6 mo; no immunosuppression reduction	4-log decrease in BK virus viremia. No drug-related adverse events. Stable kidney function, and did not require dialysis
Caruso Brown *et al*[28], 2015	Open-label, non-randomized, single-dose, pilot study	12 pediatric patients (ages 6-18) with a hematopoietic stem cell transplant within 2 yr, with symptomatic infection of adenovirus, nucleoside-resistant CMV, human polyomavirus (BK or JC virus), and/or nucleoside-resistant HSV diagnosed by viral culture or PCR; all patients received cidofovir	2/12 acute kidney injury after the first dose 2/12 developed nephrotoxicity. Mean drug half-life 9.5 h (longer than documented half-life for adults based on other studies). No correlation between nephrotoxicity and plasma maximum concentration, clearance, or half-life. Cidofovir was well- tolerated in majority of patients
Kuten *et al*[27], 2014	Single-center, retrospective review; Jan 2007 to	75 kidney and kidney-pancreas transplant recipients who received cidofovir combined with reduced immunosuppression	32 (43%) had short-term BK (≤ 6 mo); 43 (57%) had long-term BK. 53 (71%) eventually cleared BK at a median of 4.2 mo (interquartile range 2.1-9.3 mo). Factors associated with long-term BK: older age

	Jun 2012		(OR 1.1, P = 0.02), Delayed graft function (OR 31.4, P = 0.01); higher peak BK (OR 12.8, P = 0.02. This group was associated with a 15% decline in estimated glomerular filtration rate. Factor associated with short-term BK: BK reduction by at least 1 $\log_{10}$copies/mL at 1 mo of treatment (OR 49.3, P < 0.01). This group maintained stable graft function and no graft loss was noted
Reisman, *et al* [31], 2014	Case report	pediatric patient who received kidney transplant for bilateral dysplastic kidneys, developed BKVAN; did not respond to decreased immunosuppression, ciprofloxacin, leflunomide; given brincidofovir	BK viral load decreased, but still detectable. Urine viral load declined but still elevated. Creatinine declined to baseline level and was stable for 2 yr. No drug-related adverse events
Kuypers *et al* [26], 2009	Single-center study	41 adult renal transplant recipients with biopsy-proven BKVIN; 26 received cidofovir at 1 mg/kg for a maximum duration of 10 wk and without probenecid; 15 did not receive cidofovir; All patients had immunosuppression reduction	Graft loss: 4/26 (15.4%) in cidofovir group, 11/15 (73.3%) in no cidofovir group (P = 0.0002). Percentage of patients who completely cleared the virus from the blood was not different between the 2 groups. 3 patients in the cidofovir group developed severe anterior uveitis at 6, 7 and 8 doses, respectively (later switched to leflunomide). No BM or renal toxicity was observed in the cidofovir group. One patient developed a skin rash during infusion of cidofovir
IVIg			
Naef *et al*[39], 2021	Retrospective analysis; Jan 2009-Mar 2019	Kidney transplant recipients with high level BK viremia; 79 transplanted before 2014 and had immunosuppression reduction alone; 52 transplanted after 2014 and had immunosuppression reduction + IVIg	IVIg group showed lower eGFR (44 mL/min *vs* 52 mL/min). IVIg did not shorten duration of BK viremia
Kable *et al*[38], 2017	Retrospective, single-center cohort study	50 BKVAN patients received IVIg 1 g/kg	Better clearance of BK viremia and fewer graft loss (not statistically significant)
Vu *et al*[37], 2015	Retrospective analysis; 2008-2012	30 kidney transplant recipients with BKVAN received IVIg 2 g/kg	90% of patients showed positive response in clearing viremia. Graft survival rate was 96.7% at 12 mo follow-up
Sener *et al*[34], 2006	Case series; Jul 2000-Jul 2003	8 kidney transplant recipients with IVIg 2 g/kg	88% of patients showed functioning graft at 15 mo follow-up
Monoclonal antibodies			
In the study	Ongoing RCT (NCT 04294472)	30 kidney transplant recipients with BK viremia; 22 received MAU868; 8 received placebo	Better BK viral clearance in MAU group
Virus-specific T-cell therapy			
Pfeiffer *et al* [44], 2023	Open-label, phase II trial; Apr 2014-Jul 2021	27 pediatric and adult HSCT recipients with BK infection; 25 with hemorrhagic cystitis; 2 with nephritis	100% had partial response at 6 weeks of treatment. 74% of patients who developed hemorrhagic cystitis had symptom resolution. 9/24 (37.5%) had increase in IFN-γ ELISpot counts
Koldehoff *et al* [1], 2023	Sequential analysis	17 HSCT recipients with BK hemorrhagic cystitis; 7 received VST; 10 did not receive VST (immunosuppression reduction or cidofovir)	6/7 from the VST group *vs* 6/10 from the non-VST group had T-specific cellular response, in most cases parallel to decrease in BK viral load
Olson *et al*[43], 2021	Single-arm, phase II clinical trial; Oct 2015-Sept 2019	59 HSCT recipients with BK hemorrhagic cystitis; received single IV infusion of partially HLA-matched BKV-CTL	Response rate and clinical improvement following the off-the-shelf BK-specific cytotoxic T-cells: 67.7% at day 14; 81.6% at day 45
Nelson *et al* [51], 2020	Phase II study; Jun 2017-Dec 2019	38 HSCT recipients; 3 solid organ transplant recipients: 1 kidney transplant recipient; 1 heart transplant recipient; 1 heart-kidney transplant recipient	Response rates: 86% in patients with BK viremia, 100% in patients with hemorrhagic cystitis, 87% in patients with BK viremia and hemorrhagic cystitis. Of the 3 solid organ transplant recipients, 1 had complete response and 2 had partial response
Tzannou *et al* [42], 2017	Phase II study	16 HSCT recipients; 14 with BK hemorrhagic cystitis; 2 with BKVAN	Decrease in urine BK viral load following VST: 85.5% at week 6, 96% at week 12. 13/14 with hemorrhagic cystitis had resolution of hematuria. 1/2 with BKVAN had improved renal function
Jahan *et al*[50], 2020	Case report; Sept 2018	1 kidney transplant recipient with BKVAN who failed other treatments	BK viral load decreased significantly following T-cell therapy, but allograft eventually failed due to interstitial fibrosis and tubular atrophy

BKVAN: BK virus-associated nephropathy; BKVIN: BK virus interstitial nephritis; CMV: Cytomegalovirus; eGFR: Estimated glomerular filtration rate; FK: Tacrolimus; GFR: Glomerular filtration rate; HLA: Human leukocyte antigen; HSCT: Hematopoietic stem cell transplant; IVIg: Intravenous immunoglobulin; MMF: Mycophenolate mofetil; MPA: Mycophenolic acid; mTOR: Mechanistic target of rapamycin; PCR: Polymerase chain reaction; RCT: Randomized controlled trial; VST: Virus-specific T-cell therapy.

FOOTNOTES

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MINIREVIEWS

Focus on limbal stem cell deficiency and limbal cell transplantation

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Abstract

Limbal stem cell deficiency (LSCD) causes severe vision impairment and can lead to blindness, representing one of the most challenging ocular surface disorders. Stem cell deficiency can be congenital or, more often, acquired. The categorization of ocular surface transplantation techniques is crucial to achieving treatment homogeneity and quality of care, according to the anatomic source of the tissue being transplanted, genetic source, autologous or allogenic transplantation (to reflect histocompatibility in the latter group), and cell culture and tissue engineering techniques. The aim of this minireview is to provide a summary of the management of LSCD, from clinical characteristics and therapeutic outcomes to the development of novel therapeutic approaches. The manuscript also briefly summarizes recent findings in the current literature and outlines the future challenges to overcome in the management of the major types of ocular surface failure.

Key Words: Limbal stem cell deficiency; Conjunctival limbal autograft; Conjunctival limbal allograft; Keratolimbal allograft; Cultivated limbal epithelial transplantation; Simple limbal epithelial transplantation

Core Tip: Limbal cell transplantation has been developed for the management of limbal stem cell (LSC) deficiency, to improve this condition and related complications, ameliorating visual acuity and quality of life of affected patients. Some of the limitations include the lack of specific markers and standardized methods to identify LSCs, as well as the need to standardize the choice of therapeutic options which have diversified over the years and have evolved in terms of technology, efficacy, and safety. This clinical update review is to enable clinicians with the best evidence and current recommendations for managing their patients within the most advanced limbal cell transplant techniques.

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INTRODUCTION

The primary function of the cornea is to refract light, and its function directly depends on its transparency. One of the factors that is implied in the cornea's transparency is epithelium integrity. The corneal epithelium is a non-keratinized multilayer cuboid epithelium that covers the cornea starting from the limbus, where the junction between the conjunctiva and cornea is. It is capable of self-renewing thanks to the presence of stem cells. Corneal epithelial limbal stem cells (LSCs) reside preferentially in the basal layer of the peripheral cornea in the limbal zone[1,2]. There are no current specific markers for these cells. The research methods used for the identification of these cells tend to be indirect. The presence of stem cell-associated markers such as p63 and the absence of differentiated cell markers such as chemokine (CK) 12 (for corneal epithelium) or CK19 (for conjunctiva) indicate the putative stem cells[3,4].

With regards to the predominant theory about corneal epithelium regeneration, LSCs asymmetrically divide into transient amplifying cells that migrate centripetally and anteriorly and differentiate into squamous cells[5]. Several current studies suggest that, in experimental models, some stem cells could reside outside the limbus[5-7].

The presence of LSCs is crucial to inhibit the proliferation of the conjunctival epithelium on the corneal surface, and the reduction of their number leads to conjunctivalisation of the corneal surface, persistent and recurrent epithelial defects, scarring, and ulceration of the cornea. This condition is called LSC deficiency (LSCD). LSCD can be primary or secondary. Primary causes can occur for genetic pathologies or idiopathically, while the acquired ones can occur for traumas or autoimmune pathologies (Table 1)[8]. LSCD presents nonspecific symptoms such as discomfort, pain, photophobia, and decreased vision in more severe cases. The signs of LSCD depend upon pathology severity, starting from focal areas with a stippled staining pattern, loss of clarity, epithelium hyperreflectivity on Anterior Segment Optical Coherence Tomography (AS-OCT), and flattening of Vogt palisades. In severe cases, it is possible to have conjunctivalisation of the cornea, whorl pattern in fluorescein staining, superficial corneal neovascularization, persistent epithelium defect, stromal scarring, or sterile melts[9].

These signs can affect just some portion of the cornea with a clear demarcation between normal and abnormal areas, accordingly with the extension of LSC damage. In the cases of traumatic etiologies, LSCD is commonly asymmetrical. In autoimmune and congenital etiologies, LSC damage is commonly symmetrical. LSCD diagnosis is clinical in frank cases, but it can be confirmed by diagnostic investigation in subtle situations. There are several reliable tests in the diagnosis of LSCD. Understanding the underlying cause of LSC damage and starting adequate therapy are fundamental to ensuring good outcomes of LSCD treatments.

The aim of this minireview is to briefly summarize the important issues regarding the clinical characteristics and management of patients with LSCD, in addition to summarizing the therapeutic outcomes to the development of novel therapeutic approaches, future challenges, and recent findings in the current literature.

METHODS

We conducted a search of the literature published between January 1, 2002, to December 1, 2022, using MEDLINE (PubMed). The database was first searched using the following keywords: "Limbal Stem Cell Deficiency; Limbal Cell Transplantation; Limbal Stem Cell Deficiency and/or Limbal Cell Transplantation; LSCD, and/or Conjunctival limbal autograft (CLAU); LSCD and/or Conjunctival limbal allograft (CLAL); LSCD and/or Cultivated limbal epithelial transplantation (CLET); and, LSCD and/or Simple limbal epithelial transplantation (SLET)". We considered only studies in English and those referring to humans and with an abstract, thus reducing the count to 301 papers. The reference lists of all retrieved articles were assessed to identify additional relevant studies. The research of articles was performed using PubMed (https://pubmed.ncbi.nlm.nih.gov) and *Reference Citation Analysis* (https://www.referencecitationanalysis.com) Only articles with an abstract were considered. A quality score was calculated for each article using a checklist. Each study was independently assessed by at least two reviewers (Tonti E and Zeppieri M), and rating decisions were based on the consensus of the reviewing authors. The most common surgical techniques highlighted in the most relevant studies are shown in Table 1.

Table 1 Causes of limbal stem cell deficiency

Genetic disease	Acquired immune-mediated	Acquired nonimmune-mediated	Others
Congenital aniridia	Steven-Johnson syndrome	Chemical/thermal injury	Ocular surface tumors
Keratitis ichthyosis deafness syndrome	Toxic necrolysis	Radiation injury	Drug-induced LSCD
Xeroderma pigmentosum	Mucous membrane pemphigoid	Contact lens wear	Idiopathic
Ectrodactyly-ectodermal dysplasia-clefting syndrome	Vernal/atopic keratoconjunctivitis	Multiple limbal surgeries	
Dyskeratosis congenita	Graft-*vs*-host disease	Bullous keratopathy	
Peter's anomaly		Chronic lid diseases	
		Infectious ocular diseases	

LSCD: Limbal stem cell deficiency.

DIAGNOSIS AND STAGING OF LSCD

Impression cytology

A filter paper of nitrocellulose or acetate cellulose is applied over the cornea or the conjunctiva to obtain cells from the ocular surface. Repeating the sampling on the same area allows us to obtain cells from the deeper layers, and this makes the sampling more reliable. The specimens are processed with various stains searching for goblet cells[9-11]. The presence of these cells indicates the invasion of the conjunctival epithelium over the cornea, but their absence does not exclude LSCD. In fact, in some cases, such as Stevens-Johnson syndrome or chronic inflammatory diseases, the number of conjunctival goblet cells can be markedly reduced, and their identification can be difficult[11]. Differentiating corneal epithelial cells from conjunctival epithelial cells, instead, is possible only by immunohistochemistry. As mentioned before, CK2 and CK12 are specific for the mature corneal epithelium, CK3 for the conjunctiva and corneal epithelium, and CK7, CK13, and CK19 are specific for the conjunctival epithelium. Another used marker is mucin 5AC, but it has a low sensitivity. Impression cytology is also useful for analyzing the results of LSCD therapies[12].

In-vivo confocal microscopy (IVCM)

With this exam, it is possible to acquire pictures of the corneal microstructures without collecting specimens. The presence of goblet cells in a corneal IVCM, as seen in impression cytology, confirms the diagnosis of LSCD, but their absence cannot exclude the diagnosis because this exam scans just a small area and the morphology of goblet cells can be difficult to recognize in IVCM[13]. In LSCD, the density of the basal cells of the corneal epithelium is decreased and the mean size of cells is increased, and these findings correlate with the severity of the pathology[14]. Other findings are intraepithelial cystic lesions surrounded by goblet cells and the decrease in the density of the sub-basal nervous plexus [15].

AS-OCT

This is a non-invasive imaging tool with low operator dependence. LSCD has been associated with epithelial thinning at the cornea and limbus, but these signs are not specific to LSCD[16]. With volumetric scans, it is possible to study the status of Vogt palisades, and their thinning (or absence) is associated with areas with a thinned epithelium. The analysis of the reflectivity of the epithelium and stroma in LSCD shows that epithelial reflectivity varies more than stromal reflectivity, and the ratio between them could be a diagnostic tool for LSCD. Furthermore, this ratio tends to return to normal values after LSC transplantation, even if it does not return to normal values[11,17].

LSCD staging is based on the clinical presentation, and 5 mm central cornea involvement and limbal involvement are the two parameters evaluated (Table 2). The most important factor is corneal involvement; in the first stage central 5 mm is not involved, in the second it is partially affected, and in the final stage the entire corneal surface is involved. Every stage is divided into A when limbus involvement is less than 50%; B if it is more than 50% but non-complete; and C if the limbus is completely affected. Correct staging is useful for therapeutic decisions, but it is also important to evaluate palpebral and adnexa status and to control the underlying pathology if the LSCD is secondary to other pathologies[18]. Figures 1-3 show different stages of stem cell deficiency.

OCULAR SURFACE STEM CELL TRANSPLANTATION: CLINICAL OUTCOMES

Over the past two decades, a variety of ocular surface rehabilitation treatments have been developed. The ocular surface is rehabilitated by improving the ocular surface environment, ensuring control of inflammation, good lubrication, and lid closure, and eliminating keratinization and symblepharon. A favorable environment is crucial for restoring the normal corneal phenotype and proper corneal clarity[18]. Corneal transplantation can be considered for corneal clarity restoration in patients with LSCD, but results and visual outcomes tend to be limiting over time because of the inability of

Table 2 Stages of limbal stem cell deficiency

Stage		A	B	C
I	Central 5 mm of cornea not involved	Limbus involvement < 50%	Limbus involvement > 50% but < 100%	Limbus entirely involved
II	Central 5 mm of cornea involved	Limbus involvement < 50%	Limbus involvement > 50% but < 100%	
III	The entire corneal surface involved			

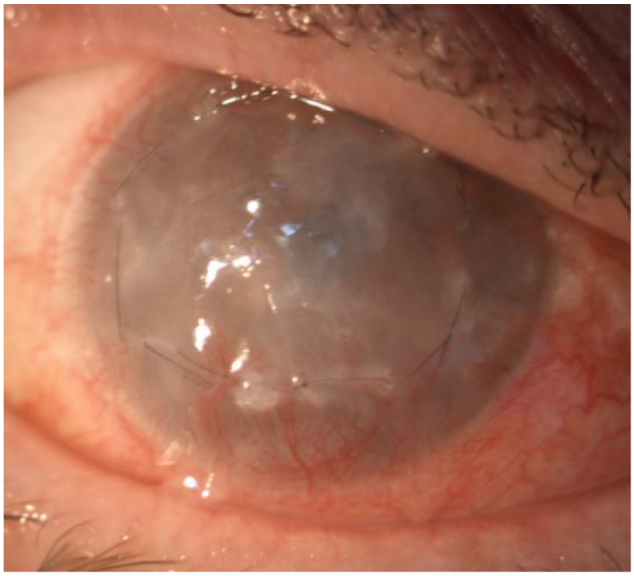

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Figure 1 Limbal stem cell deficiency stage III, in which the entire corneal surface is involved.

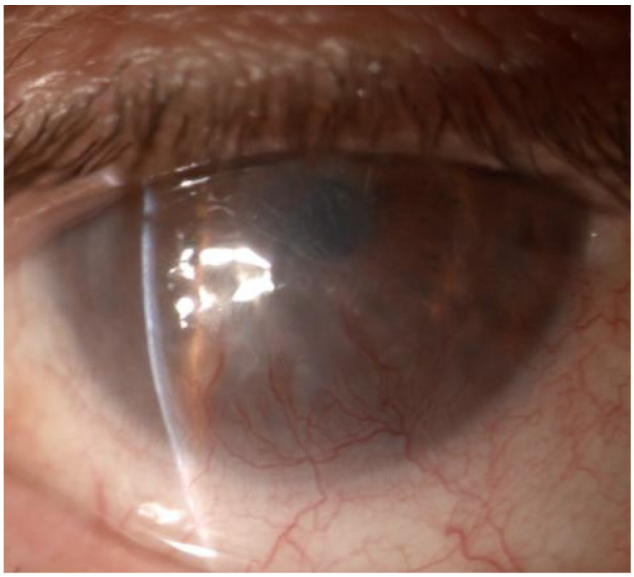

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Figure 2 Partial stem cell deficiency (stage II-A).

the LSC to regenerate and maintain the transparency of the epithelium[19].

Several transplantation procedures have been used over the past years, and many of them have been labeled using different terminologies. These procedures include autologous and allograft conjunctival transplantation[20-22], keratoepithelioplasty[23], homotransplantation of limbal cells[24], limbal transplantation[25], homotransplantation of limbal cells [26], and autologous and allograft limbal transplantation (Table 3)[27-31].

Table 3 Common surgical techniques for limbal stem cell deficiency

Procedure	Abbreviation	Tissue origin
Direct transplantation		
Conjunctival limbal autograft	CLAU	Patient
Living-related conjunctival allograft	lr-CLAL	Relative donor
Keratolimbal allograft	KLAL	Cadaveric donor
Cincinnati procedure		Relative/cadaveric donor
Modified Cincinnati procedure		Patient/cadaveric donor
Stem cells transplantation		
Simple limbal epithelium transplantation	SLET	Patient
Tissue engineering		
Cultured limbal epithelial transplantation	CLET	Patient/living donor
Autologous conjunctival epithelial cells cultivated *ex vivo*	EVCAU	Patient
Cultivated oral mucosa epithelial transplantation	COMET	Patient

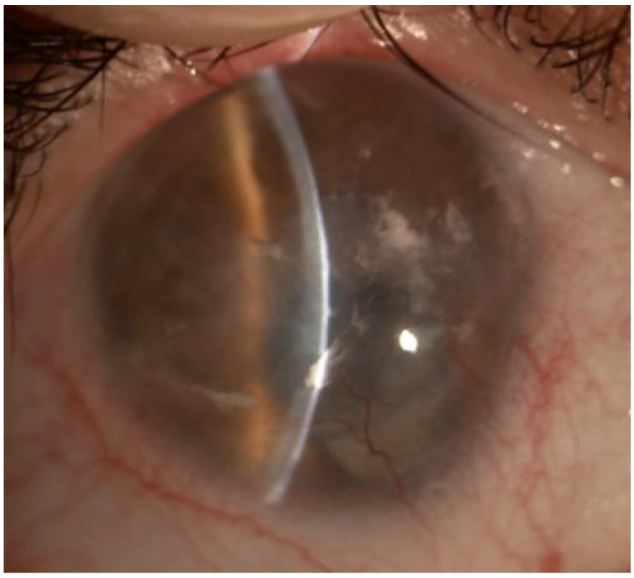

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Figure 3 Post-traumatic limbal stem cell deficiency with central corneal scarring.

CLAU

CLAU, first described by Tseng and Kenyon[26] in 1989, is one of the most used techniques for LSC transplantation in unilateral LSCD. It consists of taking a portion of limbal conjunctiva (usually from 3 to 6 o'clock) from the fellow eye and implanting it in the affected eye, with or without amniotic membrane (AM) transplantation. CLAU has been successfully used to treat different pathologies and LSCD of different etiologies and different severities, even in cases of total corneal involvement[26]. The donor eye should be examined with particular attention to exclude any sign of LSCD to avoid an iatrogenic LSCD, even if this represents a remote possibility[32,33].

The inflammatory status of the graft can reduce the transplantation success rate, so topical steroids could be useful before the graft harvesting[34]. There is not a universally accepted consensus about the size of limbal grafts, generally harvested at 12 and 6 o'clock, where Vogt palisades are more developed. The first technique used a wide graft (8 o'clock), but successful results have been achieved with smaller grafts and new promising techniques use two grafts each at about 1 o'clock combined with AM transplantation (AMT)[35-37].

AM could be transplanted even in the donor's eye when large grafts are taken to reduce the risk of LSCD due to its capacity to facilitate the *in-vivo* expansion of LSCs[38,39]. Generally, the recipient bed is prepared by doing a peritomy 4-5 mm from the limbus and dissecting the corneal pannus, the dissection of corneal layers should be avoided, and stromal opacities are better treated afterward by keratoplasty[40]. The limbal epithelial graft is fixed around the cornea and the posterior margin is sutured to the conjunctiva. Between the graft and the ocular surface could be interposed a layer of AM

that seems to increase the rates of success and the rapidity of the healing process, especially with little grafts, but a study with wide grafts showed no significant differences between the AMT group and the other without[41,42].

The great advantage of autologous transplantation is the immunosuppressive therapy sparing, so, generally, the only medication needed are topical antibiotics and topical corticosteroids. A scleral lens is applied to protect the graft from the mechanical stress of winking, and sometimes a temporary tarsorrhaphy can be considered[43]. A meta-analysis in 2020 found that the overall success rate of CLAU was 83.2%, with a 95%CI. In this study, "success" was defined as the reconstruction of an intact epithelium and a stable ocular surface. These data involved 16 articles, and 505 eyes, most of which had chemical or thermal injury[44].

Living-related (lr-CLAL) transplantation

In this technique, the surgical procedure is the same as CLAU, but the graft is harvested from a living related, and this makes it suitable even for severe bilateral LSCD. To decrease the chances of rejection, the donor must be the best human leukocyte antigen (HLA) matched available relative, generally a parent or a sibling. In 100% HLA compatibility cases, immunosuppressive therapy is not needed; in other cases, the administration of 6-12 mo of oral corticosteroids 10 mg/kg/d and oral cyclosporin A 10 mg/kg/d is required, subsequently tapered for maintenance dose during all the follow-up period. Some protocols added azathioprine to previous drugs and others used tacrolimus and mycophenolate mofetil [45,46]. Other authors administered oral cyclosporin for more than 6 mo and topical cyclosporin continued indefinitely unless toxic effects onset[47]. Patients under immunosuppressive therapy must be checked often for liver and kidney function.

Keratolimbal allograft (KLAL)

KLAL is an allogenic transplant from a cadaveric donor. The graft is prepared by dissecting and removing the limbus and a peripheral portion of the cornea of the donor eye, then the stromal portion is dissected carefully to preserve the conjunctival and limbal epithelium. The graft is then sutured to the peripheral cornea and a patch of AM is generally transplanted to ensure better outcomes[48-50]. Tissue from the youngest possible donor with an upper limit of 50 years is recommended. Surgery should be performed within 72 h as the cells are expected to be more active and vital[51-53]. The recipient bed is prepared the same way as CLAU and lr-CLAL. HLA matching is recommended, and an immunosuppressive therapy, similar to lr-CLAL, is generally needed.

Holland[29] developed the Cincinnati procedure combining lr-CLAL and KLAL. In this technique, two portions of healthy limbus conjunctiva are harvested from an HLA-matched living donor, and the corneoscleral rim is taken from a cadaveric donor. The conjunctival tissue is placed at 12 and 6 o'clock in the same anatomical orientation, and the corneoscleral tissue is placed at 3 and 9 o'clock. With this procedure, ocular surface stability was achieved in 54.2% and an improvement was achieved in 33.3%, and 75% had an improvement in the visual acuity[54]. The same authors described even the modified Cincinnati procedure that combines CLAU with KLAL, achieving ocular surface stability in 82% of patients and ocular surface improvement in 18%[55]. Both techniques require an immunosuppressive therapy like that for KLAL[54,55].

Tissue engineering for the reconstruction of the corneal epithelium

In this group of techniques, a small portion of the corneal epithelium is taken from a donor, cultivated to expand its surface, and then transplanted. The advantage of these techniques is that with a limited amount of harvested tissue, it is possible to generate a considerable amount of epithelium to transplant. Thus, even in severe bilateral LSCD, it is possible to conduct autologous transplantations. However, the cultivation process needs an advanced laboratory and a relevant amount of resources, so just a few centers perform these kinds of surgeries. The harvested corneal tissue can belong to a living donor (the patient itself, a living relative, or a living nonrelative person) or from a cadaveric donor, but some techniques use other epithelia such as the oral one (*ex vivo* oral mucosa autograft, also called cultivated oral mucosa epithelial transplantation).

However, most of the techniques are still experimental procedures non-suitable for routine application except for Holoclar (*ex vivo* expanded autologous human corneal epithelial cells containing stem cells), first described in 1997 by Pellegrini *et al*[56] that achieved EMA authorization for commercial purposes in 2015.

Holoclar is a CLET procedure that starts with the enzymatical dissociation of the sample and the seeding of the cells in a layer of irradiated mouse feeder cells with growth factors and antibiotics. After this step, cells are cryopreserved and samples are tested; some of them are stored in case of failure of the first graft. Primary cultures are then seeded into antibiotic-free fibrin matrix discs and cultivated again. Epithelized discs are then shipped to the clinic, shaped by the surgeon, and implanted like in the CLAU technique. There is no standard procedure for CLET, and in fact, the cultivation procedure in the literature varies for the substrate used.

AM and cultivating milieu are mostly used, but the overall success rate of this technique is 71.8%. When cultivated cells are autologous, the ocular surface stability is maintained for long follow-up periods[57-65]. Most patients are typically affected by LSCD for chemical injury, but this technology is used also to treat LSCD due to autoimmune and congenital pathologies.

Another technique is autologous conjunctival epithelial cells cultivated *ex vivo*, in which the cultivated tissue is fornical conjunctiva. The specimen is placed on a denuded human AM and submerged in a culture medium with grow factors and antibiotics. The cultivated tissue is then shaped and transplanted to the prepared corneal surface. A study with 12 eyes reported a success rate of 66.6% and 16.6% of partial success (conjunctival epithelial ingrowth recurred in 2 corneal quadrants), but we have no other data about the clinical outcomes in humans[66].

SLET

SLET is a recent procedure for unilateral disease and seeds donor stem cells directly on an AM placed on the recipient's ocular surface, completely obviating any need for laboratory conditions of expansion[67]. Although CLET reduced the complications of CLAU, cell expansion required a clinical-grade lab with regulatory approvals, which was and continues to be very expensive to build and maintain. SLET, introduced by Sangwan *et al*[67] in 2012, combining the benefits of CLAU and CLET while avoiding the limitations of both strategies.

Unilateral LSCD is the primary indication for autologous SLET. Ocular burns are the most common cause of unilateral LSCD, so it is not surprising that this indication is covered by almost all of the published literature on autologous SLET. More recently, the first case reports of allogenic SLET in cases of bilateral LSCD have been proposed and involved patients with severe chemical burns and dry eyes, respectively[68,69]. In the SLET technique, in the superior limbal district of the unaffected contralateral eye, a portion of 2 mm × 2 mm of limbal tissue is removed under topical anesthesia and placed in a balanced saline solution.

The corneal surface is exposed by removing the fibrovascular corneal pannus after 360-degree conjunctival peritomy and peribulbar anesthesia is induced. With the epithelial side up, human AM is grafted over the cornea and secured with fibrin glue, and the margins are trimmed to fit the external conjunctival borders. Eight to ten tiny pieces of the limbal sclerocorneal tissue are cut into pieces and adhered to the AM in a circular pattern using fibrin glue, sparing the optical zone[67]. In a study involving six patients with total unilateral LSCD, visual acuity improved in four of the recipients' eyes (66.6%), going from 20/200 or worse before SLET surgery to 20/60 or better afterward. None of the donor eyes experienced any complications. It took 9.2 mo on average to follow up[67].

CONCLUSION

Patients with severe ocular surface disease need to be treated in a methodical, step-by-step manner. To achieve the best results in the rehabilitation of the ocular surface, it is crucial to select the patient's most appropriate strategy of treatment. The underlying pathology, the extent, and severity of ocular surface disease, including the degree of stem cell damage, unilaterality or bilateralism of the condition, the presence or absence of conjunctival inflammation, and whether tear production is normal (significantly altered or absent), the patient's age, and systemic co-morbidities are important factors in the choice of regimen among the various surgical procedures proposed for the treatment of LSCD.

The development of xenobiotic-free culture systems and the standardization of culture conditions are two improvements that must be made in order to advance the therapeutic approach. Additionally, to guarantee the functionality and long-term regeneration of the transplants, tissue engineering strategies must incorporate a kind of quality control, verifying the preservation of stem cells during the culture process.

Clarifying the signaling pathways that control stem cell function and fate *in vivo* and *in vitro* is one of the remaining challenges. Future trends include the creation of biomimetic scaffolds that can deliver drugs, growth factors, or signaling molecules to help further promote cell function and tissue regeneration in addition to acting as structural supports for living cells.

FOOTNOTES

Author contributions: Tonti E wrote the outline, did the research, wrote the paper, and provided the final approval of the version of the article; Manco GA assisted in the editing and making critical revisions of the manuscript; Spadea L assisted in the writing, editing, and making critical revisions of the manuscript; Zeppieri M assisted in the conception and design of the study, and writing, outline, and final approval of the version of the article to be published and completed the English and scientific editing (a native English speaker).

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ORIGINAL ARTICLE

Retrospective Cohort Study

Predictors of graft function and survival in second kidney transplantation: A single center experience

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Abstract

BACKGROUND

The increasing kidney retransplantation rate has created a parallel field of research, including the risk factors and outcomes of this advanced form of renal replacement therapy. The presentation of experiences from different kidney transplantation centers may help enrich the literature on kidney retransplantation, as a specific topic in the field of kidney transplantation.

AIM

To identify the risk factors affecting primary graft function and graft survival rates after second kidney transplantation (SKT).

METHODS

The records of SKT cases performed between January 1977 and December 2014 at a European tertiary-level kidney transplantation center were retrospectively reviewed and analyzed. Beside the descriptive characteristics, the survivals of patients and both the first and second grafts were described using Kaplan-Meier curves. In addition, Kaplan-Meier analyses were also used to estimate the survival probabilities at 1, 3, 5, and 10 post-operative years, as well as at the longest follow-up duration available. Moreover, bivariate associations between various predictors and the categorical outcomes were assessed, using the suitable biostatistical tests, according to the predictor type.

RESULTS

Out of 1861 cases of kidney transplantation, only 48 cases with SKT were eligible for studying, including 33 men and 15 women with a mean age of 42.1 ± 13 years.

The primary non-function (PNF) graft occurred in five patients (10.4%). In bivariate analyses, a high body mass index (P = 0.009) and first graft loss due to acute rejection (P = 0.025) were the only significant predictors of PNF graft. The second graft survival was reduced by delayed graft function in the first (P = 0.008) and second (P < 0.001) grafts. However, the effect of acute rejection within the first year after the first transplant did not reach the threshold of significance (P = 0.053). The mean follow-up period was 59.8 ± 48.6 mo. Censored graft/patient survival rates at 1, 3, 5 and 10 years were 90.5%/97.9%, 79.9%/95.6%, 73.7%/91.9%, and 51.6%/83.0%, respectively.

CONCLUSION

Non-immediate recovery modes of the first and second graft functions were significantly associated with unfavorable second graft survival rates. Patient and graft survival rates of SKT were similar to those of the first kidney transplantation.

Key Words: Graft failure; Graft function; Kidney; Kidney retransplantation; Primary non-function graft; Second kidney transplantation

Core Tip: Second kidney transplantation (SKT) is a viable option for patients with failed first kidney transplantation (FKT). Although the first primary nonfunction graft is a common contributor to SKT, it is also a potential outcome among a major proportion of those populations. Also, it is a significant risk factor for graft survival among those patients with functioning SKTs. Hence, the non-immediate recovery of the first graft function and delayed graft function in the second graft are significantly associated with unfavorable second graft survival rates. Inspite of this wide spectrum of risk factors, patient and graft survival rates in SKT seemed to be similar to those of FKT. SKT should be recommended for patients with failed FKT.

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INTRODUCTION

Kidney transplantation is the optimal treatment of end-stage renal disease (ESRD), because it provides better outcomes in survival rates, quality of life, and economic saving[1,2]. However, the expected survival of renal allografts is relatively lower than the patients' survival. This discrepancy between the patient and graft survival rates resulted in a progressively increasing number of patients who may need kidney retransplantation (KRT)[3-5]. Rates of KRT represent more than 15% of patients on the waiting lists[2,3,5], where the second kidney transplantation (SKT) is the most frequent form[5,6]. The numbers of KRT being still relatively far less than that of the first kidney transplantation (FKT) has resulted in persistent debates about the risk factors that may affect KRT and its controversial survival benefits. The magnitude of the reported outcomes of KRT has been shown to be either inferior or acceptable relative to those of FKT[5,7,8]. Beside the potential exposure to the same risk factors of FKT, recipients of KRT are prone to additional factors that may evolve from the repeated process such as sensitization and technical difficulties[5,6,9].

The unresolved debates about the risk factors and survival rates represented our rationale to present the current single center experience of SKT and explore the predictors for the graft function and survival of SKT.

MATERIALS AND METHODS

Study design

The electronic and manual records of the cases of KRT which were performed between January 1977 and December 2014 at Urology Department, Martin-Luther University, Halle (Saale), Germany were reviewed for the characteristics of the FKT and SKT processes. The effects of these variables on the primary graft function and the survival of both graft and patient were evaluated in SKT.

The target population was the adult patients who received SKT. Exclusion criteria were blood grouping or human leucocytic antigen (HLA) incompatible transplants; immunosuppression protocols other than basiliximab or anti-thymoglobulin for induction, and steroid, tacrolimus or cyclosporine, and mycophenolate mofetil for maintenance; missing data; and SKT within the year just before data collection.

Ethical approval

The authors confirm that all the experimental protocols of this study were approved by the Ethical Committee (Institutional Review Board; IRB) of the Faculty of Medicine, Assiut University, Egypt and Martin-Luther University, Germany (IRB approval number: 17200548/2015).

Statistical analysis

The statistical methods were implemented using IBM® SPSS® Statistics 23 and GraphPad Prism® 6. Two-tailed *P* values < 0.05 were considered significant.

After excluding primary non-function grafts, the survivals of both the first and second grafts were described using Kaplan-Meier curves. The same method was also used to describe patient survival after the second transplantation for the whole study sample. Moreover, regarding the graft and the patient survivals after SKT, Kaplan-Meier analyses were also used to estimate the survival probabilities at 1, 3, 5, and 10 post-operative years, as well as at the longest follow-up duration available.

Bivariate associations between various predictors and the categorical outcomes were assessed according to the predictor type. For quantitative predictors, the independent-samples *t* test was used when all outcome groups were normally distributed. Otherwise, the independent-samples Mann-Whitney *U* test and the Kruskal-Wallis test were used for binary and multinomial outcomes, respectively. For categorical predictors, Fisher's exact test was used.

As regards the second graft survival, associations with categorical predictors were evaluated by Kaplan-Meier curves for the strata of each predictor; the similarity between these curves for each predictor was tested by the log-rank test. On the other hand, associations with quantitative predictors were evaluated by Cox regression, where testing of the proportional hazards assumption was done by correlating ranked survival times with Schoenfeld residuals.

RESULTS

Between January 1977 and December 2014, a total of 1861 kidney transplants were done, of whom 176 cases had SKT. Only 48 cases were eligible for the current study. Characteristics of patients, donors, FKT, and SKT are summarized in Table 1. Twenty-three cases (47.9%) had primary non-function (PNF) first graft, while only five cases (10.4%) had PNF second graft. Patients with PNF grafts were excluded from the graft survival analyses. The median survival time for the first graft was 36 mo, while it was undefined for the graft and the patient after SKT (Figure 1). Survival probabilities of the graft and the patient after the SKT are shown in Table 2. The follow-up period ranged from 12 to 174 mo.

PNF graft occurred in five patients (10.4%). In bivariate analyses, a high body mass index (BMI) of the recipient was the only significant quantitative predictor of PNF graft ($P = 0.009$) (Tables 3 and 4). Also, first graft loss due to acute rejection was the only significant categorical predictor of PNF graft ($P = 0.025$) (Table 5).

The second graft survival was best in cases with a PNF first graft, while it was worst in cases with a delayed graft function (DGF) of the first graft ($P = 0.008$). Also, the second graft survival was better in cases with an immediate second graft function than in those with a delayed second graft function ($P < 0.001$) (Figure 2). Finally, the occurrence of acute rejection within the first year after the FKT decreased the survival of the second graft, but didn't reach the threshold of significance ($P = 0.053$) (Tables 6 and 7; Figures 3-5).

No significant associations were found between panel reactive antibodies (PRA) categories at SKT on one hand and first graft nephrectomy ($P = 0.784$), the duration before first graft nephrectomy ($P = 0.497$), or acute rejection of the second graft in the first year after SKT ($P = 0.223$) on the other hand. Also, no significant association was found between the number of second graft arteries and the vascular complications of SKT ($P = 0.382$).

DISCUSSION

Graft loss is always a potential outcome after variable periods of FKT[3,8-10]. This outcome created an imperative need for KRT[11]. Nowadays, there is a progressive rise in the numbers of patients receiving this line of treatment. KRT entails more risk factors for unfavorable outcomes than FKT[6,12]. Also, there are substantial controversies about the differences between FKT and SKT regarding patient and graft survival rates[7]. The current study targeted the potential risk factors affecting the second graft function in a large-volume kidney transplantation center.

In our study, the mean patient age at SKT was similar to that reported in other studies[5,13]. Also, our results resembled other studies regarding the gender distribution at SKT[5,13,14]. Causes of ESRD before kidney transplantation are not the same among the different world regions. Diabetic and hypertensive nephropathies represent the main causes in the United States. However, in the current series, glomerulonephritis was the leading cause, as in other countries[5,13].

It has been reported that occurrence of certain clinical outcomes after FKT is significantly associated with more likelihood of the same outcomes after KRT which increases the chances of graft loss[13]. In general, graft loss can be classified into three major categories: PNF grafts, patient death with a functioning graft, and loss of a previously functioning graft due to different medical and surgical causes[15,16].

PNF graft is defined as the permanent absence of functions of the transplanted kidney starting immediately after transplantation. It accounts for 0.6%-8% of all renal graft loss and it is significantly associated with poor patient survival [15,17]. In our series, a slightly higher rate was observed in SKT (10.4%), while the rate was much higher in FKT (47.9%). The major cause of PNF grafts has been reported to be venous or arterial thrombosis occurring within 1-2 d after

Table 1 Characteristics of recipients, donors, first kidney transplantation, and second kidney transplantation, *n* (%)

Variable		Value[1]
Recipient age at SKT (yr)		47.5 (41.3-56; 24-70)
Recipient sex	Male	33 (68.8)
	Female	15 (31.3)
Recipient BMI (kg/m^2) at SKT		24.7 (22.13-26.95; 19-33.5)
Causes of ESRD	Glomerulonephritis	16 (33.3)
	DM	1 (2.1)
	Hypertension	4 (8.3)
	PCKD	4 (8.3)
	Others	23 (47.9)
Overall duration of dialysis (mo.)		95 (76-121.8; 29-244)
Start of first graft function	PNF	23 (47.9)
	DGF	8 (16.7)
	Immediate	17 (35.4)
GFR one year after FKT (mL/min/1.73 m^2)		0 (0-29.3; 0-78.8)
Attacks of acute rejection in first year after FKT[2]		0 (0-1; 0-6)
First graft loss due to rejection		3 (6.3)
First graft nephrectomy		37 (77.1)
SKT donor type	Living	3 (6.3)
	Deceased	45 (93.8)
SKT donor age (yr)		50 (36.3-60.8; 16-74)
Recipient age minus donor age (yr) at SKT		0 (-10-7; -39-34)
SKT donor BMI (kg/m^2)		25 (23-27; 19-37.9)
Recipient BMI minus donor BMI (kg/m^2) at SKT		-0.45 (-3.8-3.15; -16.7-9.6)
SKT PRA level	0-30%	35 (72.9)
	31-80%	10 (20.8)
	> 80%	3 (6.3)
SKT HLA mismatches		2 (1.3-3.8; 0-6)
SKT laterality relative to FKT	Ipsilateral	1 (2.1)
	Contralateral	47 (97.9)
Number of renal arteries at SKT	Single	43 (89.6)
	Double	5 (10.4)
SKT operative time (min)		140 (113-170; 82-236)
SKT ischemia time (min)		708 (531-897; 74-1319)
SKT operative revision		24 (50)
SKT vascular complications		8 (16.7)
Start of second graft function	PNF	5 (10.4)
	DGF	10 (20.8)
	Immediate	33 (68.8)
Attacks of acute rejection in first year after SKT		0 (0-1; 0-3)
GFR one year after SKT (mL/min/1.73 m^2)		36 (22.8-52.8; 0-82.4)

[1]Quantitative variables are expressed as median (IQR; range), while categorical variables are expressed as count (percentage).
[2]Two missing cases.
BMI: Body mass index; DM: Diabetes mellitus; DGF: Delayed graft function; ESRD: End-stage renal disease; FKT: First kidney transplantation; GFR: Glomerular filtration rate; HLA: Human leucocytic antigen; PCKD: Polycystic kidney disease; PNF: Primary non-function; PRA: Panel reactive antibodies; SKT: Second kidney transplantation.

Table 2 Survival probabilities of the graft and the patient after the second kidney transplantation by Kaplan-Meier analyses

Follow-up time (months)	Second graft survival			Patient survival after second kidney transplantation		
	Survival probability (%)	Upper 95% confidence limit	Lower 95% confidence limit	Survival probability (%)	Upper 95% confidence limit	Lower 95% confidence limit
12	90.53	+5.81	-13.84	97.87	+1.83	-12.04
36	79.88	+9.55	-16.22	95.60	+3.29	-12.10
60	73.71	+11.31	-17.34	91.92	+5.51	-15.82
120	51.57	+21.01	-26.12	83.04	+9.90	-20.65
174 (study max.)	51.57	+21.01	-26.12	83.04	+9.90	-20.65

Table 3 Quantitative predictors (normally distributed over both outcome groups) of primary non-function second graft by the independent-samples *t* test

	Primary non-function (*n* = 5)		Primary function (*n* = 43)		*P* value[1]
	Mean	SE	Mean	SE	
Recipient age (yr)	47.8	5.4	47.9	1.8	0.98
Donor age (yr)	49.6	7.5	48.0	2.2	0.82
Recipient BMI (kg/m^2)	28.04	0.83	24.20	0.47	0.009
Total ischemia time (min)	655	98	711	48	0.70
Operative time (min)	150	20	142	6	0.66

[1]Since Levene's test yielded no significant differences between variances of outcome groups for the five tested predictors, equal variances were assumed.
BMI: Body mass index.

Table 4 Quantitative predictors (non-normally distributed over one or both outcome groups) of primary non-function second graft by the independent-samples Mann-Whitney U test

	Primary non-function (*n* = 5)		Primary function (*n* = 43)		*P* value
	Median	Mean rank	Median	Mean rank	
Duration of first graft function (mo)	0	17.6	4	25.3	0.26
Total duration of dialysis before second transplantation (including before first transplantation) (mo)	93	19.3	96	25.1	0.39

transplantation[15]. In our series, although the odds of PNF in cases with vascular complications was 4.1 times higher than in cases without these complications, the result was statistically insignificant probably due to the small sample. However, high recipients' BMI and first graft loss due to acute rejection were significantly associated with the occurrence of PNF after SKT. This might be attributable to the same mechanisms that decrease the second graft survival[15]. To our knowledge, it seems that these factors have not yet been studied relative to PNF graft after SKT.

The third category of kidney transplantation loss outcomes is the loss of the graft which functioned for a certain period before being permanently non-functioning. The risk factors of this outcome are multiple and have different tributaries. Regarding the elements of kidney transplantation process (recipient, donor, and process) and the previously proposed categorizations in the literature[5,14,18], the potential predictors or risk factors that affect the outcome of SKT could be classified into five classes: recipient-related, donor-related, FKT process-related, SKT process-related, and common factors.

Table 5 Categorical predictors of primary non-function second graft by Fisher's exact test

Variables		Primary non-function (n = 5)	Primary function (n = 43)[1]	Odds ratio[2]	P value
DM as a cause of ESRD	No	5	42	0	1
	Yes	0	1		
First graft function	No	3	20		0.84
	Delayed	1	7		
	Instant	1	16		
Acute rejection in first year after first transplantation	No	3	24	0.94	1
	Yes	2	17		
First graft loss by acute rejection	No	3	42	28	0.025
	Yes	2	1		
First graft nephrectomy	No	0	11	Not assessed[3]	0.58
	Yes	5	32		
Living donor	No	5	40	0	1
	Yes	0	3		
PRA grouping	0% to 30%	3	32		0.33
	31% to 80%	1	9		
	Over 80%	1	2		
Number of HLA mismatches	0	1	6		0.51
	1 to 3	2	27		
	4 to 6	2	10		
Over one artery	No	5	38	0	1
	Yes	0	5		
Vascular complications	No	3	37	4.1	0.19
	Yes	2	6		

[1]Except for acute rejection in first year after first transplantation, where n = 41 because two cases are missing.
[2]Odds of primary non-function in the presence of the predictor to odds of primary non-function in its absence.
[3]Not assessed, for the calculation entails division by zero.
DM: Diabetes mellitus; ESRD: End-stage renal disease; HLA: Human leukocytic antigens; PRA: Panel reactive antibodies.

The recipient-related risk factors include patient's age, sex, BMI, race, the cause of ESRD, and the associated comorbidities like diabetes mellitus and hypertension[5,13,19,20]. The second class risk factors are the donor-related factors either in FKT and SKT processes such as donor type (living or deceased), age, sex, and relatedness[5,13,14,21]. In the current series, the studied group of these factors showed no significant effects on SKT graft survival. We examined the effect of two further potential recipient-related variables; the differences between recipients' and donors' age and BMIs. Although they have been studied previously for their effect on FKT graft survival[22,23], they haven't been tested upon KRT survival so far. However, no significant association with the second graft survival could be found. It may be better demonstrated in larger studies.

The third class includes the factors from FKT process such as duration of FKT graft function and estimated glomerular filtration rate at one year after FKT[13,21,24-26]. The fourth class of risk factors includes factors that affect only SKT process such as sensitization due to previous transplantation represented by PRA level, first graft nephrectomy, and serum creatinine at one year after SKT[5,21,25].

The fifth class consists of the common variables between FKT and SKT processes and they represent the major proportion of risk factors. They involve all the phases of the process; factors in the preoperative phase such as number of HLA mismatches[4,5,18], and duration of dialysis[13,27]; factors in the operative and perioperative phases such as ischemia time, DGF[20,28], mode of recovery of graft function[13,27], and surgical complications[14]; factors in the postoperative phase such as acute rejection[13,27]; and factors involving the whole phases such as immunosuppressive regimens[5,12,29], and volume of transplantation center[18]. The reported incidence of DGF among KRTs ranged from 26.7%-39%[5,7,20]. In our study, the non-immediate mode of recovery of first graft function and DGF of second graft were

Table 6 Categorical predictors of second graft survival by the log-rank test for Kaplan-Meier curves

Variables		Events (*n* = 13)	Censored (*n* = 30)[1]	Log-rank statistic	*P* value
DM as a cause of ESRD	No	12	30	1.218	0.270
	Yes	1	0		
First graft function	No	2	18	9.684	0.008
	Delayed	4	3		
	Instant	7	9		
Acute rejection in first year after first transplantation	No	5	19	3.757	0.053
	Yes	8	9		
First graft loss by acute rejection	No	13	29	0.369	0.543
	Yes	0	1		
First graft nephrectomy	No	3	8	0.097	0.756
	Yes	10	22		
Living donor	No	12	28	0.002	0.965
	Yes	1	2		
PRA grouping	0% to 30%	11	21	0.693	0.707
	31% to 80%	2	7		
	Over 80%	0	2		
Number of HLA mismatches	0	2	4	0.106	0.948
	1 to 3	8	19		
	4 to 6	3	7		
Over one artery	No	10	28	1.584	0.208
	Yes	3	2		
Vascular complications	No	13	24	1.723	0.189
	Yes	0	6		
Delayed second graft function	No	7	26	12.238	0.0005
	Yes	6	4		

[1]Except for acute rejection in first year after first transplantation, where $n = 28$ because two cases are missing.

BMI: Body mass index; DM: Diabetes mellitus; ESRD: End-stage renal disease; HLA: Human leukocytic antigens; PRA: Panel reactive antibodies.

the only significant predictors for low second graft survival. It has been reported that occurrence of acute rejection during their first year post FKT is significantly associated with occurrence of acute rejection during KRT[13,21]. The current results showed that the incidence of acute rejection in FKT approached the threshold of significance in affection of the graft survival of SKT. This insignificant association could be attributed to the small sample size. The significant association of the mode of recovery of FKTs and the nearly significant association of the incidence of acute rejection among FKTs with the SKT graft survival, without the same effect on the SKT, could be attributed to the more stringent immunosuppression protocols and precise donor selection. This may improve the SKT graft function recovery and decrease the incidence of acute rejections. Thus, it may improve the short-term results to some extent but, it doesn't exterminate the inherent high risk of those patients[9,30].

With controversy, rates of graft and patient survivals of KRTs have been reported as inferior[3,14] or insignificantly different from those of FKT[4,5,21]. In the current study, the long-term graft survival rates were similar to FKT. This outcome is similar to the other studies[4,21].

This study was conducted in a large-volume kidney transplantation center and extracted from a relatively large reviewed number of kidney transplantations. Also, new potential predictors including the differences in age and BMI between the recipients and donors were studied for their effect on graft survival.

Limitations of the current study were the relatively small sample size that didn't allow for adequate powerful statistical tests such as the multivariate analysis and lack of reporting of some complications as post-transplant neoplastic diseases and infections. Specifically, there were some missing data, such as the levels of the donor specific antibodies against the HLA alleles of the first graft and the pathological evaluation of the donors. In addition, the retrospective studying has its mere limitations of difficult implementation of comparison and randomization.

Table 7 Quantitative predictors of second graft survival by Cox regression

Variables	HR	95%CI for HR		*P* value	*P* value for PH testing[1]
		Lower bound	Upper bound		
Recipient age (yr)	0.976	0.930	1.023	0.306	0.074
Recipient BMI (kg/m^2)	0.980	0.810	1.185	0.833	0.787
Duration of first graft function (mo)	1.007	0.994	1.020	0.307	0.059
Total duration of dialysis before second transplantation (including before first transplantation) (mo)	1.006	0.995	1.017	0.295	0.061
Donor age (yr)	1.016	0.979	1.055	0.396	0.852
Recipient age minus donor age (yr)	0.972	0.937	1.009	0.140	0.306
Recipient BMI minus donor BMI (kg/m^2)	0.984	0.893	1.085	0.751	0.410
Total ischemia time (min)	1.001	0.999	1.003	0.284	0.579
Operative time (min)	0.995	0.979	1.010	0.497	0.363

[1]Testing of the proportional hazards assumption was done by correlating ranked survival times with Schoenfeld residuals.
BMI: Body mass index; HR: Hazard ratio; PH: Proportional hazards.

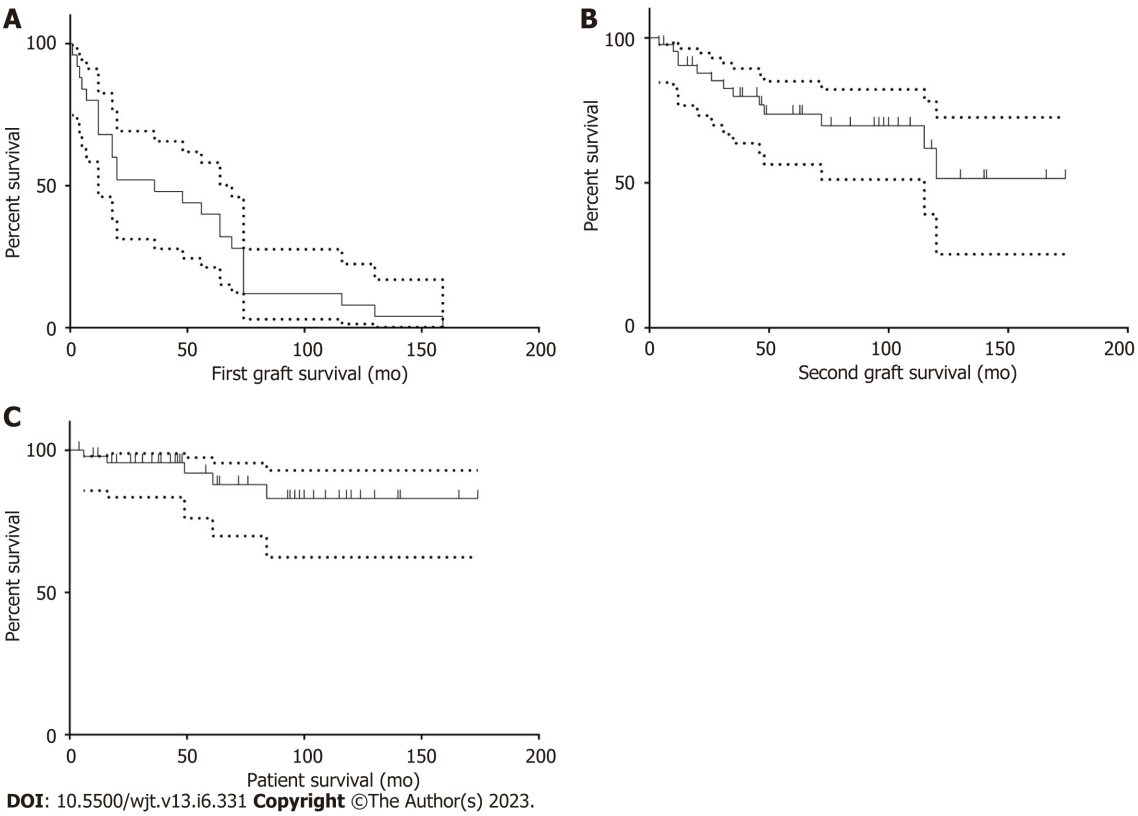

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Figure 1 Kaplan-Meier curve for overall survival rates. A: First graft survival with 95% confidence bands. Twenty-three cases were excluded from the analysis due to primary non-function grafts. All 25 cases had the event; B: Second graft survival with 95% confidence bands. Five cases were excluded from the analysis due to primary non-function grafts. Thirteen cases had the event, while 30 cases were censored; C: Patient survival after the second kidney transplantation with 95% confidence bands. Only five patients died, while 43 patients were censored.

CONCLUSION

SKT is a viable option for patients with failed FKT. Demographics and clinical characteristics of the patients accessing SKT are not significantly different from those of FKT. There are multiple potential factors that may originate from the different components and phases of SKT and could affect the survival outcomes. Although the first PNF graft is a

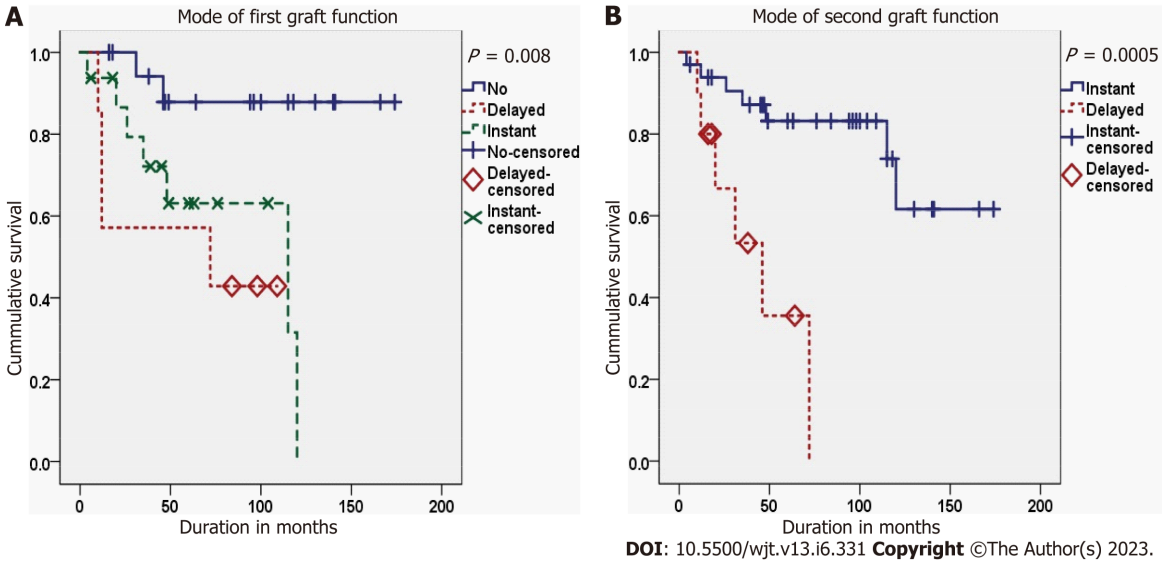

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Figure 2 Kaplan-Meier curves for the second graft survival stratified by the mode of graft function. A: In the first kidney transplantation; B: In the second kidney transplantation.

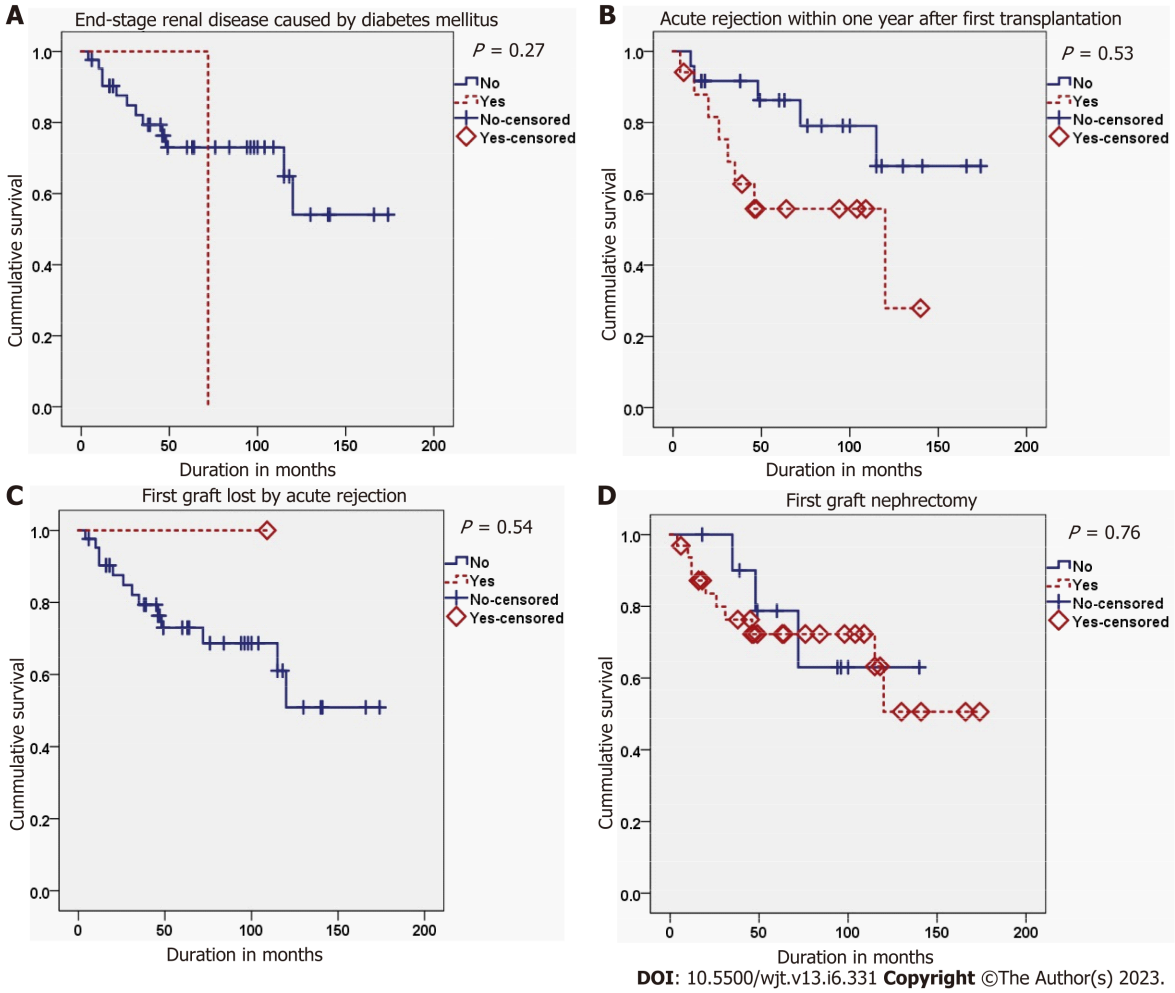

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Figure 3 Kaplan-Meier curves for the second graft survival stratified by four non-significant predictors related to the first kidney transplantation. A: End-stage renal disease caused by diabetes mellitus; B: Acute rejection within one year after first transplantation; C: First graft loss by acute rejection; D: First graft nephrectomy.

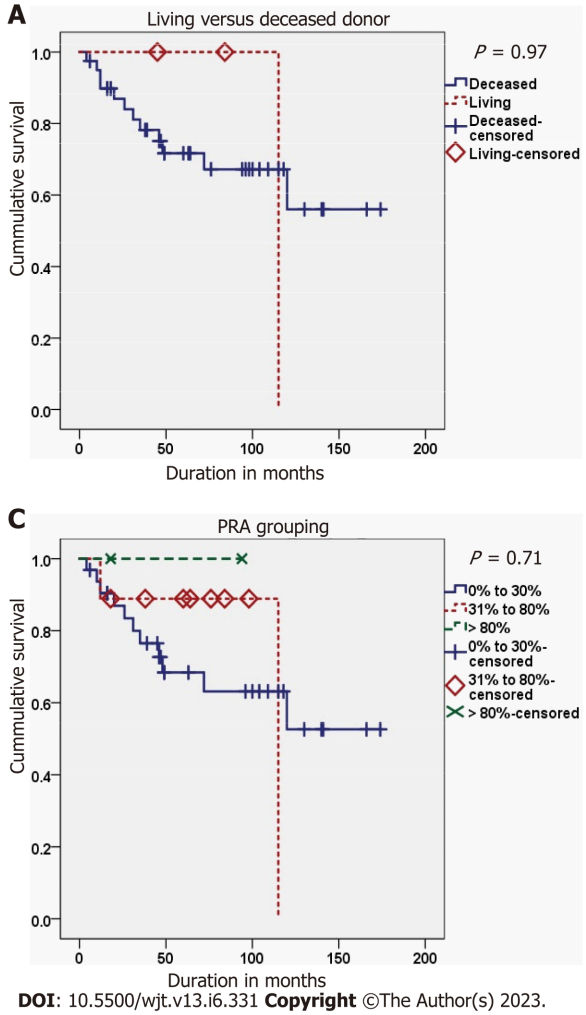

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Figure 4 Kaplan-Meier curves for the second graft survival stratified by three non-significant predictors related to the donor of second kidney transplantation. A: Living versus deceased donor; B: Number of human leukocytic antigens mismatches; C: Panel reactive antibodies. HLA: human leukocytic antigens; PRA: Panel reactive antibodies.

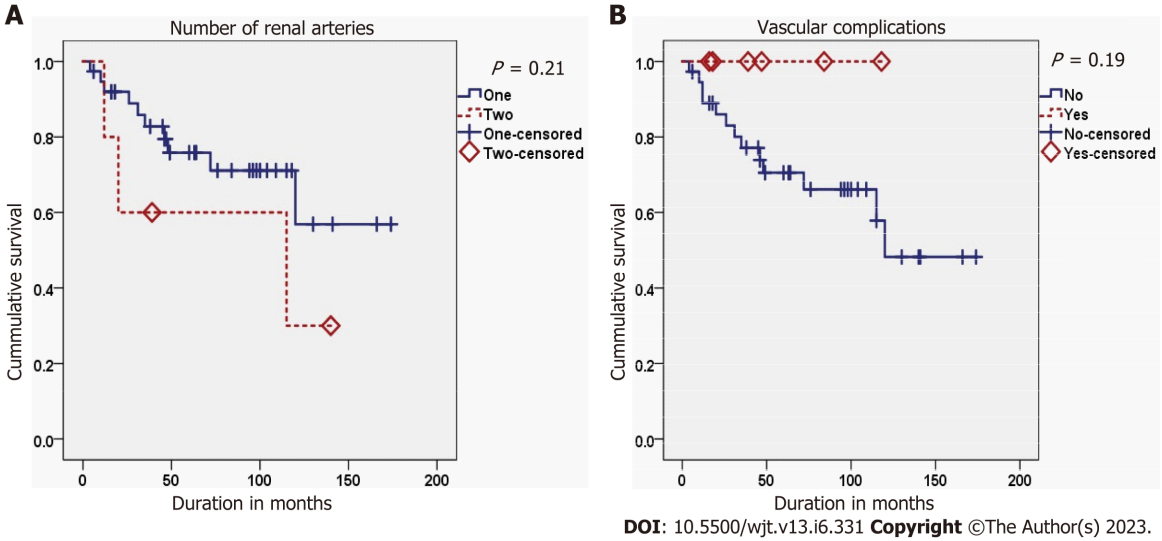

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Figure 5 Kaplan-Meier curves for the second graft survival stratified by second non-significant predictors related to the second kidney transplantation recipient. A: Number of renal arteries; B: Vascular complications.

common contributor to SKT, it is also a potential outcome among a major proportion of those populations. Also, it is a significant risk factor for graft survival among those patients with functioning SKTs. So, the non-immediate recovery of the first graft function and DGF in the second graft are significantly associated with unfavorable second graft survival rates. Inspite of this wide spectrum of risk factors, patient and graft survival rates in SKT seemed to be similar to those of FKT.

ARTICLE HIGHLIGHTS

Research background

The increasing kidney retransplantation rate has created a parallel field of research, including the risk factors and outcomes of this advanced form of renal replacement therapy. The presentation of experiences from different kidney transplantation (KT) centers may help enrich the literature on kidney retransplantation, as a specific topic in the field of KT.

Research motivation

Despite the potential high risks of repeated KT, increase of the rate of second KT (SKT) seems to be a modifiable variable and may provide better outcomes than return to dialysis in patients with failed first KT.

Research objectives

To identify the risk factors affecting primary graft function and graft survival rates after SKT.

Research methods

The records of SKT cases performed between January 1977 and December 2014 at a European tertiary-level kidney transplantation center were retrospectively reviewed and analyzed. Beside the descriptive characteristics, the survivals of patients and both the first and second grafts were described using Kaplan-Meier curves. In addition, Kaplan-Meier analyses were also used to estimate the survival probabilities at 1, 3, 5, and 10 post-operative years, as well as at the longest follow-up duration available. Moreover, bivariate associations between various predictors and the categorical outcomes were assessed, using the suitable biostatistical tests, according to the predictor type. However, associations with quantitative predictors were evaluated by Cox regression.

Research results

Out of 1861 cases of kidney transplantation, only 48 cases with SKT were eligible for studying, including 33 men and 15 women with a mean age of 42.1 ± 13 years. The primary non-function (PNF) graft occurred in five patients (10.4%). In bivariate analyses, a high body mass index (P = 0.009) and first graft loss due to acute rejection (P = 0.025) were the only significant predictors of PNF graft. The second graft survival was reduced by delayed graft function in the first (P = 0.008) and second (P < 0.001) grafts. However, the effect of acute rejection within the first year after the first transplant did not reach the threshold of significance (P = 0.053). The mean follow-up period was 59.8 ± 48.6 mo. Censored graft/patient survival rates at 1, 3, 5 and 10 years were 90.5%/97.9%, 79.9%/95.6%, 73.7%/91.9%, and 51.6%/83.0%, respectively.

Research conclusions

Non-immediate recovery modes of the first and second graft functions were significantly associated with unfavorable second graft survival rates. Patient and graft survival rates of SKT were similar to those of the first KT.

Research perspectives

Repeated kidney transplantation may provide better outcomes in patients with failed previous grafts. However, this approach may be associated with higher risks than the first time due to the surgical difficulties and immunological sensitization. Controlling of these risk factors can enhance the outcomes.

FOOTNOTES

Author contributions: Khalil M and Gadelkareem RA designed the research, collected the data, performed statistical analysis and wrote the paper; Abdallah MA, Mohammed N and Sayed MA contributed to data collection, literature review, writing and revision; and Elanany FG and Fornara P contributed to literature review, writing, revision and supervision of the work; All authors approved the paper.

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Informed consent statement: This article is a retrospective study. Hence, the patients were not required to give informed consent to the study, because the manipulated data were anonymous and were obtained after each patient, with his potential kidney donor(s), agreed

to the plan of management.

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ORIGINAL ARTICLE

Retrospective Study

Risk stratification of renal transplant recipients using routine parameters: Implication of learning from SARS-CoV-2 into transplant follow-up program

Abbas Ghazanfar, Madiha Abbas, Md Walid Hussain, Malik Kayal

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Abstract

BACKGROUND

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection is a global pandemic that is associated with a high risk of morbidity and mortality among recipients of solid organ transplantation. In the course of acute SARS-CoV-2 infection, various laboratory markers have been identified as predictors for high risk of mortality.

AIM

To risk stratify renal transplant recipients (RTxR) using general demographic parameters, comorbidities and routine laboratory markers for the severity of the disease and its outcomes. We believe that learning about these routinely monitored parameters can help us plan better strategies for the RTxR follow-up program.

METHODS

This present study includes RTxR who acquired SARS-CoV-2 infection from March 2020 to February 2021. We recorded the basic demographics, comorbidities and routine laboratory markers. We investigated the impact of SARS-CoV-2 infection on RTxRs and risk-stratified the progression of disease severity and outcomes in terms of recovery or mortality.

RESULTS

From 505 RTxRs in our renal transplant follow-up program, 29 (7.75%) RTxRs had PCR-positive SARS-CoV-2 infection. We recorded 8 deaths from SARS-CoV-2 infection giving an overall mortality rate of 1.6% but a significant 27.6% mortality in SARS-CoV-2 positive recipients. Age more than 68 years, non-Caucasian ethnicity and male gender were associated with a significant drop in survival probability; $P \leq 0.001$. < 0.001 and < 0.0001 respectively. 87.5% of the deceased were diabetic; $P \leq 0.0.0001$. Estimated glomerular filtration rate of less than 26 mL/min/1.73 m^2, serum albumin less than 20 g/L, Hemoglobin less than 9.6 g/L and serum calcium less than 1.70 mmol/L were all associated with significantly increased risk of mortality; $P = 0.0128$, < 0.001, < 0.0001 and 0.0061 respectively.

CONCLUSION

This study has identified some routinely used modifiable parameters in predicting a higher risk of mortality and morbidity. This knowledge can be used in RTxR follow-up programs by addressing these parameters early to help reduce the morbidity and mortality in RTxRs.

Key Words: SARS-CoV-2 mortality; Renal transplant recipients; Glomerular filtration rate; Anemia; Albumin; Calcium; Reducing morbidity and mortality; Renal transplant follow-up program

Core Tip: In this present study, we aim to risk stratify renal transplant recipients (RTxR) using general demographic parameters, comorbidities and routine laboratory markers for the severity of the disease and its outcomes. We believe that learning about these routinely monitored parameters can help us to plan better strategies for RTxR follow-up program.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first identified in December 2019[1] and subsequently declared a global pandemic on March 11, 2020[2]. So far it has resulted in more than 767 million cases worldwide with more than 6.9 million deaths[3]. The severity of the disease is related to various risk factors and associated comorbidities including older age, obesity, diabetes, pre-existing cardiac and pulmonary disease and conditions that affect the immune system[4-6].

The recipients of solid organ transplantation (SOT) are known to be more vulnerable to opportunistic infections[7] including several common respiratory virus infections[8], due to a weakened T-cell mediated immune response[9]. Globally, the recipients of SOT were included among patients at increased risk for severe illness from SARS-CoV-2[10, 11]. The reported mortality among Renal Transplant Recipients (RTxRs) from SARS-CoV-2 varied between 10% to 33%, in different studies across the world[12-17]. This increased risk of mortality is not only because of immunosuppression but also secondary to associated comorbidities[5,6]. In the course of acute SARS-CoV-2 infection, various laboratory markers have been identified as predictors for high risk of mortality including lymphopenia, high C-reactive protein levels, D-dimer, lactate dehydrogenase and ferritin[18-20]. However, some of the other routinely monitored parameters have not been studied in detail in RTxRs when compared with the general population. These include blood pressure control[21], Haemoglobin (Hb)[22], serum albumin[23], serum calcium levels[24] and function of the renal allograft, being measured as estimated glomerular filtration rate (eGFR)[25]. In this present study, we investigate these routine parameters with an aim to risk stratify RTxRs in high or low-risk groups using general demographic parameters, comorbidities and routine laboratory markers. The aim is to identify relevant routinely done parameters to identify high-risk RTxRs at an early stage. We believe that identification and correction of these parameters can significantly reduce long-term morbidity and mortality in RTxRs from SARS-CoV-2 as well as non-SARS-CoV-2 related infections.

MATERIALS AND METHODS

In this retrospective observational study, we analysed the data of our renal transplant follow-up program. At the time of the present study, we had 505 RTxRs registered under the follow-up program at St Georges University Hospitals NHS Foundation Trust, London, United Kingdom. Since the start of the pandemic in March 2020, on the advice of National Health Services England, National Health Services Blood and Transplant and the British Transplantation Society, we

recommended shielding for all our RTxRs in our follow-up program. In this present study, we included all RTxR who acquired SARS-CoV-2 infection from March 2020 to February 2021. It included both symptomatic and asymptomatic patients. There were 29 patients in our RTxRs follow-up program who acquired SARS-CoV-2 infection during this period which were included in study group cohort A; leaving 476 patients in the control group, cohort B. We recorded the basic demographics, comorbidities and routine laboratory markers. We compared these two groups to identify any significant factors responsible for predisposing RTxRs to the severity of SARS-CoV-2 infection. We then further investigated 29 RTxRs with SARS-CoV-2 infection to stratify the progression of disease severity and outcomes in terms of recovery or mortality. We subdivided patients in cohort A into A1 (n = 21) where they recovered from SARS-CoV-2 infection and A2 (n = 8) which resulted in mortality. We used Prism 9 and MedCalc statistical software programs for the data analyses. Baseline characteristics were compared using a t-test, Fisher exact test, Chi-square test or Mann-Whitney U-test where appropriate. Box-Whisker plots were used to describe means, standard deviations and standard error of means. Survival probabilities were recorded for individual risk factors. Univariate and multivariate analyses were performed to record the impact of various factors on each other. Survival analysis was carried out using Kaplan–Meier estimates and for differences in survival, a log-rank test was used.

RESULTS

From 505 RTxRs in our renal transplant follow-up program 29 (7.75%) RTxRs had PCR-positive SARS-CoV-2 infection (cohort A), leaving 476 patients in control cohort B. We recorded 8 deaths in cohort A giving a mortality rate of 1.6% for the overall follow-up population but a significant 27.6% mortality in SARS-CoV-2 positive patients. There was no death recorded in cohort B during the same period.

General demographic and risk of SARS-CoV-2

The patients who acquired SARS-CoV-2 infection were from a significantly older age group with a mean (SD) and median interquartile range (IQR) of 63.24 (12.57) and 65 (56-71.5) compared to rest of the group; $P \leq 0.001$ (Table 1, Figure 1). In intra-cohort A analysis, where all patients were exposed to SARS-CoV-2 infection, the mean (SD) and median (IQR) age in years in cohort A1 and A 2 were 60.85 (12.5) and 64 (64-69.5) compared with 69.5 (9.5) and 68 (68-77); $P = 0.0986$ (Figure 2). However, on further analysis of survival probability, a direct correlation was noted between older age and mortality (Figure 3). There was a significant drop in survival probability recorded once patients crossed 68 years of age; $P \leq 0.001$. When comparing gender distribution as a risk of SARS-CoV-2 infection there was no significance recorded between cohort A and B; $P = 0.3056$. However, when the risk of mortality was compared in the SARS-CoV-2 infection-positive group, there was a higher risk of mortality among male patients, with 75% of the deceased patients being male; $P \leq 0.0001$ (Table 1). Non-Caucasian ethnicity was associated with high mortality risk once infected with SARS-CoV-2; $P \leq 0.001$ (Figure 4). The survival probability was worst in older patients from Middle Eastern ethnicity followed by Black, Asian and White ethnicities (Figure 5).

Clinical comorbidities and SARS-CoV-2 risk analysis

The overall prevalence of diabetes in our RTxR follow-up patients was 20%. Of the patients who acquired SARS-CoV-2 infection, 55% were diabetic (Cohort A) with 87.5% among deceased (Cohort A2); $P \leq 0.0.0001$ (Figure 6). This suggests diabetes is a major risk factor for SARS-CoV-2-related mortality in renal transplant patients. We further analysed survival probability depending on the recipient's age and diabetic status and found a direct correlation between old age, diabetes and mortality (Figure 7). The mean (SD) body mass index (BMI) kg/m^2 of the overall RTxR population was 26.60 (4.81). There were no significant differences recorded in BMI across the RTxR population (Figure 8). There was no impact of BMI on mortality. In our RTxR follow-up cohort hypertension and history of ischemic heart disease were also not independently significant risk factors for mortality; $P = 0.8221$ and $P = 0.7622$ respectively.

Common laboratory markers and SARS-CoV-2 risk analysis

We analysed some of the routinely monitored laboratory investigations to identify the severity of SARS-CoV-2 infection. We analysed the latest laboratory markers just prior to SARS-CoV-2 infection to avoid any impact of acute infection on these markers. We investigated the patient's renal allograft function recorded as an eGFR in mL/min/1.73 m^2). The mean (SD) and median (IQR) in cohort A1 and A2 were 47 (21) and 41 (41-56.5); and 25.75 (7.5) and 26.5 (26.5-30) respectively, $P = 0.0128$ (Figure 9). The poor functional quality of the renal allograft was directly related to a higher risk of mortality (Figure 10). The second marker we investigated was serum albumin level. The mean (SD) and median (IQR) of serum albumin in g/L of all RTxR patients was 36.81 (4.36) and 38 (35-39) which was within the normal range (Figure 11). On review, there was a significant difference between serum albumin levels of patients who recovered from SARS-CoV-2 infection as compared to those who died; $P \leq 0.0001$. There was also a significant relation noted between low albumin levels and a high risk of mortality, particularly when serum albumin was less than 20g/L $P \leq 0.001$. The mean (SD) and median (IQR) (Hb g/L) of overall RTxR were 126.9 (18.07) and 127 (115-140) compared to 106.4 (20.8) in group A and 101 (93-124) in group A2; $P \leq 0.001$ (Figure 12). On further comparison between patients who recovered (A1) with patients who died (A2), a significant difference was recorded; $P \leq 0.0001$. Looking at survival probability Hb less than 7 was associated with higher mortality. Finally, we looked at serum calcium levels across our RTxR. The mean (SD) and median (IQR) values of serum calcium in mmol/L of the overall RTxR cohort were 2.39 (0.14) and 2.41 (2.3-2.4). There was a significant difference in serum calcium recorded between all RTxR and SARS-CoV-2 positive; $P \leq 0.001$ and also between patients who recovered and those who died; $P = 0.0061$ (Figure 13). We found a significant correlation between low serum

Table 1 Demographic factors and risk of severe acute respiratory syndrome coronavirus 2 infection in renal transplant recipients, (%)

Demographic	Cohort A (*n* = 29), SARS-CoV-2 infection	Cohort B (*n* = 476), no infection	Significance
Age			
Mean (SD)	63.24 (12.57)	55.70 (13.63)	< 0.001
Median (IQR)	65 (56-71.5)	57 (46-66)	
Gender			
Male	14 (48.27)	276 (57.8)	0.3056
Female	15 (51.73)	200 (42.2)	
Ethnicity			
White	7 (24.13)	252 (52.94)	
Black	8 (27.58)	90 (18.9)	
Asian	13 (44.82)	126 (26.47)	< 0.001[a]
Others	1 (3.44)	8 (1.6)	
Cohort A1		Cohort A2	
(Recovered; *n* = 21)		(Died; *n* = 8)	
Age			
Mean (SD)	60.85 (12.5)	69.5 (9.5)	0.0986
Median (IQR)	64 (64-69.5)	68 (68-77)	
Gender			
Male	8 (38)	6 (75)	< 0.001
Female	13 (62)	2 (25)	
Ethnicity			
White	5 (23.8)	2 (25)	
Black	7 (33.33)	1 (12.5)	
Asian	9 (42.85)	4 (50)	< 0.001[b]
Others	0	1 (12.5)	

[a]Asian ethnicity is associated with a significant risk of severe acute respiratory syndrome coronavirus 2 infection.
[b]Non-Caucasian ethnicities are associated with a significant risk of severe acute respiratory syndrome coronavirus 2 mortality.

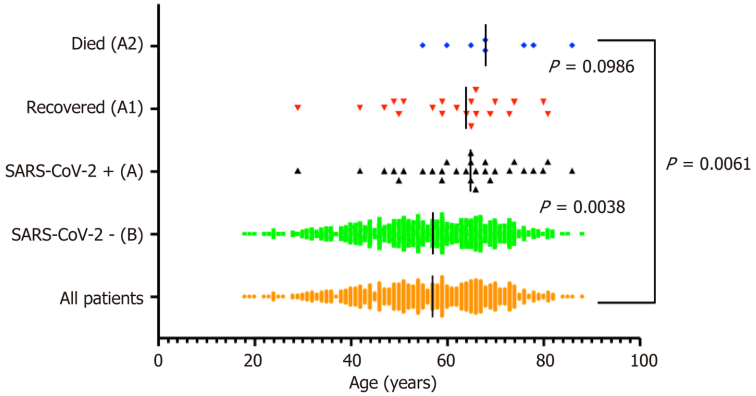

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Figure 1 Median age across various cohorts.

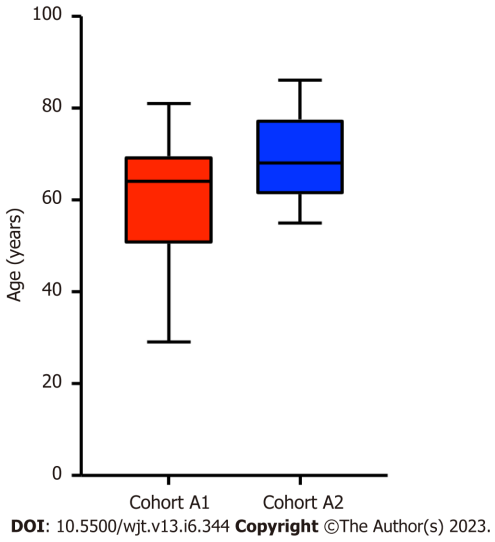

Figure 2 Intra-group age analysis A1 *vs* A2.

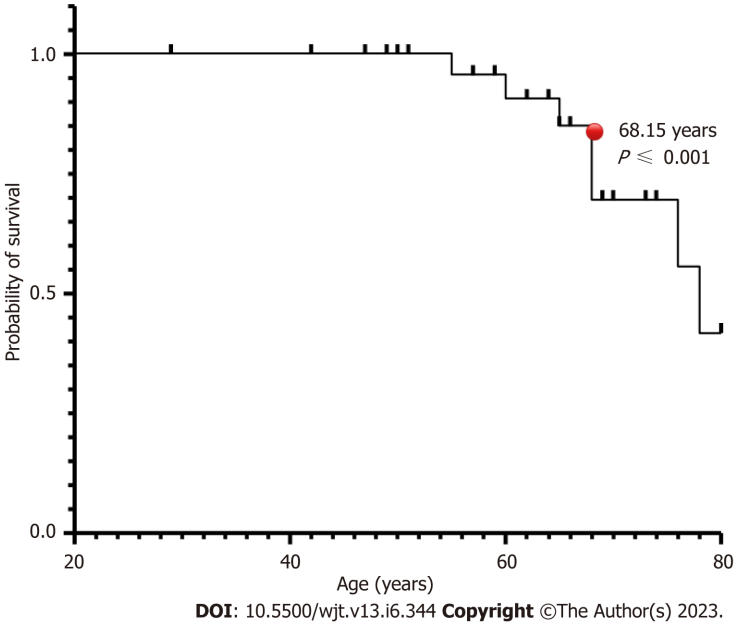

Figure 3 Age-related survival probability.

calcium levels and mortality once the level falls below 1.70 mmol/L < 0.001.

DISCUSSION

The recipients of solid organ transplantation are more vulnerable to opportunistic infections due to immunosuppressant medication. They demonstrate reduced resistance to infection, rapid progression of pathology, atypical clinical presentations and high risk of morbidity and mortality[26]. In addition to these factors, recovery from infection is dependent on various factors including the general well-being of the recipient and associated comorbidities. In renal transplant recipients, the functional status of the renal allograft also plays a vital part in the recovery phase, particularly when medication dosage is dependent on renal function. In addition to this, treatment regimens may be complicated by drug interactions and the need to maintain immunosuppression to prevent rejection. This complex interconnection between high-risk of infection, allograft function, limited treatment choices and associated comorbidities makes post-transplant infections the leading cause of morbidity and mortality[27]. During SARS-CoV-2 pandemic, the reported mortality among renal transplant recipients was as high as 33%[17]. In various general population studies, greater than 75 years of age, male gender and BMI greater than 40 are associated with significant mortality[28]. In our study, the mean (SD) age of recipient mortality was 69.5 (± 9.5) with a significantly increased risk of mortality after age 68 years and above. This

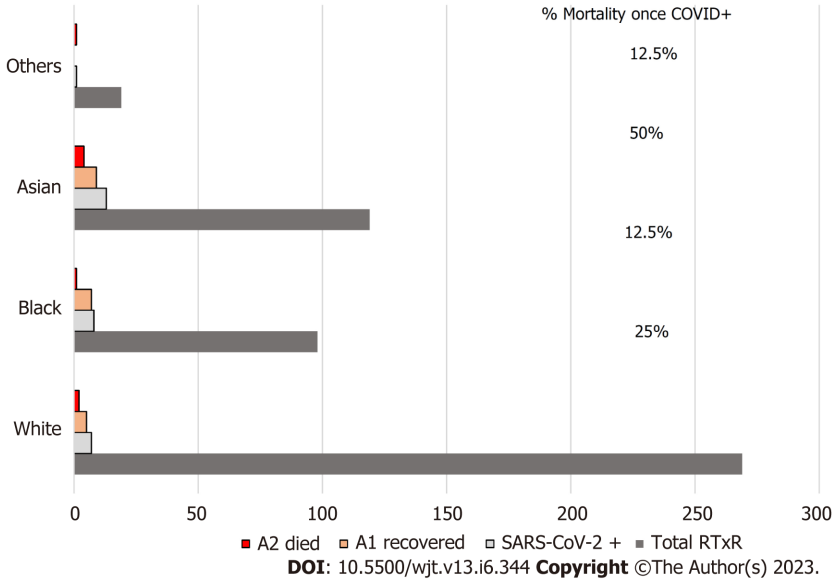

Figure 4 Ethnicity across various cohorts.

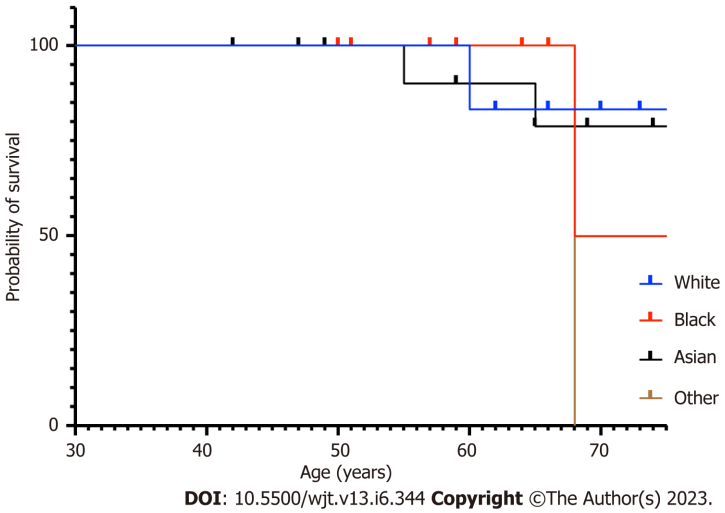

Figure 5 Survival probability with age and ethnicity.

finding is comparable to other studies in the renal transplant population[11,17,28] (Figure 14). These findings confirm that renal transplant recipients are at high risk of mortality at an earlier age when compared to the general population. We recorded a higher rate of mortality among male patients. This is also comparable with other published data[11,17,27,29]. Comparing BMI, there was no significant difference recorded in various groups in our study. The mean (SD) BMI in our mortality group was 27.20 (4.97) which is comparable to other published studies in the transplant population[11,17,29]. In general population studies, there was a high risk of mortality recorded in patients with a BMI greater than 40, but this BMI range is uncommon in renal transplant recipients. Surprisingly, we did not find any significant impact of hypertension and ischemic heart disease on mortality in our study population. This finding contrasts with general population studies[28,30,31] as well as other renal transplant studies[11,17,29] where there is a high risk of SARS-CoV-2-related mortality. The mean (SD) and median (IQR) systolic BP in our RTxR population were 130.8 (14.56) and 128 (120-139) with diastolic BP 79.22 (9.9) and 80 (71-87). This showed good BP control of our RTxR population. We had a higher number of patients with diabetes compared to other studies. This demonstrates that the demographics of renal transplant recipients vary widely across the globe and even within the United Kingdom. In contrast to other studies where acute inflammatory markers were studied in RTxR, we looked at routine laboratory parameters in predicting the outcome of RTxR after getting SARS-CoV-2 infection. We compared this to routine laboratory parameters when RTxR were free of SARS-CoV-2 infection. This helped to determine baseline parameters. There are limited studies of such parameters in the transplant population, so we compared our results with published data on the non-transplant population[14]. We noted that anaemia, hypercalcemia and hypoalbuminemia are associated with high-risk mortality in our study. These findings are similar to other published studies in non-transplant patients (Figure 15). We also noted that low GFR in RTxRs is also associated with high mortality risk. When we compared the impact of these parameters on mortality using multiple

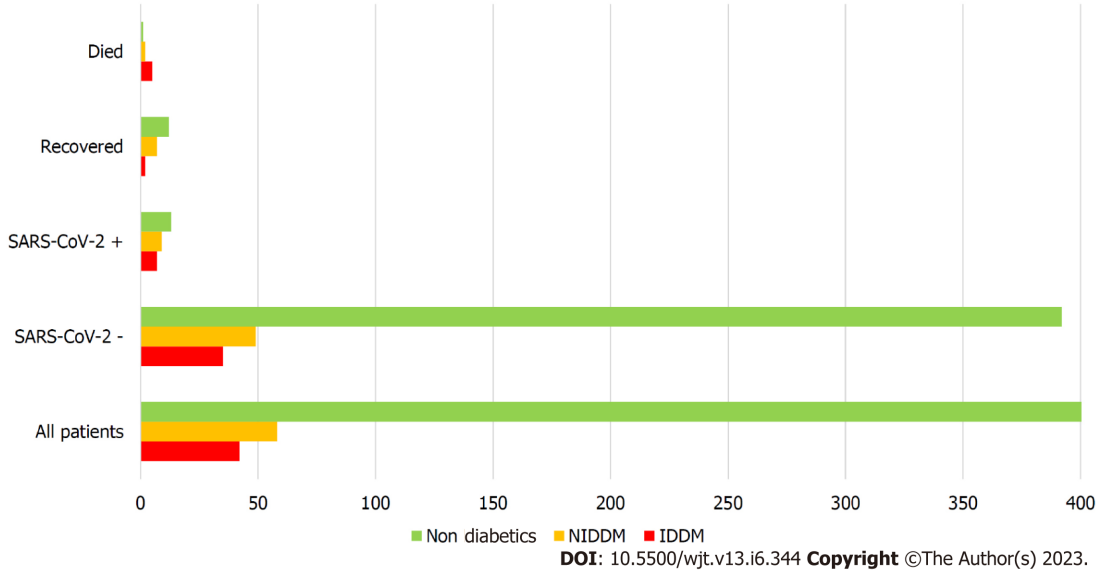

Figure 6 Diabetic status across various cohorts.

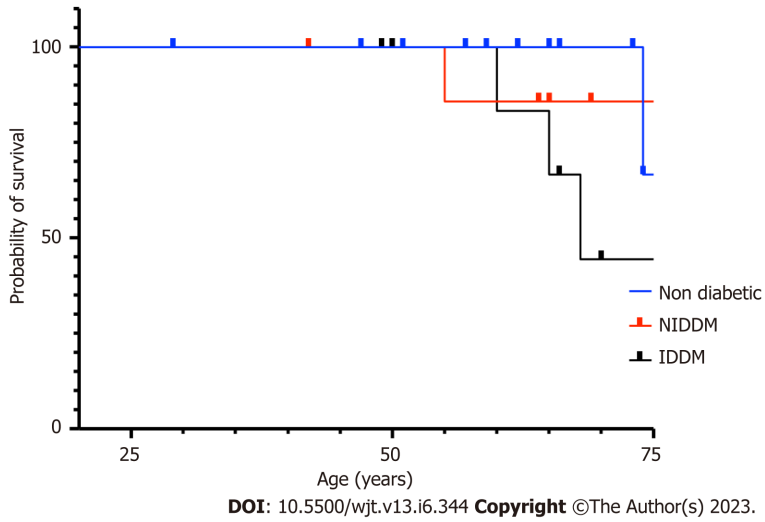

Figure 7 Survival probability with age and diabetic status.

logistic regression, we found a direct correlation (Figure 16). Identifying the impact of these parameters on mortality may be an important finding. The majority of these factors can be picked up on simple routine tests and do not require specialist investigations. Some of these factors are correctable with simple interventions. These can be addressed at an early stage during the RTxRs follow-up program with an aim to bring them to the normal range, where possible. This in return can significantly reduce morbidity and mortality in RTxRs.

CONCLUSION

It would be very easy and cost-effective to incorporate the findings of this study into any post-operative follow-up pathway and protocol for RTxRs. These simple parameters can help to risk stratify RTxRs into high and low-risk categories. In addition to this, despite having a failing renal transplant, early intervention to improve a patient's anaemia, hypercalcemia and hypoalbuminemia could reduce their risk of morbidity and mortality. Early identification of at-risk sub-groups within those already identified as being high-risk, can further reduce the risk of infection-associated mortality, with timely interventions.

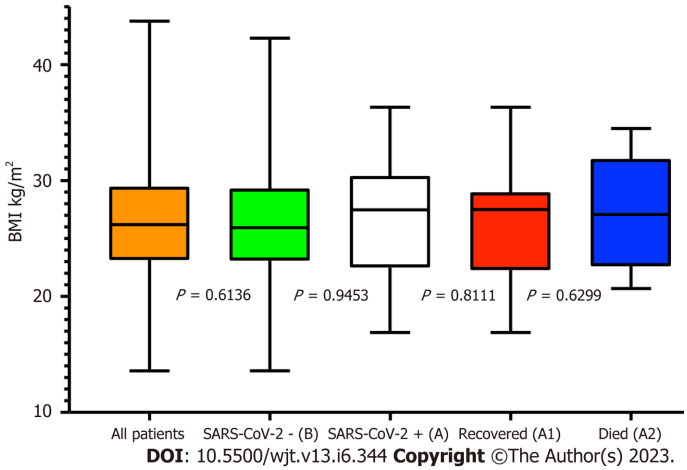

Figure 8 Body mass index range across various cohorts. BMI: Body mass index; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

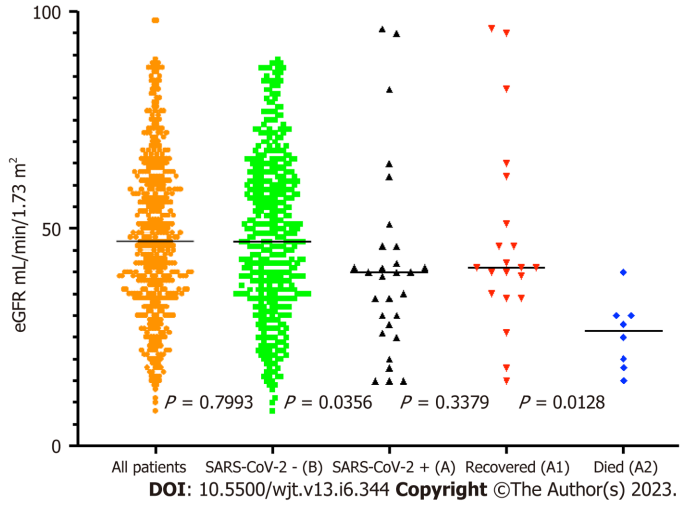

Figure 9 Median estimated glomerular filtration rate across various cohorts. eGFR: Estimated glomerular filtration rate; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

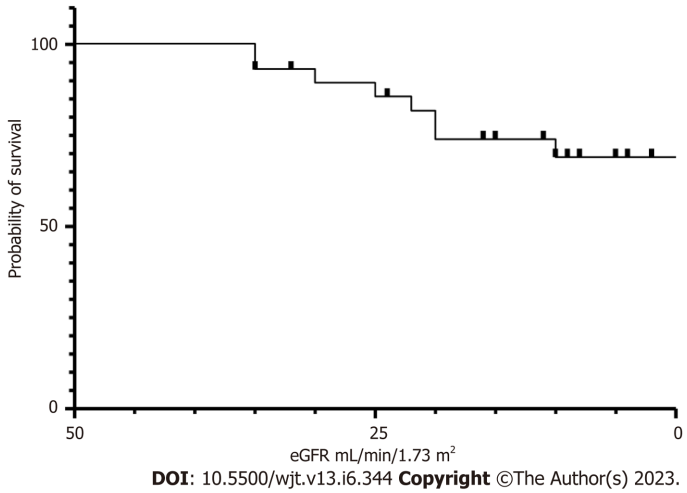

Figure 10 Survival probability dependent on graft estimated glomerular filtration rate. eGFR: Estimated glomerular filtration rate.

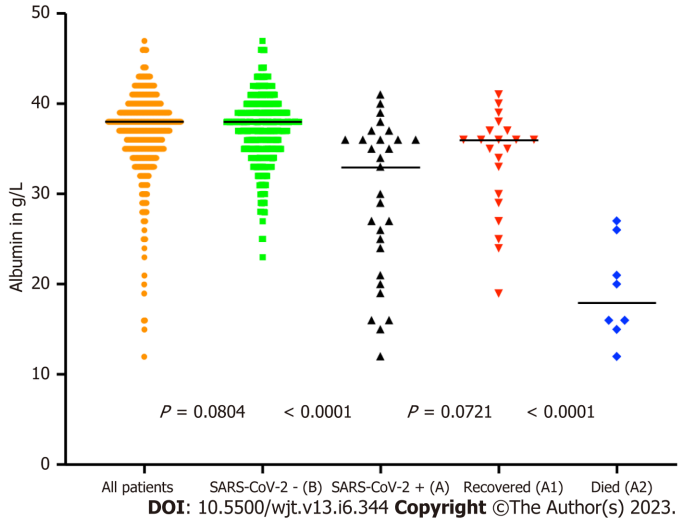

Figure 11 Albumin (g/L) across various cohorts. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

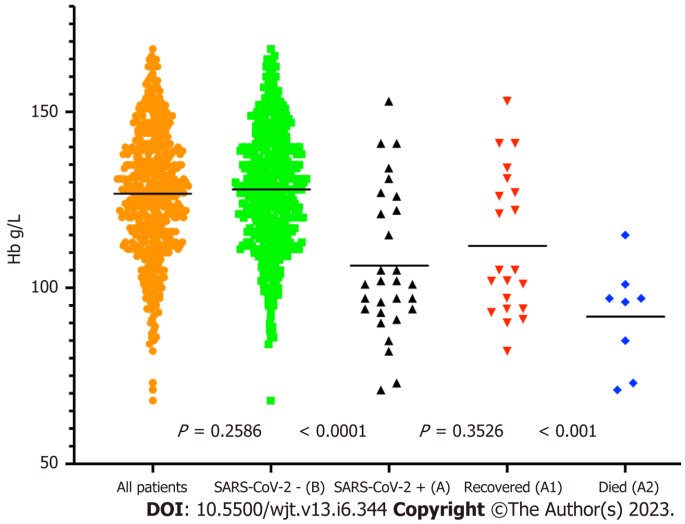

Figure 12 Hb distribution among various cohorts. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

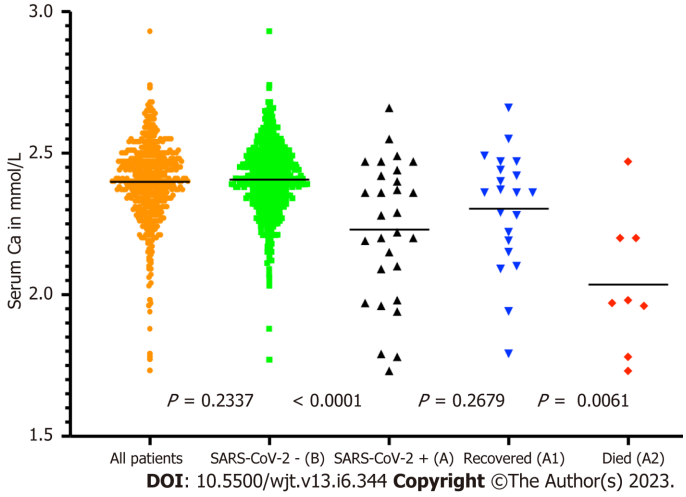

Figure 13 Serum Ca distribution across various cohorts. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

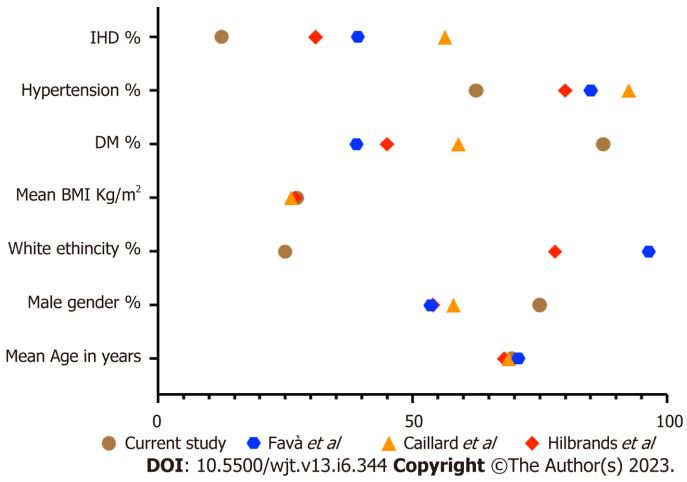

Figure 14 Comparison of current study demographics with published data. IHD: Ischemic heart disease; DM: Diabetes mellitus; BMI: Body mass index.

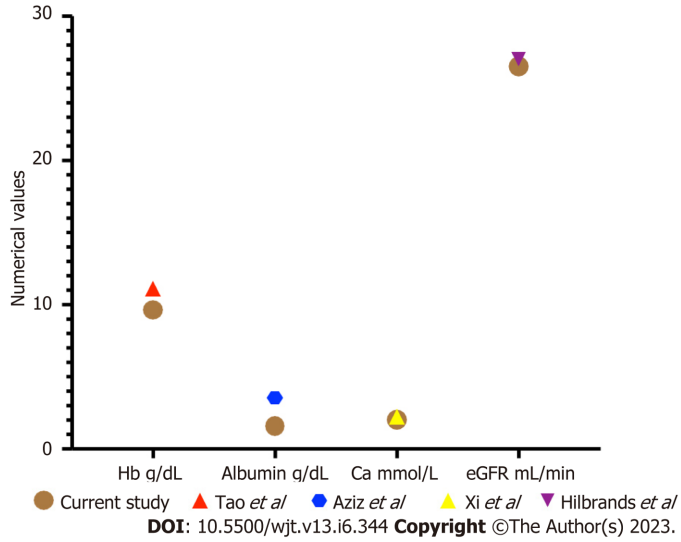

Figure 15 Comparison of current study routine lab parameters with published data. eGFR: Estimated glomerular filtration rate.

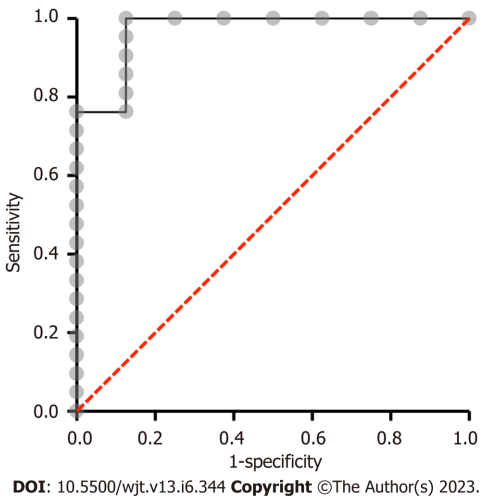

Figure 16 Multiple logistic regression: Mortality with Haemoglobin, Estimated glomerular filtration rate, Albumin and Ca.

ARTICLE HIGHLIGHTS

Research background

Various studies have been done to separately study routine laboratory markers to stratify patients with high risk of morbidity and mortality but very little is known in renal transplant patients.

Research motivation

This study provides a new way of looking at the significance of routine laboratory tests with an aim to risk stratify renal transplant recipients into high-risk sub-groups.

Research objectives

This study will help in shaping new policies and guidelines by providing individualized shielding advice, self-isolation guidance and booster coronavirus disease 2019 vaccination. Moreover, this will also help to plan better follow-up strategies for transplant patient. Addressing and correcting these parameters during a follow-up program can reduce the risk of morbidity and mortality in renal transplant recipients (RTxR).

Research methods

Retrospective observational study to analyze the data of our renal transplant follow-up program for various routine parameters and their impact of patient outcomes.

Research results

This study has identified some routinely used modifiable parameters in predicting a higher risk of mortality and morbidity.

Research conclusions

This knowledge can be used in RTxR follow-up programs by addressing these parameters early to help reduce the morbidity and mortality in RTxRs.

Research perspectives

This knowledge can be used in RTxR follow-up programs by addressing these parameters early to help reduce the morbidity and mortality in RTxRs.

FOOTNOTES

Author contributions: Ghazanfar A and Abbas M contributed to study design, data analysis, manuscript writing; Ghazanfar A and Hussain Md W contributed to data collection; Kayal M contributed to manuscript writing support.

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Informed consent statement: Online assessment confirmed that no patient consent or ethical approval from NHS HRA/REC was required.

Conflict-of-interest statement: Authors have no conflict of interest related to the content of this publication.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at email address a.ghazanfar@nhs.net.

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ORIGINAL ARTICLE

Observational Study

Outcomes of early hospital readmission after kidney transplantation: Perspectives from a Canadian transplant centre

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Abstract

BACKGROUND

Early hospital readmissions (EHRs) after kidney transplantation range in incidence from 18%-47% and are important and substantial healthcare quality indicators. EHR can adversely impact clinical outcomes such as graft function and patient mortality as well as healthcare costs. EHRs have been extensively studied in American healthcare systems, but these associations have not been explored within a Canadian setting. Due to significant differences in the delivery of healthcare and patient outcomes, results from American studies cannot be readily applicable to Canadian populations. A better understanding of EHR can facilitate improved discharge planning and long-term outpatient management post kidney transplant.

AIM

To explore the burden of EHR on kidney transplant recipients (KTRs) and the Canadian healthcare system in a large transplant centre.

METHODS

This single centre cohort study included 1564 KTRs recruited from January 1, 2009 to December 31, 2017, with a 1-year follow-up. We defined EHR as hospitalizations within 30 d or 90 d of transplant discharge, excluding elective procedures. Multivariable Cox and linear regression models were used to examine EHR, late hospital readmissions (defined as hospitalizations within 31-365 d for 30-d EHR and within 91-365 d for 90-d EHR), and outcomes including graft function and patient mortality.

RESULTS
In this study, 307 (22.4%) and 394 (29.6%) KTRs had 30-d and 90-d EHRs, respectively. Factors such as having previous cases of rejection, being transplanted in more recent years, having a longer duration of dialysis pretransplant, and having an expanded criteria donor were associated with EHR post-transplant. The cumulative probability of death censored graft failure, as well as total graft failure, was higher among the 90-d EHR group as compared to patients with no EHR. While multivariable models found no significant association between EHR and patient mortality, patients with EHR were at an increased risk of late hospital readmissions, poorer kidney function throughout the 1st year post-transplant, and higher hospital-based care costs within the 1st year of follow-up.

CONCLUSION
EHRs are associated with suboptimal outcomes after kidney transplant and increased financial burden on the healthcare system. The results warrant the need for effective strategies to reduce post-transplant EHR.

Key Words: Kidney; Transplantation; Early hospital readmissions; Incidence and trends; Post-transplant outcomes

Core Tip: Early hospital readmissions post-transplant are associated with suboptimal patient outcomes and increased financial burden on the healthcare system. The 90-d window for defining early hospital readmissions, in addition to the frequently used 30-d period, provides a novel opportunity to evaluate the risks for kidney transplant recipients.

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INTRODUCTION

Kidney transplantation is widely accepted as the best treatment option for the majority of patients with end-stage renal disease[1]; however, it carries a risk of complications and subsequent hospital readmissions in the post-transplant period [2]. Early hospital readmissions (EHRs), commonly defined as any new hospitalization occurring within 30 d after initial transplant discharge, is an indicator of healthcare quality and an important outcome measure after transplantation[2,3]. In the United States, approximately 30% of kidney transplant recipients (KTRs) have EHR, with rates ranging from 18% to 47% between transplant centres[4,5]. More recently, a single-centre Brazilian study reported an EHR incidence of 27% among 1175 KTRs from 2011 to 2012[3], while a population-based Canadian study reported a cumulative EHR incidence of 21% among 5437 KTRs from 2002 to 2014[2].

The relatively high incidence of post-transplant EHR is concerning since EHRs have been associated with a severe reduction in health status and substantial healthcare costs. Several kidney transplant studies observed an increased risk of graft failure, patient mortality, and suboptimal graft function with EHR[6-10]. EHRs were also associated with more late hospital readmissions (LHRs), defined as subsequent readmissions within the 1st year of transplantation after the EHR time frame. Furthermore, EHRs had a mean cost of approximately 10000 USD per KTR, which can create a significant burden on healthcare delivery systems[2].

Factors that interfere with post-transplant recovery and increase the risk of EHR include patient demographics (*e.g.*, older age, African American race), pre-existing comorbidities (*e.g.*, obesity, diabetes, heart disease, chronic obstructive pulmonary disease), transplant characteristics (*e.g.*, expanded criteria donor transplants, lack of induction therapy, longer initial hospital stay, surgical complications), and frailty, a measure of physiologic reserve in aging populations[7,8,11-13]. Alternatively, EHR could potentially reflect deficits in discharge planning and outpatient management, calling for improvements in transplant care practices[8].

While EHRs have been studied extensively in American transplant settings, there is a paucity of EHR data collected in Canadian transplant populations. One Canadian study recently examined secular trends in post-transplant EHR incidence; however, it did not report on the impact of these findings on patients and healthcare delivery systems[2]. Due to significant differences in the delivery of healthcare services and patient outcomes between American and Canadian transplant centres[2], results from American studies cannot be readily extrapolated to Canadian populations[2].

The objectives of our study were to examine the impact of EHR on graft outcomes, patient mortality, LHR, and hospital costs in a Canadian transplant setting. We also considered how the impact on outcomes would change with an expanded EHR definition that included hospitalizations within 90-d of transplant discharge. With this information, we hoped to generate knowledge that may be useful in developing strategies to reduce post-transplant EHR.

MATERIALS AND METHODS

Design and setting

We conducted a single-centre observational cohort study at the University Health Network (UHN) in Toronto, Ontario. Approval was obtained from the institutional Research Ethics Board.

Population and sample

We included all adult (age ≥ 18 years) KTRs who received a kidney transplant from January 1, 2009 to December 31, 2017 (with follow-up until December 31, 2018) at the Toronto General Hospital, UHN. KTRs were excluded if they: (1) Were multiorgan transplant recipients; (2) Were transplanted at another transplant facility; (3) Experienced primary graft non-function; or (4) Experienced graft loss, death, or had their last follow-up before the study origin (*i.e.* 30 d after discharge from their transplant hospitalization).

Data collection

Patient data was obtained from electronic hospital health records in the Organ Transplant Tracking Record and subsequently stored in the in-centre research database, the Comprehensive Renal Transplant Information System (CoReTRIS)[14]. CoReTRIS consists of recipient, donor, transplant, treatment, and follow-up data for all KTRs at UHN since January 2000 and has been audited for completeness and accuracy. All participants provided informed written consent for their health record information to be stored, collected, and used in CoReTRIS.

Exposure and outcome classification and assessment

The main exposure of interest was EHR, defined as any hospitalization occurring within 30 d after discharge from the transplant hospitalization. We also examined an extended window of 90 d after discharge. The hospitalization must have been documented, either as an electronic summary in the Organ Transplant Tracking Record or as a paper discharge summary faxed from a non-UHN hospital. Hospitalization data was captured by a team of research assistants using a systematic review of medical records. Any discrepancies during data collection were later validated and resolved by a trained clinician.

The primary clinical outcome of interest was the composite of graft failure or death with graft function. Graft failure and death with graft function were also examined separately as our secondary outcomes. The time of origin for the analyses was defined as either 30 d or 90 d after transplant discharge. Therefore, we excluded KTRs who experienced death or graft failure or were lost to follow-up prior to this time. Other clinical outcomes included: (1) Graft function, which was measured using estimated glomerular filtration rate, calculated using the Chronic Kidney Disease Epidemiology Collaboration equation, at 6-mo and 1-year post-transplant; and (2) LHR, defined as any hospitalization occurring between 31 and 365 d for 30-d EHR or 91 to 365 d for 90-d EHR. The financial outcome of interest was the average cost of hospital-based care (inpatient and outpatient) per KTR over the 1st year of follow-up. This included all billed patient expenditures at each department of all hospitals that are part of UHN. Inpatient and outpatient cost data were provided by the UHN Accounting Centre and evaluated using a single-centre perspective.

Potential confounders

To assess the independent association between the exposure and outcomes, covariates were chosen based on the literature and clinical experience. Recipient factors (*i.e.* age, sex, race, body mass index at time of transplant discharge, smoking history, diabetes mellitus, chronic lung disease, cardiovascular disease, baseline estimated glomerular filtration rate, and time on dialysis), donor factors (*i.e.* donor age, body mass index at time of donation, donation type, and expanded-criteria status), and transplant factors (*i.e.* peak panel reactive antibody, delayed graft function, acute rejection within 30 d of discharge, and transplant era) were considered in multivariable analyses.

Statistical analysis

Categorical variables were described using frequencies and percentages. Continuous variables were described using mean ± standard deviation if normally distributed and median [interquartile range (IQR)] if skewed. Baseline characteristics were compared between patients who experienced EHR and patients who did not experience EHR, using the χ^2 test for categorical variables, the Student *t*-test for normally distributed continuous variables, and Wilcoxon rank-sum test for skewed continuous variables. The Kaplan-Meier product limit method was used to assess time from 30 d post-discharge to graft failure, death, or the composite by EHR status. Multivariable Cox proportional hazard models were used to estimate the independent association of EHR with graft failure, mortality, and LHR. Linear regression models were used to estimate the association between EHR and graft function during 1-year of post-transplant follow-up. Multiple imputation by chained equations method was used to address the degree of absence of all outcome variables[15]. Two-tailed *P* values < 0.05 were considered statistically significant. Data management and analyses were performed using Stata/MP 12.0 (StataCorp, College Station, TX, United States). Statistical review of the study was performed by a biomedical statistician (Li Y from Toronto General Hospital).

RESULTS

A total of 1564 KTRs were eligible for inclusion in the study cohort. Application of the prespecified exclusion criteria

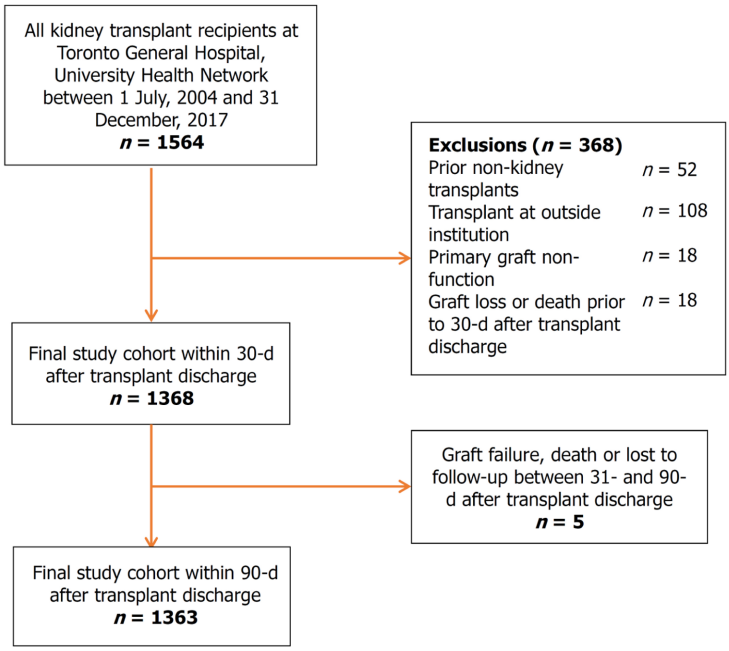

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Figure 1 Study flow diagram.

resulted in a final study cohort of 1368 KTRs for 30-d EHR analyses (Figure 1). A final study cohort of 1333 KTRs was used for the 90-d EHR analyses, as 5 KTRs experienced death or graft failure or were lost to follow-up between 31-d and 90-d post-transplant. For the 30-d EHR analysis, the median follow-up time was 5.11 years (IQR: 3.16, 7.59), with 329 cases of graft failure, 145 cases of death, and 439 cases of LHR in the 1st year starting from 30 d after transplant discharge. For the 90-d EHR analysis, the median follow-up time was 5.05 years (IQR: 3.12, 7.52), with 324 cases of graft failure, 140 cases of death, and 368 cases of LHR in the 1st year starting from 90 d after transplant discharge.

Baseline recipient, donor, and transplant characteristics of both study cohorts are summarized in Table 1. The 30-d EHR study population was 60.0% male and 46.7% White. The 90-d EHR study population was 60.2% male and 47.4% White. A total of 307 (22.4%) and 394 (29.6%) KTRs experienced 30-d and 90-d EHRs, respectively. KTRs who experienced an EHR were more likely to have a longer duration of dialysis, an expanded donor criteria donor, and a previous case of biopsy-proven acute rejection and received transplantation between 2015-2017. Particularly, KTRs with 90-d EHR were older, more likely to have a history of diabetes, and more likely to have a previous case of rejection, spent a longer time on dialysis before transplant, had older donors, and received transplantation between 2015-2017. Other characteristics were similar between the EHR and non-EHR groups.

The 30-d EHR group did not have a higher cumulative probability of death-censored graft failure as compared to non-EHR KTR (Figure 2A); in contrast, the 90-d EHR group did show a greater probability of death-censored graft failure (log rank $P = 0.01$, Figure 2B). The 30-d and 90-d EHR groups had greater cumulative probabilities of LHR within 1 year (Log rank $P < 0.001$, Figure 2C and D) compared to the non-EHR group. The 30-d EHR group did not have a higher probability of death *vs* the non-EHR group (Figure 2E), whereas the 90-d EHR group displayed a higher probability of death (log rank $P = 0.02$, Figure 2F). Neither EHR group had a higher probability of the composite outcome of graft failure or death *vs* the non-EHR group (Figure 2G and H).

The 30-d and 90-d EHRs were independent predictors of LHR hazard ratio (HR): 1.73; 95% confidence interval (CI): 1.40, 2.13 for 30-d EHR and HR: 1.58; 95%CI: 1.27 to 1.97 for 90-d EHR (Table 2). Neither 30-d nor 90-d EHRs were associated with the other outcomes of interest in the multivariable Cox models. In multivariable linear regression models (Table 3), 30-d and 90-d EHRs were associated with lower graft function at 3 mo (HR: -2.60; 95%CI: -4.90 to -0.30) and 12 mo (HR: -3.11; 95%CI: -5.62 to -0.60) for 30-d EHR and lower function at 3 mo (HR: -3.08; 95%CI: -5.17 to -0.99), 9 mo (HR: -2.81; 95%CI: -5.24 to -0.39), and 12 mo (HR: -3.77; 95%CI: -6.15 to -1.38) for 90-d EHR.

The mean cost of hospital-based care per KTR in the 1st year post-transplant is shown in Figure 3. In the first 3 mo, the mean cost of care for KTR with an EHR was nearly three times higher than for those without EHR (Figure 3A). After 3 mo, the mean cost of care for the EHR group declined to levels comparable to the non-EHR group, with an exception at month 7. Similarly, the mean number of readmissions for the EHR group decreased after the first 3 mo post-transplant, though the EHR group had more readmissions than the non-EHR group overall (Supplementary Figure 1). When the cost of hospital-based care was examined cumulatively, the mean post-transplant cost was consistently higher for the EHR group than the non-EHR group (Figure 3B).

Table 1 Baseline recipient, donor, and transplant characteristics by early hospital readmission status

Variables	EHR within 30 d after transplant discharge				EHR within 90 d after transplant discharge			
	Number of patients, *n* = 1368	Yes, *n* = 307	No, *n* = 1061	*P* value	Number of patients, *n* = 1333	Yes, *n* = 394	No, *n* = 939	*P* value
Recipient characteristics								
Recipient age at transplant in yr, mean (± SD)	1368	52.4 (13.4)	51.4 (13.6)	0.26	1333	52.7 (13.4)	51.1 (13.6)	0.05
Recipient sex								
Male	821	185 (60.3%)	636 (59.9%)	0.92	802	235 (59.6%)	567 (60.4%)	0.80
Female	547	122 (39.7%)	425 (40.1%)		531	159 (40.4%)	372 (39.6%)	
Recipient race								
White	639	134 (56.3%)	505 (59.1%)	0.44	632	176 (57.1%)	456 (59.1%)	0.56
Non-white	454	104 (43.7%)	350 (40.9%)		448	132 (42.9%)	316 (40.9%)	
History of smoking								
Smoker	584	137 (44.8%)	447 (42.3%)	0.42	569	174 (44.5%)	395 (42.1%)	0.42
Non-smoker	780	169 (55.2%)	611 (57.8%)		760	217 (55.5%)	543 (57.9%)	
History of diabetes mellitus	467	111 (36.2%)	356 (33.6%)	0.40	455	151 (38.3%)	304 (32.4%)	0.04
History of chronic lung disease	57	14 (4.6%)	43 (4.1%)	0.70	56	21 (5.3%)	35 (3.7%)	0.19
History of cardiovascular disease	377	82 (26.7%)	295 (27.8%)	0.70	368	118 (30.0%)	250 (26.7%)	0.22
Recipient body mass index at transplant discharge in kg/m^2, mean (± SD)	1074	27.2 (6.0)	26.9 (5.9)	0.49	1333	52.7 (13.4)	51.1 (13.6)	0.05
Recipient eGFR at baseline in mL/min, mean (± SD)	1085	62.7 (26.5)	63.0 (28.0)	0.88	1072	60.7 (27.0)	64.0 (27.7)	0.08
Time on dialysis in yr, mean (± IQR)	1368	3.4 (1.2, 5.6)	3.2 (1.2, 5.3)	0.46	1333	3.5 (1.6, 5.8)	3.1 (1.1, 5.3)	0.02
Donor characteristics								
Donor age at donation in yr, mean (± SD)	1359	48.9 (14.2)	47.3 (15.0)	0.10	1325	49.3 (14.4)	47.0 (14.9)	0.01
Donor body mass index in kg/m^2, (mean ± SD)	1339	26.9 (5.6)	26.7 (5.4)	0.56	1305	26.7 (5.4)	26.8 (5.4)	0.70
Type of donation								
Deceased	766	178 (58.0%)	588 (55.4%)	0.43	742	234 (59.4%)	508 (54.1%)	0.08
Living	602	129 (42.0%)	473 (44.6%)		591	160 (40.6%)	431 (45.9%)	
Expanded criteria donor	256	70 (22.8%)	186 (17.5%)	0.04	249	94 (23.9%)	155 (16.5%)	0.002
Transplant characteristics								
Peak PRA								
0%	709	161 (52.6%)	548 (51.7%)	0.78	686	211 (53.7%)	475 (50.6%)	0.31
> 0%	657	145	512		645	182	463	

		(47.4%)	(48.3%)			(46.3%)	(49.4%)	
Delayed graft function	276	57 (18.6%)	219 (20.6%)	0.43	265	79 (20.1%)	186 (19.8%)	0.92
Biopsy-proven acute rejection	85	46 (15.0%)	39 (3.7%)	< 0.001	98	59 (15.0%)	39 (4.2%)	< 0.001
Transplant era								
2009-2011	429	94 (30.6%)	335 (31.6%)	0.002	429	131 (33.3%)	298 (31.7%)	< 0.001
2012-2014	431	75 (24.4%)	356 (33.6%)		430	96 (24.4%)	334 (35.6%)	
2015-2017	508	138 (45.0%)	370 (34.9%)		474	167 (42.4%)	307 (32.7%)	

Mean and median values were reported with standard deviation and interquartile range, respectively. IQR: Interquartile range; eGFR: Estimated glomerular filtration rate; EHR: Early hospital readmissions; PRA: Panel reactive antibody; SD: Standard deviation.

Table 2 Association of early hospital readmission with graft failure or death, graft failure, death, and late hospital admissions (within 1 yr after the first 30 d or 90 d post-transplant discharge)

Outcome	30-d EHR			90-d EHR		
	Number of outcomes	Full model[1]		Number of outcomes	Full model[1]	
		HR (95%CI)	*P* value		HR (95%CI)	*P* value
Graft failure or death	237	1.06 (0.77-1.45)	0.73	232	1.21 (0.91-1.61)	0.19
Graft failure	92	1.05 (0.62-1.77)	0.86	92	1.45 (0.92-2.29)	0.11
Death	145	1.08 (0.73-1.60)	0.71	140	1.09 (0.75-1.57)	0.66
LHR	439	1.73 (1.40-2.13)	< 0.001	368	1.58 (1.27-1.97)	< 0.001

[1]This Cox proportional hazard model for the association of 30-d early hospital readmissions on outcomes included early hospital readmission status, recipient characteristics (age, sex, race, body mass index at transplant discharge, history of smoking, diabetes, chronic lung disease and cardiovascular disease, estimated glomerular filtration rate at baseline, and time on dialysis), donor characteristics (age, body mass index at time of donation, donation type, and expanded-criteria status), and transplant characteristics (peak panel reactive antibodies, delayed graft function, acute rejection within 30 d of discharge, and transplant era). CI: Confidence interval; EHRs: Early hospital readmissions; HR: Hazard ratio; LHRs: Late hospital readmissions.

Table 3 Association of early hospital readmission with estimated glomerular filtration rate at 6-mo and 12-mo after transplant discharge

Outcomes	30-d HER, Full model[1]		90-d HER, Full model[1]	
	Coefficient (95%CI)	*P* value	Coefficient (95%CI)	*P* value
eGFR at 6 mo after transplant discharge	-1.38 (-3.87 to 1.12)	0.28	-2.18 (-4.40 to -0.03)	0.054
eGFR at 12 mo after transplant discharge	-3.11 (-5.62 to -0.60)	0.02	-3.77 (-6.15 to -1.38)	0.002

[1]The linear regression model for the association of 30-d early hospital readmissions on estimated glomerular filtration rate included early hospital readmissions status, recipient characteristics (age, sex, race, body mass index at transplant discharge, history of smoking, diabetes, chronic lung disease and cardiovascular disease, estimated glomerular filtration rate at baseline, and time on dialysis), donor characteristics (age, body mass index at time of donation, donation type, and expanded-criteria status), and transplant characteristics (peak panel reactive antibody, delayed graft function, acute rejection within 30 d of discharge, and transplant era). CI: Confidence interval; eGFR: Estimated glomerular filtration rate; EHR: Early hospital readmissions.

DISCUSSION

In our patient cohort, the incidence of 30-d and 90-d EHRs was 22.4% and 29.5%, respectively. The 30-d EHR incidence was lower than those reported in the American studies by Luan *et al*[8] (36%) and McAdams-DeMarco *et al*[9] (31%). This may be related to differences in the study population as well as specific threshold and institutional criteria for admitting KTRs rather than outpatient care. Our results were comparable with the 30-d EHR incidence of 21% that was reported in a recent population-based Canadian study[2]. However, Naylor *et al*[2] found that 30-d EHR rates can vary even across different transplant centres within the province of Ontario, ranging from 16% to 27%.

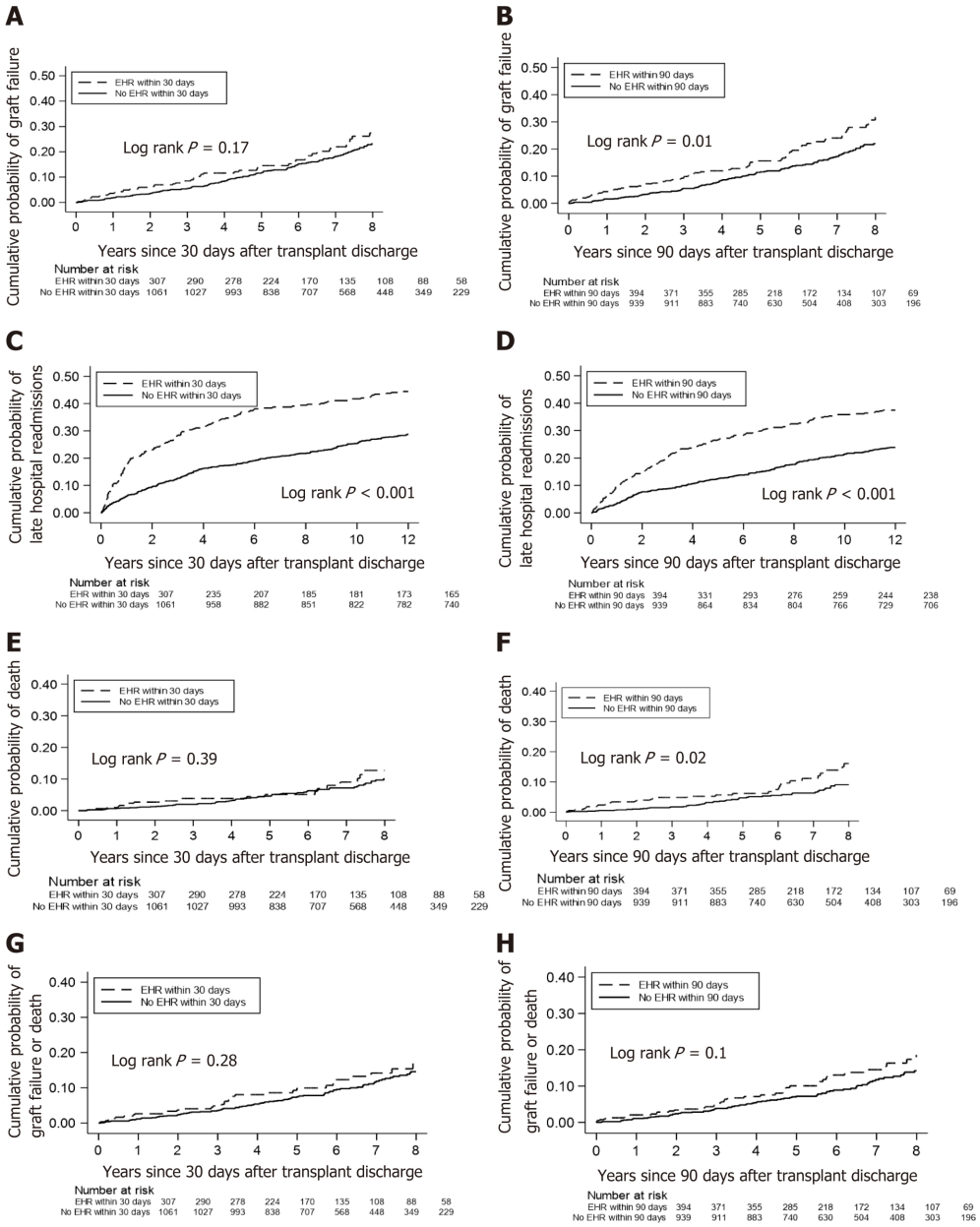

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Figure 2 Cumulative probability of clinical outcomes, separated by early hospital readmission status, over 5 years of follow up. A: Graft failure for 30-d early hospital readmission (EHR) group; B: Graft failure for 90-d EHR; C: Late hospital readmissions for 30-d EHR; D: Late hospital readmissions for 90-d EHR: E: Death for 30-d EHR; F: Death for 90-d EHR; G: Composite of graft failure and death for 30-d EHR; H: Composite of graft failure and death for 90-d EHR.

After accounting for potential confounders, 30-d and 90-d EHRs were shown to be an independent predictor of LHR at 1-year post-transplant and poorer graft function. Our results corroborated recent observational studies that associated EHR with negative clinical outcomes among KTRs and other high-risk patient populations[7,16-19,20]. More specifically, Luan *et al*[8] and McAdams-DeMarco *et al*[9] also demonstrated that EHRs are associated with a higher risk of LHR and graft failure in KTRs. However, contrary to these studies, we did not find a statistically significant association between EHR and patient mortality. Additionally, while most studies focused on the impact of 30-d EHR[9,21], we expanded our EHR definition and were able to demonstrate that hospitalizations occurring within the first 3 mo after transplantation discharge are also associated with rehospitalizations up to 1 year post-transplant.

The relationship between EHR and inferior patient outcomes can be explained in several ways. Post-transplant conditions or complications that necessitate EHR could directly result in clinical events like graft failure or frequent hospital readmissions[8]. Alternately, KTRs with EHR may already possess pre-existing medical comorbidities (*e.g.*, diabetes mellitus, cardiovascular disease) that increase the likelihood of adverse clinical events after transplantation. In our study, the patients in the EHR group were more likely to have an expanded criteria donor and a history of acute rejection, which have been previously linked with mortality, graft failure, and hospitalizations after kidney transplant[5,22]. EHRs are also associated with frailty, which is a marker of suboptimal transplant outcomes[12,23-26]. This factor may become increasingly important over time with an aging and subsequently a KTR population with more comorbidities[7].

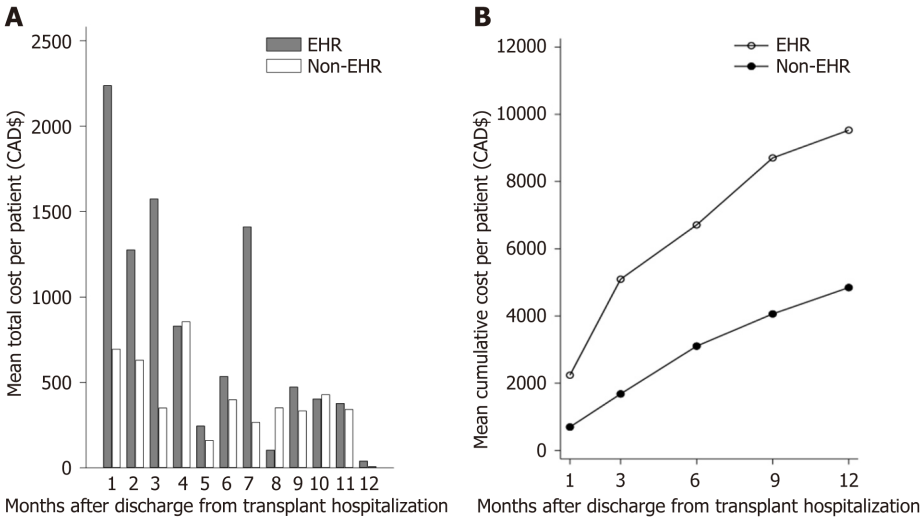

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Figure 3 Costs of hospital-based care per kidney transplant recipient over 1-year after transplant discharge. A: Mean cost of hospital-based care per kidney transplant recipient per month; B: Cumulative cost of hospital-based care over 1-year after transplant discharge. EHR: Early hospital admission.

Moreover, patients transplanted in the more recent era (2015-2017) were more likely to have EHRs as compared to earlier transplant years. The transplant program at our centre has been expanding its pool of patients among both recipients and donors in more recent years to include more medically complicated patients such as expanded criteria donors.

EHR not only affects patient outcomes but is detrimental from a financial perspective. We observed that, on average, twice as much money was spent on EHR patients as compared to non-EHR patients. Hospital readmissions increase the financial burden on the healthcare system, costing 1.8 billion CAD annually (11% of annual inpatient costs)[27]. Moreover, the average cost of a second hospitalization is often greater than the first[27], which is particularly relevant to our finding that EHR increase the risk of LHR. Our analysis only focused on costs at a single transplant centre, thus the financial consequence might have been more significant if expenditure at other tertiary care centres and community-based hospitals were also taken into consideration.

Due to the risks and costs associated with EHR, there is considerable interest in clinical monitoring and prevention of EHR. However, despite the growing evidence in the literature, there are no specific clinical practice guidelines to manage and monitor KTRs with EHR. After transplant discharge, KTRs at the UHN Kidney Transplant Program are followed weekly for the 1st month, biweekly from months 2 to 3, monthly from months 4 to 6, bimonthly from months 7 to 12, every 3 to 4 mo from 13 to 24 mo, and then every 6 to 12 mo beyond 24 mo[28]. Like many other centres, a number of KTRs with stable kidney function from the UHN program are transferred from the hospital-based transplant unit to community-based general nephrology centres within the 1st year post-transplant. Thus, although there are standard practices in place for KTR management in general, there are no standardized strategies that are tailored specifically for those KTRs at risk of EHR.

KTRs who are at increased risk of EHR may benefit from multifaceted interventions that include: (1) Better educational strategies to improve medication knowledge and support capacity for self-care; (2) Collaborative care provided by transplant and general nephrologists; and (3) More frequent follow-up visits for an extended period of time[2,9,29]. Further investigation of these interventions would be required to determine the feasibility and efficiency of reducing EHR in KTRs. Previous studies have suggested that up to half of hospital readmissions for KTRs are preventable and can be reduced by early intervention[30]. Exploring the characteristics of KTRs with preventable EHR can inform the development and evaluation of prediction tools, which will aid clinicians in identifying high-risk patients[31].

With this study, we were able to extend the previous work on EHR and long-term outcomes of KTRs to a Canadian healthcare context. Our methodology involved a standardized and comprehensive collection of patient and hospitalization data for a relatively large study population of over 1000 KTRs[14]. Moreover, we benefited from exploring the use of 90-d EHR (in addition to the previously used 30-d EHR definition) for the assessment of outcomes. Nevertheless, some limitations to our study also warrant discussion. First, the generalizability of our findings may be limited by the single-centre study design. Second, this study was based on observational data. Therefore, we cannot confirm that changes in EHR would improve long-term outcomes. Third, non-UHN readmissions may have been missed since we relied on UHN clinical notes to determine readmissions within the 1st year. However, it is unlikely that many events were missed since patients are instructed to contact the transplant centre if they are admitted to any facilities outside of UHN, and hospitalization events are checked with each patient at every clinic visit. Finally, while patient and hospitalization data could be verified with patient charts and electronic records, our cost data was solely obtained from the UHN financial services and was difficult to verify independently. However, these cost data are used for hospital planning and budgeting and were sufficient for their intended purpose in our study.

CONCLUSION

In summary, EHR after kidney transplantation was associated with a greater risk of LHR at 1-year post-transplant, suboptimal kidney function, and higher hospital-based care costs. The 90-d window after discharge from transplant hospitalization, in addition to the frequently used 30-d post-transplant period, marks a novel opportunity to evaluate the risks for KTRs. Further studies are required to determine which EHRs are preventable and implement reliable tools that can reduce EHR after kidney transplantation.

ARTICLE HIGHLIGHTS

Research background

Early hospital readmissions (EHRs) post-kidney transplantation adversely impact clinical outcomes such as graft function and patient mortality as well as healthcare costs. A better understanding of EHR can facilitate improved discharge planning and long-term outpatient management post kidney transplant.

Research motivation

Associations between EHR and suboptimal clinical outcomes post kidney transplant have not been extensively studied in a Canadian healthcare setting. We sought to explore the burden of EHR on kidney transplant recipients (KTRs) and the Canadian healthcare system in a large transplant centre.

Research objectives

The objectives of our study were to examine the impact of EHR on graft outcomes, patient mortality, late hospital readmissions (LHRs), and hospital costs in a Canadian transplant setting.

Research methods

This was a single centre cohort study of 1564 KTRs transplanted between 2009-2017. Analyses were separated by patients with no EHRs, patients with EHRs within 30 d of transplant, and those with EHRs within 90 d of transplant. Multivariable Cox and linear regression models were used to examine EHR, LHR, and outcomes including graft function and patient mortality.

Research results

EHRs post kidney transplant were associated with subsequent LHRs, suboptimal kidney function, and a higher burden on the healthcare system.

Research conclusions

EHRs post kidney transplant are associated with suboptimal patient outcomes and higher burdens on the healthcare system. Expanding the window of readmissions to 90 d post-transplant revealed an important target for reducing the risk of suboptimal outcomes.

Research perspectives

A better understanding of EHR can contribute to the development of prediction tools to identify those KTRs at risk of EHR and thus a standardized approach to manage and target these patients.

FOOTNOTES

Author contributions: Famure O and Kim SJ formulated the research question; Famure O, Kim ED, Huang J, Zyla R, Au M, Chen PX, Sultan H, Ashwin M, and Kim SJ designed the study; Kim ED, Huang J, Zyla R, Au M, Chen PX, Sultan H, Ashwin M, and Minkovich M performed the research; Li Y analysed the data; Famure O, Kim ED, Li Y, Huang J, Zyla R, Au M, Chen PX, Sultan H, Ashwin M, Minkovich M, and Kim SJ interpreted the data; Famure O, Kim ED, Huang J, Zyla R, Au M, Chen PX, Sultan H, Ashwin M, Minkovich M, and Kim SJ wrote the paper.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at michelleminkovich@gmail.com. Consent was not obtained but the presented data are anonymized and risk of identification is low.

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ORIGINAL ARTICLE

Prospective Study

Dosing strategies for *de novo* once-daily extended release tacrolimus in kidney transplant recipients based on *CYP3A5* genotype

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Abstract

BACKGROUND

Tacrolimus extended-release tablets have been Food and Drug Administration-approved for use in the *de novo* kidney transplant population. Dosing requirements often vary for tacrolimus based on several factors including variation in metabolism based on *CYP3A5* expression. Patients who express *CYP3A5* often require higher dosing of immediate-release tacrolimus, but this has not been established for tacrolimus extended-release tablets in the *de novo* setting.

AIM

To obtain target trough concentrations of extended-release tacrolimus in *de novo* kidney transplant recipients according to *CYP3A5* genotype.

METHODS

Single-arm, prospective, single-center, open-label, observational study (Clinical-

Trials.gov: NCT037-13645). Life cycle pharma tacrolimus (LCPT) orally once daily at a starting dose of 0.13 mg/kg/day based on actual body weight. If weight is more than 120% of ideal body weight, an adjusted body weight was used. LCPT dose was adjusted to maintain tacrolimus trough concentrations of 8-10 ng/mL. Pharmacogenetic analysis of *CYP3A5* genotype was performed at study conclusion.

RESULTS

Mean time to therapeutic tacrolimus trough concentration was longer in *CYP3A5* intermediate and extensive metabolizers *vs* *CYP3A5* non-expressers (6 d *vs* 13.5 d *vs* 4.5 d; $P = 0.025$). Mean tacrolimus doses and weight-based doses to achieve therapeutic concentration were higher in *CYP3A5* intermediate and extensive metabolizers *vs* *CYP3A5* non-expressers (16 mg *vs* 16 mg *vs* 12 mg; $P = 0.010$) (0.20 mg/kg *vs* 0.19 mg/kg *vs* 0.13 mg/kg; $P = 0.018$). *CYP3A5* extensive metabolizers experienced lower mean tacrolimus trough concentrations throughout the study period compared to *CYP3A5* intermediate metabolizers and non-expressers (7.98 ng/mL *vs* 9.18 ng/mL *vs* 10.78 ng/mL; $P = 0$ 0.008). No differences were identified with regards to kidney graft function at 30-d post-transplant. Serious adverse events were reported for 13 (36%) patients.

CONCLUSION

Expression of *CYP3A5* leads to higher starting doses and incremental dosage titration of extended-release tacrolimus to achieve target trough concentrations. We suggest a higher starting dose of 0.2 mg/kg/d for *CYP3A5* expressers.

Key Words: Immunosuppression; Kidney transplant; Dosing; Tacrolimus; Therapeutic drug monitoring; Genotype

Core Tip: In this single-arm, prospective, observational study we study once-daily, extended release tacrolimus dosing. Here we find that the expression of the cyctrochrome P450 enzyme, *CYP3A5*, is an important clinical factor to determine optimal dosage requirements after kidney transplantation. In kidney transplant recipients who express *CYP3A5* activity, higher doses of extended-release tacrolimus are required to attain therapeutic trough concentrations. Delays in achieving therapeutic trough concentrations has been linked to increase rates of acute rejection which highlights the importance of this research in identifying dosing considerations for extended-release tacrolimus in the *de novo* kidney transplant setting.

Citation: Diamond A, Karhadkar S, Chavin K, Constantinescu S, Lau KN, Perez-Leal O, Mohrien K, Sifontis N, Di Carlo A. Dosing strategies for *de novo* once-daily extended release tacrolimus in kidney transplant recipients based on *CYP3A5* genotype. *World J Transplant* 2023; 13(6): 368-378

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INTRODUCTION

Outcomes after kidney transplantation have been significantly improved with advances in immunosuppressive therapies. Tacrolimus is currently marketed in various formulations that have proven to be highly effective in preventing acute rejection after kidney transplantation[1-3]. Compared to immediate release tacrolimus, once daily extended-release formulations have demonstrated similar efficacy and safety in the *de novo* kidney transplant setting leading to increased utilization[1,2,4,5]. Life cycle pharma tacrolimus (LCPT) (Envarsus®; Veloxis Pharmaceuticals) was designed to enhance the bioavailability of tacrolimus[6]. In published studies, utilization of LCPT has been shown to provide rapid achievement of target trough concentrations following kidney transplantation[1,2,7]. In addition, a once daily LCPT dosing regimen results in lower peak concentrations with equivalent overall exposure compared to immediate-release and other extended-release tacrolimus formulations[3]. Similar efficacy and safety profiles have been demonstrated when comparing LCPT to other available tacrolimus formulations[1-3,7,8].

The use of LCPT has been Food and Drug Administration (FDA)-approved for the prophylaxis of organ rejection in *de novo* kidney transplant patients and in kidney transplant patients converted from tacrolimus immediate-release formulations[6]. The recommended FDA-approved dosing for *de novo* kidney transplant recipients is 0.14 mg/kg/d, however various starting doses have been evaluated in clinical trials[1,2,6]. Some kidney transplant recipients are known to metabolize tacrolimus at a higher or lower rate due to the presence of genetic polymorphisms that affect its metabolism [9]. The metabolism of LCPT occurs primarily within the cytochrome P450 (CYP) system, of which approximately 55 different genes have been identified in the human genome[10]. A multitude of CYP enzymes exist, including *CYP3A5* which is known to be an integral component of tacrolimus metabolism. In addition, genetic variation affecting *CYP3A5* function is known to impact overall tacrolimus exposure as well as dosing requirements to attain therapeutic concentrations[11,12]. The most common genetic variants (*CYP3A5**3* and *CYP3A5**6*) of *CYP3A5* in the general population

produce non-functional versions of the enzyme[13,14]. On the other hand, the presence of at least one *CYP3A5*1* allele would confer activity to *CYP3A5* (commonly known as an expresser) which has been shown to lead to higher dosage requirements of tacrolimus to attain therapeutic concentrations[9]. Previous data has illustrated that *CYP3A5* expressers can require up to 2-fold higher tacrolimus doses to achieve similar trough concentrations compared to *CYP3A5* non-expressers[15]. *CYP3A5* genetic variation may also lead to delays in time to achievement of target trough concentrations, which has been linked to higher rates of acute rejection. Furthermore, knowledge of *CYP3A5* genetic variants in transplant patients may lead to prevention of subtherapeutic and supratherapeutic concentrations in the early post-transplant period potentially lowering the risks of acute rejection and drug toxicities[16,17].

The primary objective of this study was to identify the time to therapeutic trough concentration of *de novo* once-daily LCPT in kidney transplant recipients according to *CYP3A5* expresser status. Secondary objectives include the description of the distribution of common *CYP3A5* variants in our population and to identify the dose required (total and weight-based) to obtain target trough concentrations according to expresser status.

MATERIALS AND METHODS

Study design and patient population

We conducted a single-arm, prospective, open-label, single-center, observational study (ClinicalTrials.gov: NCT03-713645). Adult *de novo* recipients of a living or deceased donor kidney transplant capable of providing consent from November 15, 2018 to April 23, 2021 were consented for inclusion in the study. Patients who were scheduled for multiple organ transplants at enrollment, non-English speaking, pregnant, or diagnosed with moderate to severe hepatic impairment (Child Pugh > 10 or bilirubin > 2 mg/dL) were excluded from the study. In addition, patients who had existing contraindications to tacrolimus-based products including hypersensitivity to tacrolimus or any other component of the formulation or who were receiving concomitant medications known to have strong drug-drug interaction potential with tacrolimus were excluded from the study. The post-transplant observation period was 30 d.

Intervention

All patients received LCPT tablets orally once daily at a starting dose of 0.13 mg/kg/day based on actual body weight. If a patient weighed more than 120% of their ideal body weight, an adjusted body weight was calculated for initial drug dosing[18]. All doses were rounded to the nearest 1 mg increment and adjusted to maintain a tacrolimus trough concentration of 8-10 ng/mL for the first 30 d after kidney transplant. No dose adjustments were performed during the first 48 h after the initial dose or subsequent dose adjustments to allow steady state concentrations to be achieved. All patients received additional immunosuppression with antithymocyte globulin (Thymoglobulin®; Sanofi Pharmaceuticals) or basiliximab (Simulect®; Novartis Pharmaceuticals) induction and mycophenolate sodium 720 mg by mouth every 12 h. Antithymocyte globulin dosing ranged between 4-6 mg/kg based on immunologic risk and was dosed by actual body weight unless the patient was greater than 120% of their ideal body weight, for which an adjusted body weight was utilized. Adjustments to mycophenolate sodium dosing was at the discretion of the treating physician, based on adverse effects, lab abnormalities, and other clinical considerations. All patients received daily pulse-dose methylprednisolone for 5 d according to institutional protocol. Prednisone maintenance immunosuppression was utilized in some recipients based on immunologic risk and the presence of an automimmune kidney disease at the time of kidney transplant. Patients requiring prednisone received a maintenance dose of prednisone 5-10 mg by mouth daily.

CYP3A5 genotype sample collection and analysis

Two buccal swab samples were collected from each patient using DNA/RNA Shield™ collection tubes (Zymo Research Corporation). The samples were stored frozen at -20 °C until obtaining samples from all patients included in the study. The DNA extraction was performed with NucleoMag® DNA Swab extraction kit (Takara Bio Inc.) following the manufacturer's recommendations. A Tecan Spark Plate Reader was used to determine the DNA concentration using the NanoQuant Plate™. The DNA concentration was normalized at 5 ng/L for all the samples with molecular biology grade water for real-time PCR analysis.

We processed all the patient's DNA samples on the same day for DNA genotyping. We performed DNA single nucleotide polymorphism (SNP) analysis of these CYP3A5 variants: *CYP3A5*3* (rs776746), *CYP3A5*6* (rs10264272), and *CYP3A5*7* (rs41303343). We used three TaqMan™ probes for variant detection *via* real-time PCR (Catalog ID 4362691, 4362691, 4362691) following the manufacturer's recommendations (Thermo Fisher Scientific). As positive controls, we used nine commercially available DNA samples containing all possible combinations of the *CYP3A5* variants analyzed in this study. The positive control samples were obtained from the Coriell Institute with the following catalog numbers: *CYP3A5 *1*1* (HG01190), **1*3* (NA07000), **1*6* (NA19226) **1*7* (NA19035), **3*3* (NA17660), **3*6* (NA18855), **3*7* (NA19207), **6*7* (NA19143), **7*7* (NA19920). The real-time PCR assay was performed at the Genetics Core Facility of the University of Arizona. The variant detection data analysis was done single-blinded to corroborate the correct identification of the control samples' genotypes. Upon receipt of *CYP3A5* genotype results, patients were then classified by *CYP3A5* phenotype as a *CYP3A5* non-expresser (individual carries two non-functional alleles), *CYP3A5* intermediate metabolizer (individual carrying one functional allele and one non-functional allele), or *CYP3A5* extensive metabolizer (individual carrying two functional alleles)[19].

Clinical and safety endpoints

The primary efficacy endpoint was the time to therapeutic tacrolimus trough concentration during the first 30 d after kidney transplantation. Therapeutic tacrolimus trough concentration was defined as tacrolimus trough concentration 8 ng/mL. Secondary efficacy endpoints included the tacrolimus dose and weight-based tacrolimus dose required to achieve an initial therapeutic trough concentration. Safety outcomes measured included incidence of hyperkalemia (serum potassium > 5.5 mEq/L) and incidence of tremor. Tremor was assessed utilizing the quality of life in essential tremor (QUEST) questionnaire and was completed at 30 d post-kidney transplant[20]. Incidence of serious adverse events (SAEs) and drug discontinuation due to adverse events (AEs) were also reported.

Statistical methods and analysis

Descriptive statistics were used to characterize the baseline demographics of the entire cohort (intent to treat population). Continuous parametric data are presented as mean ± SD while continuous non-parametric data are presented as median (25%-75% interquartile range). Analysis of outcomes according to *CYP3A5* expresser status within the modified intent-to-treat (ITT) population were completed using the Kruskal-Wallis or ANOVA test for continuous data and the chi-squared test for categorical data. Tests were corrected for multiple comparisons as necessary utilizing the Bonferroni method. All tests were two-tailed, and $P < 0.05$ was used to represent statistical significance. Time to therapeutic tacrolimus trough concentration was analyzed using a Kaplan-Meier time-to-event analysis. All analyses were performed using SPSS, version 26 for windows (Armonk, NY; IBM Inc.).

RESULTS

A total of 36 patients (ITT population) were enrolled and 35 patients completed the entire 30-d treatment period. One patient withdrew prior to the end of the study time period due to neurologic toxicity and tremors. Patients who were able to complete genotype testing were included in the final analysis [$n = 34$; modified ITT (mITT) population]. All 34 patients were included in the mITT analysis and patients were stratified based on *CYP3A5* phenotype. Of the 34 total patients, 15 (44.1%) were found to be non-expressers of *CYP3A5*, while 13 (38.2%) and 6 (17.6%) were found to be intermediate and extensive metabolizers, respectively. The population was predominantly black (66.7%), male (55.6%), and recipients of a deceased donor kidney transplant (69.4%) with a mean age of 55.5 years (Table 1). Baseline characteristics were similar between groups except for a higher percentage of black patients (92.3% *vs* 83.3% *vs* 46.7%; $P = 0.026$) in the CYP3A5 intermediate and extensive metabolizer groups compared to *CYP3A5* non-expressers (Table 2).

Mean time to therapeutic tacrolimus trough concentration was longer in *CYP3A5* intermediate and extensive metabolizers compared to *CYP3A5* non-expressers ($P = 0.025$). A Kaplan Meier analysis demonstrated that the highest incidence of patients not achieving therapeutic tacrolimus trough concentration by 7 d post-transplant were *CYP3A5* extensive metabolizers followed by *CYP3A5* intermediate metabolizers. Only 13.3% of *CYP3A5* non-expressers failed to achieve a therapeutic tacrolimus trough by 7 d post-transplant compared to approximately 30.8% of *CYP3A5* intermediate metabolizers and 83.3% of *CYP3A5* extensive metabolizers (Figure 1). Mean tacrolimus doses to achieve therapeutic concentration were higher in *CYP3A5* intermediate and extensive metabolizers compared to *CYP3A5* non-expressers (16 mg *vs* 16 mg *vs* 12 mg; $P = 0.010$). Mean weight-based tacrolimus doses to achieve therapeutic tacrolimus trough concentrations were also higher in *CYP3A5* intermediate and extensive metabolizers compared to *CYP3A5* non-expressers (0.20 mg/kg *vs* 0.19 mg/kg *vs* 0.13 mg/kg; $P = 0.018$) (Table 3).

Mean daily tacrolimus dose, daily weight-based tacrolimus dose, and tacrolimus trough concentrations throughout the 30-d study period were compared amongst the three groups. A higher mean daily tacrolimus dose was seen in *CYP3A5* intermediate and extensive metabolizers compared to poor metabolizers (12.5 mg *vs* 13.8 mg *vs* 9.6 mg; $P = 0.011$). While not statistically significant, a higher daily weight-based tacrolimus dose was seen in *CYP3A5* intermediate and extensive metabolizers compared to *CYP3A5* non-expressers (0.136 mg/kg *vs* 0.176 mg/kg *vs* 0.128 mg/kg; $P = 0.074$). *CYP3A5* extensive metabolizers experienced lower mean tacrolimus trough concentrations throughout the study period compared to *CYP3A5* intermediate metabolizers and non-expressers (7.98 ng/mL *vs* 9.18 ng/mL *vs* 10.78 ng/mL; $P = 0.008$). No statistically significant differences in kidney graft function at 30-d post-transplant were observed between *CYP3A5* extensive metabolizers, intermediate metabolizers, and non-expressers measured by mean serum creatinine (1.94 mg/dL *vs* 1.76 mg/dL *vs* 1.76 mg/dL; $P = 0.906$) and mean estimated glomerular filtration rate (31.5 mL/min/1.73 m^2 *vs* 46 mL/min/1.73 m^2 *vs* 40 mL/min/1.73 m^2; $P = 0.701$) (Table 3).

Safety endpoints were evaluated as part of the ITT analysis. SAEs were reported for 13 (36%) patients with 1 SAE (2.8%) attributed to study drug. The one patient who experienced a SAE attributed to study drug resulted in neurotoxicity which led to study drug discontinuation. Assessment of tremor using the QUEST questionnaire revealed that the majority of patients experienced no significant impact of tremor on their quality of life. A further description of patient responses to the QUEST questionnaire are summarized in Figure 2. A total of 11 (31%) patients enrolled experienced at least one potassium value above 5.5 mEq/L. Mean potassium values did differ throughout the 30-d study period between extensive metabolizer, intermediate metabolizer, and non-expresser groups, but were not clinically significant (4.35 *vs* 4.68 *vs* 4.29; $P = 0.041$).

Table 1 Baseline Characteristics (intent to treat population)

All patients (*n* = 36)	
Age (yr), mean ± SD	55.5 ± 13.7
Male, *n* (%)	20 (55.6)
Race, *n* (%)	
White	9 (25.0)
Black	24 (66.7)
Hispanic	2 (5.6)
Transplant type, *n* (%)	
Deceased donor	25 (69.4)
Living donor	11 (30.6)
Hypertension, *n* (%)	28 (77.8)
Diabetes mellitus, *n* (%)	9 (25.0)
Focal segmental glomerulosclerosis, *n* (%)	1 (2.8)
Polycystic kidney disease, *n* (%)	1 (2.8)
HIV-associated nephropathy, *n* (%)	1 (2.8)
Lupus nephritis, *n* (%)	1 (2.8)
Transplant number, *n* (%)	
One	35 (97.2)
Two	1 (2.8)
cPRA (%), median (IQR)	0 (0-10.0)
Actual body weight (kg), mean ± SD	87.4 ± 18.4
Dosing weight, *n* (%)	
Actual	16 (44.4)
Adjusted	20 (55.6)
BMI (kg/m^2), mean ± SD	30.0 ± 5.5

BMI: Body mass index; cPRA: Calculated panel reactive antibody; IQR: Interquartile range; HIV: Human immunodeficiency virus.

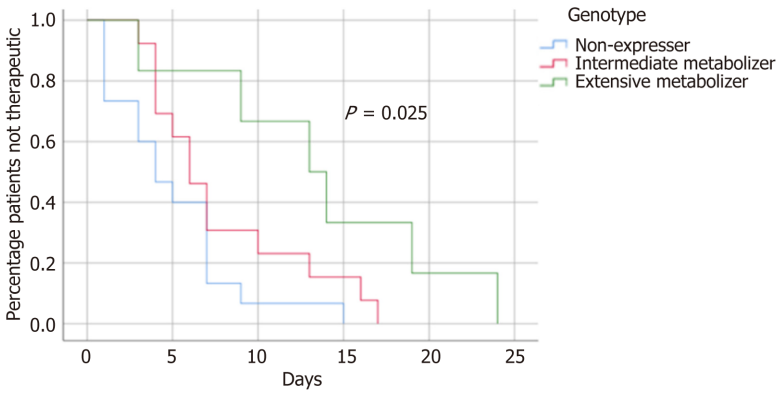

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Figure 1 Time to therapeutic tacrolimus trough concentration (Kaplan Meier analysis).

Table 2 Baseline characteristics (modified intent to treat population)

	Non-expresser (*n* = 15)	Intermediate metabolizer (*n* = 13)	Extensive metabolizer (*n* = 6)	*P* value
Age (yr), mean ± SD	53.8 ± 12.6	49.0 ± 14.0	58.3 ± 14.1	0.354
Male, *n* (%)	9 (60.0)	8 (61.5)	2 (33.3)	0.470
Race, *n* (%)				0.026
White	7 (46.7)	1 (7.7)	0	
Black	7 (46.7)	12 (92.3)	5 (83.3)	
Hispanic	1 (6.7)	0	0	
Transplant type, *n* (%)				0.550
Deceased donor	12 (80.0)	8 (61.5)	4 (66.7)	
Living donor	3 (20.0)	5 (38.5)	2 (33.3)	
Hypertension, *n* (%)	12 (80.0)	9 (69.2)	6 (100.0)	0.304
Diabetes mellitus, *n* (%)	2 (13.3)	4 (30.8)	2 (33.3)	0.457
Focal segmental glomerulosclerosis, *n* (%)	1 (6.7)	0	0	0.521
Polycystic kidney disease, *n* (%)	0	1 (7.7)	0	0.435
HIV-associated nephropathy, *n* (%)	1 (6.7)	0	0	0.521
Lupus nephritis, *n* (%)	0	1 (7.7)	0	0.435
Transplant number, *n* (%)				0.435
One	15 (100.0)	12 (92.3)	6 (100.0)	
Two	0	1 (7.7)	0	
cPRA (%), median (IQR)	0 (0-20.8)	10.0 (0-10.0)	0 (0-20.8)	0.732
Actual body weight (kg), mean ± SD	85.5 ± 16.8	91.8 ± 22.2	77.4 ± 10.5	0.286
Dosing weight, *n* (%)				0.987
Actual	7 (53.3)	6 (53.8)	3 (50.0)	
Adjusted	8 (46.7)	7 (46.2)	3 (50.0)	
BMI (kg/m^2), mean ± SD	29.68 ± 5.0	30.68 ± 6.9	28.88 ± 4.5	0.805

BMI: Body mass index; cPRA: Calculated panel reactive antibody; IQR: Interquartile range; HIV: Human immunodeficiency virus.

DISCUSSION

To our knowledge, this is the first prospective observational study to provide outcomes data for the *de novo* dosing of extended-release tacrolimus, LCPT, in a predominant *CYP3A5*1* expresser kidney transplant population. This research evaluates a lower initial LCPT dose of 0.13 mg/kg/d compared with the FDA-approved initial LCPT dosing of 0.14 mg/kg/d. A starting LCPT dose of 0.17 mg/kg/d was commonly evaluated in other *de novo* kidney transplant populations[1,2]. Genetic polymorphisms have been shown in numerous studies to directly affect dosage requirements of extended-release tacrolimus preparations, including LCPT[8,12]. In addition, several other patient specific factors may affect tacrolimus absorption including age, ethnicity, body weight, hepatic function, drug-drug interactions, and oral intake[15,21]. The incorporation of a *CYP3A5* genotype testing variable provides a clearer understanding of LCPT dosage requirements in this study given its significant impact on individual metabolism and tacrolimus interpatient variability[12,19].

Delayed time to therapeutic tacrolimus trough concentrations results in higher rates of acute cellular rejection[15-17]. The expression of at least one *CYP3A5*1* allele is associated with a delayed time to achieve initial therapeutic tacrolimus trough concentration as well as a decreased time within therapeutic tacrolimus trough concentration range after kidney transplantation[12,15]. The significant impact of *CYP3A5* activity on tacrolimus metabolism warrants investigation into dosing of once daily tacrolimus formulations in *CYP3A5* expressers. Participants in this study who expressed at least one *CYP3A5*1* allele or two *CYP3A5*1* alleles had significant increases in LCPT dosing requirements compared to those who did not express any *CYP3A5*1* alleles. Higher dosing requirements in *CYP3A5* expressers noted in this study leads to the consideration of a need for higher initial LCPT *de novo* dosing in this population to avoid delays in attainment of therapeutic tacrolimus trough concentrations.

Table 3 Primary and secondary efficacy endpoints

	Non-expressers (*n* = 15)	Intermediate metabolizer (*n* = 13)	Extensive metabolizer (*n* = 6)	*P* value
Time (d) to therapeutic tacrolimus concentration, median (IQR)	4.5 (1.0-7.0)	6.0 (4.0-11.5)	13.5 (7.5-20.25)	0.025
Tacrolimus dose (mg) at therapeutic concentration, median (IQR)	12 (10-14)	16 (13-20)	16 (11-20.5)	0.010
Weight-based tacrolimus dose (mg/kg) at therapeutic concentration, median (IQR)	0.13 (0.12-0.165)	0.20 (0.125-0.25)	0.19 (0.138-0.265)	0.018
Tacrolimus dose (mg), median (IQR)	9.6 (9.2-10.1)	12.5 (10.6-14.5)	13.8 (10.4-14.4)	0.011
Weight-based tacrolimus dose (mg/kg), median (IQR)	0.128 (0.102-0.142)	0.136 (0.108-0.169)	0.176 (0.128-0.217)	0.074
Tacrolimus trough concentration (ng/mL), mean ± SD	10.78 ± 2.1	9.18 ± 1.6	7.98 ± 1.3	0.008
Weight-based tacrolimus dose at day 30 (mg/kg), mean ± SD	0.103 ± 0.429	0.154 ± 0.620	0.167 ± 0.590	0.022
Potassium (mEq/L), mean ± SD	4.29 ± 0.36	4.68 ± 0.35	4.35 ± 0.59	0.041
Serum creatinine (mg/dL) at day 30, median (IQR)	1.76 (1.29-2.62)	1.75 (1.27-2.65)	1.94 (1.2-3.0)	0.906
eGFR (mL/min/1.73 m^2) at day 30, median (IQR)	40.0 (27.0-58.0)	46.0 (30.0-58.5)	31.5 (25.0-56.3)	0.701

eGFR: Estimated glomerular filtration rate; IQR: Interquartile range.

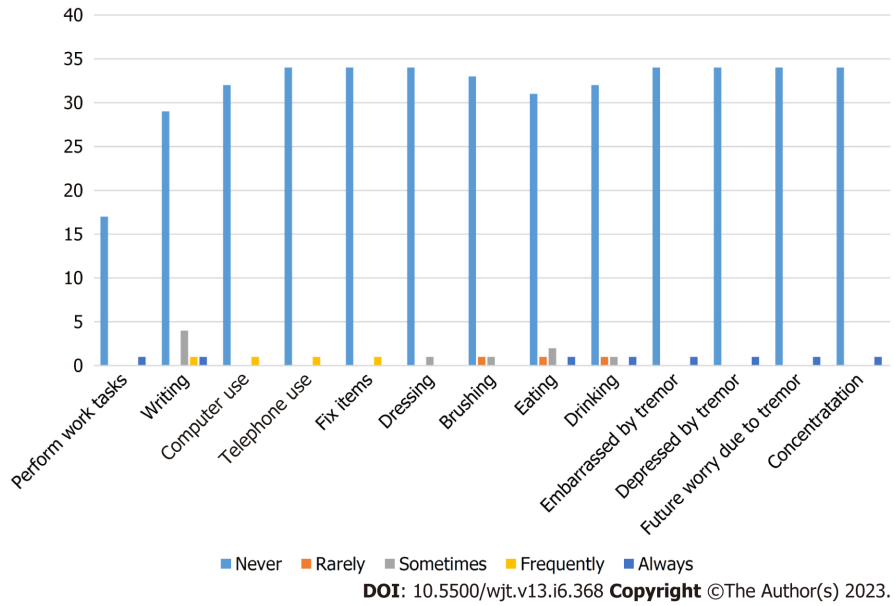

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Figure 2 Quality of life in essential tremor questionnaire lifestyle self-assessment.

Since LCPT dosing has not been evaluated in prior studies based on *CYP3A5* genotype, it is worthwhile to compare the results of our study to a different once daily tacrolimus formulation (Advagraf®, Astagraf®; Astellas Pharmaceuticals). De Meyer *et al*[12] showed that *CYP3A5* expressers, both intermediate and extensive metabolizers, required higher tacrolimus extended-release doses by day 30 compared to *CYP3A5* non-expressers (*CYP3A5* intermediate metabolizer: 0.30 mg/kg/d *vs* *CYP3A5* extensive metabolizer: 0.46 mg/kg/d *vs* *CYP3A5* non-expresser: 0.15 mg/kg/d; $P < 0.001$). We identified a similar trend as De Meyer *et al*[12] in which *CYP3A5* expressers regardless of intermediate or extensive metabolizer phenotype, require higher LCPT dosing than *CYP3A5* non-expressers. However, lower doses of LCPT appear to be required for *CYP3A5* expressers in our study compared to other available once daily tacrolimus formulations (Astagraf®) when comparing dosing at day 30 post-kidney transplant (*CYP3A5* intermediate metabolizer: 0.17 mg/kg/d *vs* 0.30 mg/kg/d; *CYP3A5* extensive metabolizer: 0.16 mg/kg/d *vs* 0.46 mg/kg/d). One limitation to this comparison is the goal tacrolimus trough concentration, since in De Meyer *et al*[12] it was 8-12 ng/mL at day 30 compared to our study goal of 8-10 ng/mL, although median trough concentrations were similar at day 30 for both studies.

Guidelines from the Clinical Pharmacogenetics Implementation Consortium for *CYP3A5* Genotype and Tacrolimus Dosing provide clinical recommendations for dosing based on *CYP3A5* genotype[19]. These guidelines provide clinical

evidence for dose adjustments required for immediate-release tacrolimus dosing, but do not discuss the implications for dose adjustments for tacrolimus extended-release formulations, such as LCPT. Recommendations for all *CYP3A5* expressers is to provide initial tacrolimus dosing of 1.5-2 times the recommended starting dose and to not exceed a starting dose of 0.3 mg/kg/d. *CYP3A5* expressers in our study required approximately 1.5 times the recommended FDA-approved 0.14 mg/kg/d starting dose for LCPT to achieve therapeutic tacrolimus trough concentrations[6]. The majority of *CYP3A5* expressers in this study required an approximate 20% reduction in the mean LCPT dose at day 30 from the time of attainment of therapeutic tacrolimus trough concentrations. The delayed time to achieve therapeutic tacrolimus trough concentration for *CYP3A5* expressers compared to *CYP3A5* non-expressers leads to *CYP3A5* expressers requiring higher initial starting doses of LCPT. However, patients may require dose reduction over time to maintain therapeutic tacrolimus concentrations. Patients in this study identified as *CYP3A5* intermediate metabolizers required a mean of 0.2 mg/kg/d to achieve therapeutic tacrolimus concentration *vs* 0.19 mg/kg/d for *CYP3A5* extensive metabolizers. Given the similarity in doses required to achieve therapeutic tacrolimus concentrations between these two groups, a similar dosing strategy for all *CYP3A5* expressers could be utilized. Two large sample size studies evaluated clinical outcomes associated with *de novo* use of LCPT in kidney transplant recipients. Budde *et al*[1] evaluated the incidence of biopsy-proven acute rejection, graft failure, patient survival, and AEs at 12 mo while Rostaing *et al*[2] evaluated similar outcomes at both 12 and 24 mo after kidney transplantation. Both of these studies evaluated an initial starting dose of 0.17 mg/kg/d, which is the maximum dose of LCPT evaluated in clinical studies with at least 12 mo efficacy and safety outcomes. *CYP3A5* genotype was not performed for these studies and our study provides additional data regarding initial *de novo* LCPT dosing based on *CYP3A5* genotype. Based on these findings, we suggest that *CYP3A5* expressers may require higher initial starting doses of approximately 0.2 mg/kg/d. In order to avoid delays in attaining therapeutic tacrolimus trough concentrations, 0.2 mg/kg/d may be used as an initial starting LCPT dose for *CYP3A5* expressers barring no other clinical barriers to higher starting doses.

This study has several limitations to the interpretation and generalizability of its findings. The single-center design of this study reflects the clinical approach of one institution which may not be applicable to all kidney transplant recipients. *CYP3A5* genotype may not be the only genetic consideration when determining an individual's genetic predisposition to the metabolism of tacrolimus. Genetic differences in *CYP3A4* activity and other SNPs within the *CYP* system could play a role in determining the metabolic rate of tacrolimus which is not captured in our study[12]. Our study evaluated dose requirements and other clinical outcomes through the first 30 d after kidney transplantation. This follow-up period only provides information on short-term LCPT dosing outcomes and future studies with long-term follow-up periods should be performed. This study is also limited by its open-label design and relatively smaller number of patients enrolled. In addition, the single-arm design of this study did not allow for a comparator arm and future randomized controlled studies should be performed to further evaluate dosing requirements of LCPT in the *de novo* kidney transplant population.

CONCLUSION

Expression of *CYP3A5* metabolic activity is an important clinical factor needed to determine optimal LCPT dosage requirements in the *de novo* kidney transplant recipient. It is expected that *CYP3A5* expressers would require a higher initial starting dose as well as higher incremental dosage titration to achieve therapeutic tacrolimus trough concentrations in a reasonable timeframe. Prospective identification of *CYP3A5* genotype may lead to optimized dosing of LCPT in the *de novo* kidney transplant setting. Future, randomized, larger-scale studies should be conducted to determine the optimal *de novo* dosing of LCPT after kidney transplantation.

ARTICLE HIGHLIGHTS

Research background

Tacrolimus has been extensively studied and shown to require significant dose adjustments in *CYP3A5* expressers compared to non-expressers. Data regarding the impact of *CYP35* expresser status on the dosing of tacrolimus extended-release tablets has not been published and is important to understand given the vastly different pharmacokinetic profile of this tacrolimus formulation. There is an increased use of tacrolimus extended-release tablets in the *de novo* setting warranting further investigation into this clinical question.

Research motivation

The main concerns when initiating tacrolimus in the *de novo* kidney transplant setting is to achieve therapeutic tacrolimus trough concentrations in a reasonable timeframe while also avoiding drug toxicity. The rationale behind this research is to identify dosing strategies that should be considered when initiating tacrolimus extended-release immediately after kidney transplant. Particular, research evaluating dosing strategies in patients known to have higher tacrolimus dose requirements (*i.e*. *CYP3A5* expressers) will provide data for transplant centers to make educated clinical decisions surrounding dosing and dosing adjustments for tacrolimus extended-release tablet formulations.

Research objectives

The main objectives of this research was to identify the time to therapeutic tacrolimus trough concentration as well as the dose required to obtain that trough concentration. These objectives were realized as well as the differences in dosing requirements amongst CYP3A5 expressers compared to non-expressers. The significance of these objectives warrant further investigation towards linking clinical outcomes such as acute rejection and graft function outside of the first month after transplant in patients receiving tacrolimus extended-release tablets in the *de novo* kidney transplant setting.

Research methods

Patients (n = 36) were consented to receive tacrolimus extended-release tablets at a dose of 0.13 mg/kg/d at the time of kidney transplantation. Dosing was adjusted to maintain therapeutic trough concentrations of 8-10 ng/mL which assisted in identifying the primary objective of time to therapeutic concentration. In addition, all patients that consented to *CYP3A5* genotype testing (n = 34) were included in additional data analysis to describe dosing requirements for tacrolimus extended-release tablets in patients that were *CYP3A5* expressers compared to *CYP3A5* non-expressers. These methods allowed the authors to describe initial dosing requirements as well as the impact of *CYP3A5* metabolism on tacrolimus extended-release dosing and attainment of target trough concentrations.

Research results

This research demonstrated that kidney transplant recipients who are expressers of *CYP3A5* exhibited higher dose requirements for tacrolimus extended-release tablets and also experienced delays in attaining therapeutic trough concentrations compared to *CYP3A5* non-expressers. These findings are pertinent to the field of solid organ transplant since transplant centers that utilize tacrolimus extended-release tablets in the *de novo* setting should be aware of the higher dosing needs in this patient population. In addition, transplant recipients suspected to or known to be *CYP3A5* expressers may require more aggressive dose titration to achieve and maintain target tacrolimus trough concentrations. Future research in this area should focus on clinical outcomes beyond our study period of 30 d to determine the impact on acute rejection and kidney graft function.

Research conclusions

Overall, this study provides additional clinical information regarding the dosing requirements of tacrolimus extended-release tablets in the *de novo* kidney transplant setting. To our knowledge, this is the first prospective observational study to provide outcomes data for the *de novo* dosing of tacrolimus extended-release tablets. The findings from this research validate that the impact of *CYP3A5* expression has a clinical impact on the pharmacokinetic profile of tacrolimus extended-release tablets similar to findings published with tacrolimus immediate-release. New approaches to dosing and dose titration for tacrolimus extended-release tablets have been proposed by this research in the *de novo* kidney transplant setting and can be used as a guide when making clinical decisions in this patient population.

Research perspectives

Future research should aim to randomize patients to various doses of tacrolimus extended-release tablets to offer a more advanced comparison of different initial dosing strategies. Conducting *CYP3A5* genotype analyses prior to study drug initiation would be beneficial in future studies in order to further assess the impact of pharmacokinetic variations in metabolism on tacrolimus extended-release tablets.

FOOTNOTES

Author contributions: Diamond A and Di Carlo A participated in design and oversight of the study, data collection, data analysis, and drafted and finalized the manuscript; Karhadkar S and Lau KN, participated in design and oversight of the study, and assisted with data analysis; Chavin K drafted the manuscript and assisted with data analysis; Constantinescu S participated in design and oversight of the study, drafted the manuscript, and assisted with data analysis; Mohrien K performed statistical analysis in conjunction with Temple University Center for Biostatistics & Epidemiology, participated in data analysis, and drafted the manuscript; Perez-Leal O conducted DNA analysis for genotype samples and analyzed genotype data, and drafted the manuscript; Sifontis N participated in design and oversight of the study, assisted with data analysis, and drafted the manuscript; all authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Basic Study

Pathophysiology of acute graft-versus-host disease from the perspective of hemodynamics determined by dielectric analysis

Masayuki Nagasawa

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Abstract

BACKGROUND

Numerous reports have demonstrated that the pathophysiology of graft-versus-host disease (GVHD) during hematopoietic stem cell transplantation (HSCT) is closely related to vascular endothelial disorders and coagulation abnormalities. We previously presented the discovery of a principle and the development of a novel instrument for measuring whole blood coagulation. This was achieved by assessing the variations in the dielectric properties of whole blood.

AIM

To investigate how GVHD affects the changes of dielectric properties of whole blood in patients with HSCT.

METHODS

We examined the changes of dielectric properties of whole blood and erythrocyte proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis sequentially in patients with HSCT and compared it with clinical symptoms and inflammatory parameters of GVHD.

RESULTS

During severe GVHD, the dielectric relaxation strength markedly increased and expression of band3 decreased. The dielectric relaxation strength normalized with the improvement of GVHD. *In vitro* analysis confirmed that the increase of relaxation strength was associated with severe erythrocyte aggregates, but not with decreased expression of band3.

CONCLUSION

Severe erythrocyte aggregates observed in GVHD may cause coagulation abnor-

malities and circulatory failure, which, together with the irreversible erythrocyte dysfunction we recently reported, could lead to organ failure.

Key Words: Graft-versus-host disease; Dielectric relaxation; Erythrocyte; Stem cell transplantation; Coagulation

Core Tip: The pathophysiology of graft-versus-host disease (GVHD) is complex. Examination of changes in the dielectric properties of whole blood revealed that erythrocytes formed risky levels of rouleaux and aggregates in severe GVHD. In severe GVHD, oxidative stress causes degradation of erythrocyte band3 and truncation of the C-terminus of peroxiredoxin 2, resulting in decreased plasticity, increased fragility, and reduced oxygen-carrying capacity. These phenomena may underlie persistent refractory coagulopathy and circulation disorder, leading to organ damage in severe GVHD.

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INTRODUCTION

The graft-versus-host (GVH) reaction is the most serious complication of hematopoietic stem cell transplantation (HSCT) and the most important reaction that eradicates malignant cells[1,2]. When the GVH reaction is excessively induced leading to organ dysfunction, it is designated as graft-versus-host disease (GVHD) and is a target for treatment. Moreover, GVHD progresses in three phases[3]. The first is cell damage due to pretreatment with anticancer drugs and radiation, which induces inflammatory reactions and the production of inflammatory cytokines. The second mechanism involves the recognition and activation of host antigens by transplanted immune cells. Inflammatory cytokines produced in the first phase promote the response in phase 2 by inducing an increased expression of alloantigens. In phase 3, immunocompetent cells activated in phase 2 attack the host cells, induce new inflammation and initiate a vicious cycle of excessive inflammatory responses. The treatment for GVHD involves suppressing and controlling activated immunocompetent cells induced in phases 2 and 3[3]. Uncontrolled and persistent GVHD leads to the insidious progression of severe coagulation disorders and microcirculation disturbances, resulting in irreversible organ failure and tissue damage directly induced by the attack of activated immunocompetent cells[4]. Early and persistent coagulopathy has been reported to be associated with HSCT prognosis[4].

Coagulation abnormalities and circulatory failure in the peripheral circulation are not always caused by GVHD after HSCT. In some cases, the main causes of coagulation disturbances stem from treatment with anticancer drugs, radiation, or vascular endothelial damage caused by calcineurin inhibitors, which are used as immunosuppressants to prevent GVHD[1,5,6]. Furthermore, cytomegalovirus infection/reactivation induced in an immunodeficient state may also be involved in the aforementioned disturbances[7,8]. Additionally, clinically determining whether coagulation abnormalities and vascular endothelial disorders that develop after HSCT are caused by GVHD is often difficult[9,10]. The assessment of peripheral blood circulation abnormalities and coagulation disorders following HSCT, specifically concerning pathological alterations in erythrocytes, has received limited attention in the context of GVHD.

The measurement of the complex dielectric properties of biomaterials at radio frequency has gained increasing importance not only in material science, microwave circuit design, and absorber development but also in biological research[11-13]. Dielectric measurement is important for providing the electrical or magnetic characteristics of materials in a noninvasive manner and has proven useful in many research and development fields. We performed dielectric measurements of the coagulation process in whole human blood, clarified the principle, and reported its usefulness as a new whole-blood coagulation measurement method[14,15]. Moreover, we examined the changes in the dielectric properties of whole blood after HSCT in 16 patients and discussed the relationship between the changes in the dielectric properties of whole blood and GVHD.

MATERIALS AND METHODS

Principles of dielectric properties of whole blood

The principle of the dielectric measurement of whole blood is displayed in Figure 1A-D. Whole blood comprises plasma and blood cells (mainly erythrocytes), each of which is electrically charged. When an alternating electric field is applied to whole blood, sufficient ionization is achieved at low frequencies. However, as the frequency increases, ionization fails to keep pace sufficiently. Beyond a certain frequency, ionization is no longer possible and the dielectric constant does not change (Figure 1B-D). The dielectric properties of each material can be represented by the relaxation strength and

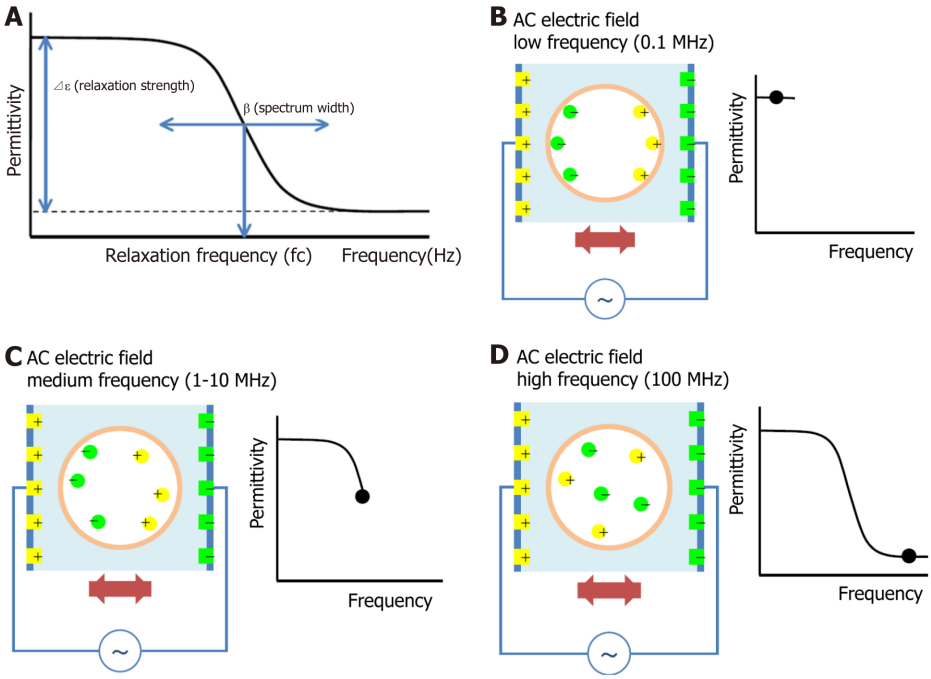

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Figure 1 The principle of the dielectric measurement of whole blood is displayed. A: Brief description of dielectric parameters; B: Sufficient ionization is achieved when a low frequency alternating voltage is applied; C: As the frequency of the AC voltage increases, the ionization cannot keep up with the frequency, and the dielectric strength gradually decreases; D: When the frequency of the AC voltage exceeds a certain level, no dielectric occurs and the dielectric strength stabilizes at a low value.

frequency, as displayed in Figure 1A. The dielectric constant of whole blood was determined as the sum of the plasma (solvent) and blood cell components. As the electrode polarization of the solution component had a strong effect in the low-frequency region, the interfacial polarization of the blood cell component was calculated by correcting for this effect. Theoretically, the dielectric properties of blood cell components are considered to be significantly affected in the high-frequency range (Figure 2).

Dielectric measurement of whole blood

Venous blood was drawn in a blood collection tube containing Ethylenediamine-N, N, N', N'-tetra acetic acid dipotassium salt dihydrate (EDTA-2K·2H_2O) and diluted to 10% hematocrit (Hct) with phosphate buffer saline (PBS). After 30 min of incubation at room temperature with gentle stirring, the dielectric properties were measured by our developed equipment. Diluted blood samples (200 L) were adjusted to 10% Hct using PBS and measured at 57 frequencies from 100 Hz to 40 MHz[14]. The measurement time for each frequency was < 0.2 s and the total frequency scan time for one dielectric relaxation measurement was 10 s. Measurements were performed within 1 h of blood sample collection.

Clinical samples

Clinical samples were obtained from 16 patients who underwent allogeneic HSCT. The clinical information for each patient is summarized in Table 1. A portion (1 mL) of blood collected during routine clinical examinations performed 2–3 times per week after HSCT was used for the research. Information such as clinical symptoms and blood data were collected as anonymized information from medical records.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Cells were washed with PBS and then lysed at 4 °C in a buffer containing 10 mmol/L Tris-hydrochloric acid, 50 mmol/L sodium chloride, 0.5% sodium deoxycholate, 0.2% SDS, 1% Nonidet P-40, 1 mmol/L phenylmethylsulfonyl fluoride, 50 mg/mL aprotinin, 50 mmol/L leupeptin, and 0.1 mmol/L sodium orthovanadate. After removing cellular debris by centrifugation, lysates were prepared for electrophoresis, and PAGE was performed as described previously[16]. Further, proteins separated on the gel were stained using silver staining.

RESULTS

Typical changes in dielectric relaxation strength and frequency are depicted in patients who developed acute GVHD of grade 3 or more (patient 4) and those who did not (patient 2). During GVHD, a sharp increase in the dielectric relaxation strength and a decrease in the relaxation frequency intensity were observed (Figures 3 and 4). Overall, the dielectric

Table 1 The clinical information for each patient

Patient No	Disease	Acute GVHD	Stage			Conditioning	Stem cell source	Decreased band3	Truncated PRDX2
			Skin	Gut	Liver				
1	AML	I	1	0	0	MAC	URCB	No	No
2	HIM	0	0	0	0	RIC	URBMT	No	No
3	SCID	III	2	3	1	RIC	URCB	Yes	Yes
4	SCID	III	1	3	1	RIC	URCB	Yes	Yes
5	AML	I	1	0	0	MAC	MSBMT	No	No
6	AML	II	3	0	0	MAC	URBMT	No	No
7	DKC	III	2	3	1	RIC	URBMT	Yes	Yes
8	ALL	0	0	0	0	MAC	URBMT	No	No
9	ALL	II	3	0	0	MAC	URCB	No	No
10	ALL	II	3	0	0	MAC	MSBMT	No	No
11	AML	I	1	0	0	MAC	MSBMT	No	No
12	AML	III	1	3	1	MAC	mis-RBMT	Yes	Yes
13	ALL	III	1	2	1	MAC	URBMT	Yes	Yes
14	ALL	III	1	2	1	MAC	URBMT	Yes	No
15	ALL	II	3	0	0	MAC	mis-RBMT	No	No
16	SCN	I	1	0	0	RIC	MSBMT	No	No

AML: Acute myeloid leukemia; HIM: Hyper IgM syndrome; SCID: Severe combined immunodeficiency; DKC: Dyskeratosis congenital; ALL: Acute lymphoblastic leukemia; SCN: Severe congenital neutropenia; MAC: Myeloablative conditioning; GVHD: Graft-versus-host disease; RIC: Reduced-intensity conditioning; URCB: Unrelated cord blood; URBMT: Unrelated bone marrow transplant; MSBMT: Matched sibling bone marrow transplant; mis-RBMT: Mismatched related bone marrow transplant.

relaxation strength and frequency changed in a complementary manner.

To investigate whether the change in dielectric properties was due to blood cells or plasma components, the expression of erythrocyte membrane proteins was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). As demonstrated in Figure 3, a decrease in band 3 expression was observed in line with GVHD onset (Figure 3). Decreased expression of band 3 is one of the mechanisms of hereditary spherocytosis, in which erythrocytes are not normally concave-discoid-shaped, however, they morphologically change into spherocytes, exhibiting osmotic fragility. However, our previous study on dielectric relaxation frequency intensity changes in various erythrocyte morphologies displayed that the relaxation frequency increased during spherocytosis[12,17]. No significant changes in the dielectric properties of the plasma components were observed with or without GVHD (data not displayed). Therefore, we conducted a replacement experiment for the blood cells and plasma components. As demonstrated in Figure 5, the relaxation strength depends on the plasma components (Figure 5A). Observation of erythrocyte states under each condition using a phase-contrast microscope revealed the formation of rouleaux or aggregates in the presence of plasma components during the progression of GVHD (Figure 5B). Although the data are not displayed, rouleaux formation of erythrocytes was observed at a high frequency in the blood, in which the dielectric relaxation strength increased after HSCT.

Next, to estimate the quantitative relationship between the dielectric relaxation strength change and the rouleaux formation of erythrocytes, experiments with the addition of gamma (γ)-globulin were carried out. Blood obtained from healthy individuals was washed with PBS, a 10% Hct erythrocyte suspension solution was created, and then γ-globulin was added to measure the dielectric properties and confirm the morphology at several concentration settings. The serum concentration of γ-globulin in healthy individuals is considered to be approximately 10 mg/mL, but little change was observed in dielectric relaxation strength and morphology under conditions between 0 mg/mL and 10 mg/mL of g-globulin. A rouleaux formation of erythrocytes was observed at a g-globulin concentration of 50 mg/mL, and large erythrocyte aggregates were observed at a concentration of 150 mg/mL. For dielectric relaxation measurement, the patient's whole blood was diluted to approximately 1/3 in PBS to match 10% Hct. The observed rate of change in the dielectric constant in our study suggests that the acute phase of grade 3 or more GVHD was comparable to a state where the γ-globulin concentration exceeded 100 mg/mL, which is ten-fold higher than the normal value (Figure 6) (comparing with the data displayed in Figure 5A).

Figure 7 displays the changes in the dielectric relaxation strength and C-reactive protein (CRP), a biomarker of inflammation, in patients who developed grade 3 or more of GVHD. The dielectric relaxation strength and CRP levels demonstrated a correlation in some cases, but they did not necessarily match. In addition, the dielectric relaxation

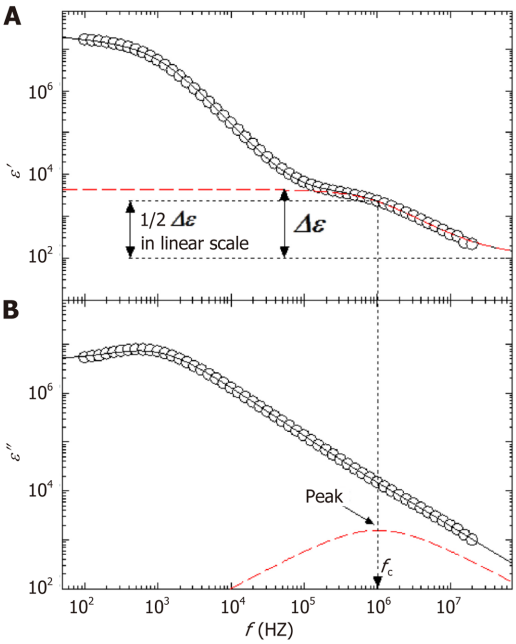

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Figure 2 Understanding of the dielectric relaxation of whole blood, which is consist of plasma and blood cells. A: Shows the real part; B: The imaginary part of the complex permittivity. The broken red lines are the result of an analysis of the interfacial polarization phenomenon according to a Cole−Cole type dielectric relaxation function. This function is characterized by the relaxation strength Δε corresponding to changes in ε' (A) and the relaxation frequency fc that corresponds to the peak of this function observed in the imaginary part of permittivity, ε" (B). The curved lines in the figure are the sum of all the assumed contributions in the analysis, and agree well with the experimental values.

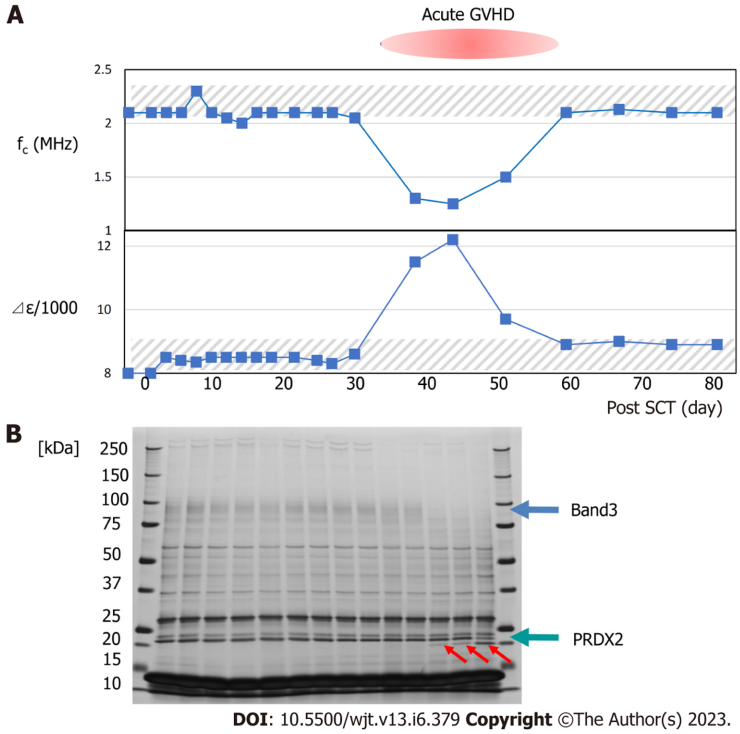

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Figure 3 Changes in the relaxation frequency and relaxation intensity of whole blood after transplantation in a transplant patient who developed severe graft-versus-host disease during the course. A: The hatched area indicates the normal range of relaxation frequency and relaxation intensity; B: The figure below shows the results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of erythrocyte proteins over time. Consistent with graft-versus-host disease, there is a decrease in band3 and the appearance of a 20KDa band (red arrows). The 20KDa band was found to be the C-terminal deleted PRDX2[37]. GVHD: Graft-versus-host disease.

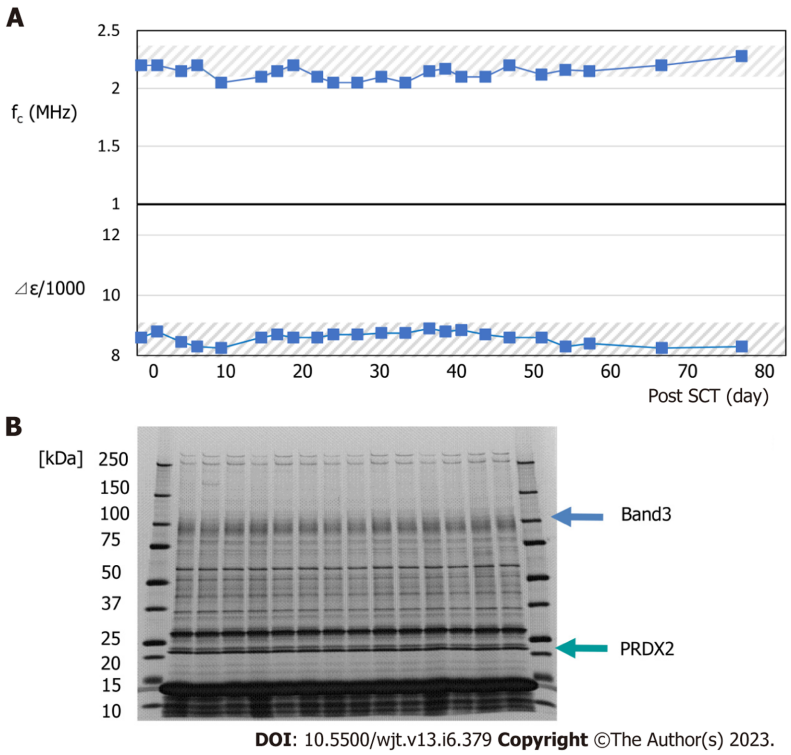

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Figure 4 Changes in the relaxation frequency and relaxation intensity of whole blood after transplantation in a transplant patient who developed no graft-versus-host disease during the course. A: The hatched area indicates the normal range of relaxation frequency and relaxation intensity; B: The figure below shows the results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of erythrocyte proteins over time. There was no significant change.

strength may fluctuate significantly, even with a slight change in the CRP. Interestingly, the dielectric relaxation strength increased gradually with minimal CRP changes in patient 3, who developed severe gastrointestinal GVHD. This indicates that the dielectric relaxation strength is affected by persistent or chronic inflammatory reactions even if the CRP level is elevated.

DISCUSSION

Erythrocytes account for two-thirds of all human cells. They are constantly produced in the bone marrow of hematopoietic stem cells and supplied to the blood[18]. The main role of erythrocytes is to transport oxygen to every corner of the body; therefore, the cell has an optimal structure for efficiently transporting oxygen. Erythrocytes are often subjected to oxidative stress due to their role in transporting high concentrations of oxygen and therefore have a strong scavenger function against reactive oxygen species[19-21]. A typical example is peroxiredoxin 2 (PRDX2), which is present in high concentrations in red blood cells and is maintained by the pentose phosphate pathway and glutathione system[22]. A concave discoid-like shape without a nucleus is the optimal structure that can efficiently take in oxygen and move inside the capillaries to every corner of the tissue with strong deformability[23]. Furthermore, erythrocytes have a strong buffering capacity to maintain the acid-base balance in body fluids[24] and abundant scavenger functions against oxidative stress[19-21].

On the other hand, the coagulation system is known to be phylogenetically closely related to inflammatory and immune responses[25,26]. The system is considered to have evolved as a biological defense mechanism that not only stops bleeding but also traps pathogens and prevents them from spreading to the surrounding area. In vivo, the coagulation system is initially considered to be activated on the cell surface[27], and evidence indicates that stagnation of blood flow can be a trigger[28].

Based on the principle of our newly developed *in vitro* whole blood coagulation measurement system utilizing whole blood permittivity measurements, this process can be divided into three stages[14]. First, an increase was observed in relaxation strength due to rouleaux formation, followed by a further increase in relaxation strength due to aggregation. Additionally, a rapid decrease in relaxation strength due to changes in erythrocyte morphology and tertiary structure caused by platelet secondary aggregation and clot retraction due to fibrin formation and polymerization was also observed. This result suggests the importance of erythrocyte rouleaux and aggregate formation as an initial reaction or precursor state for the activation of the coagulation system.

As is well known, hypergammaglobulinemia induces rouleaux formation by neutralizing the negative charge on the surface of erythrocytes. Venous thrombosis frequently occurs in multiple myeloma presenting with pathological

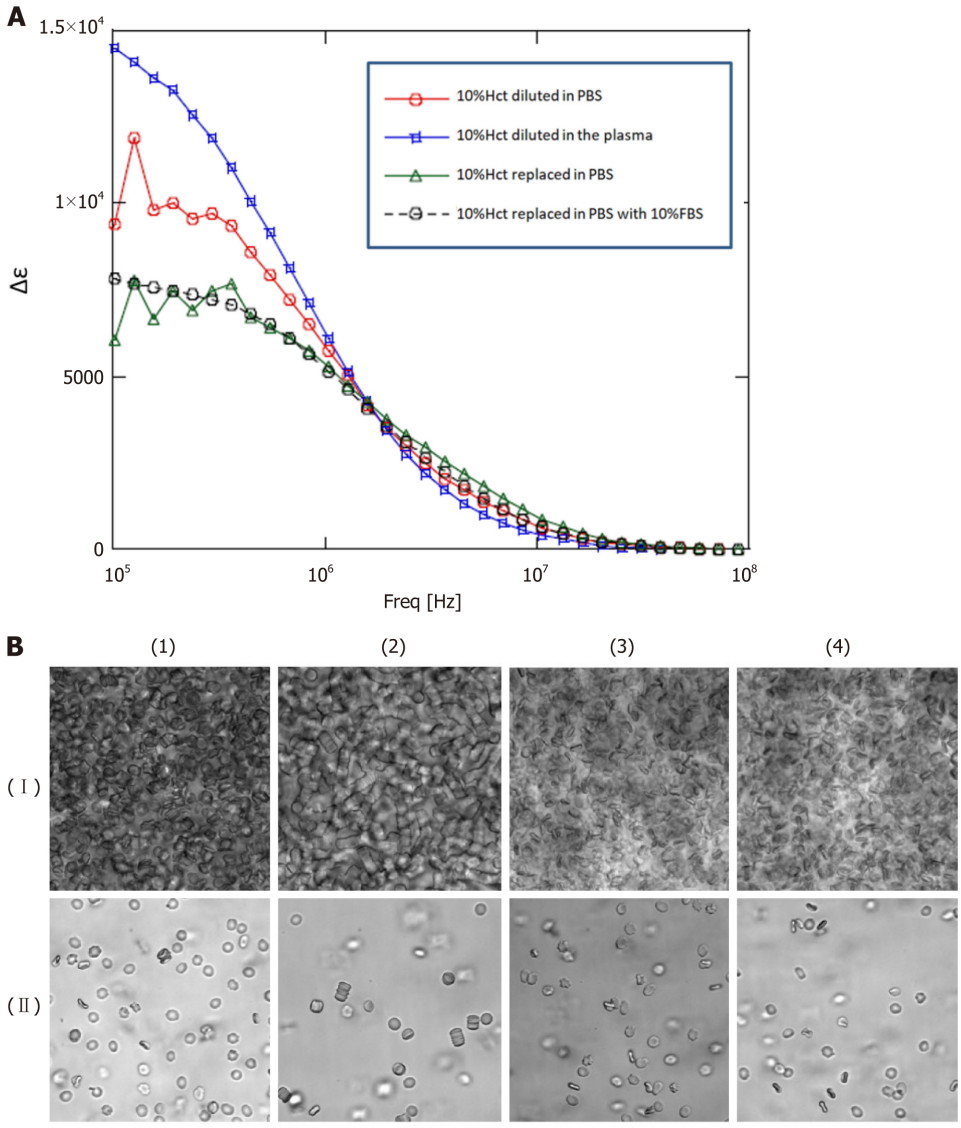

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Figure 5 We conducted a replacement experiment for the blood cells and plasma components. A: Blood sample (about 30%Hct) from the patient with graft-versus-host disease was diluted as 10%Hct diluted in phosphate buffer saline (PBS), 10%Hct diluted in the patient's plasma, 10%Hct replaced in PBS, and 10%Hct replaced in PBS with 10%FBS, and dielectric properties were measured; B: (I) Blood sample (about 30%Hct) from the patient with graft-versus-host disease was diluted as (1) 10%Hct diluted in PBS, (2) 10%Hct diluted in the patient's plasma, (3) 10%Hct replaced in PBS, and (4) 10%Hct replaced in PBS with 10%FBS, and observed by phase-contrast microscopy; (II) Sample of (I) was further diluted by 50-fold in the same way, and observed by phase-contrast microscopy. PBS: Phosphate buffer saline.

hypergammaglobulinemia[29,30]. Many reports of thrombosis, likely due to high-dose g-globulin therapy[31-33]. The high-viscosity state caused by rouleaux formation causes stagnation of blood flow and interacts with tissue factors produced by GVHD-induced inflammatory reactions to lower the threshold of initial coagulation activation. According to this study, the change in whole blood dielectric constant in acute GVHD was considered equivalent to the change in hypergammaglobulinemia from 10 to 15 mg/mL, which is a high-risk level that progresses to thrombus formation and renal failure when replaced with multiple myeloma patients.

On the other hand, oxidative stress has been reported to be enhanced in acute GVHD[34,35], in addition to the oxidative stress induced by conditioning chemotherapy and radiation therapy[36]. We recently reported that calpain activated by oxidative stress may cause the degradation of band 3 and PRDX2[37]. Degradation of band 3 causes a decrease in erythrocyte plasticity, making the cell more susceptible to hemolysis[38,39]. The degradation of PRDX2 irreversibly disrupts the scavenging function of erythrocytes and reduces the resistance of the cell against oxidative stress [40-42]. Phosphatidylserine, which appears on the cell surface due to abnormal erythrocyte membranes, acts as a procoagulant[43]. Free hemoglobin released by hemolysis scavenges nitrogen oxide (NO)[26], which has a vasodilatory effect, and the released erythrocyte arginase reduces NO production[44]. These pathological conditions overlap to form a vicious circle, and it is thought that the progression of microcirculatory failure and the accompanying organ failure result in an irreversible state leading to fatality.

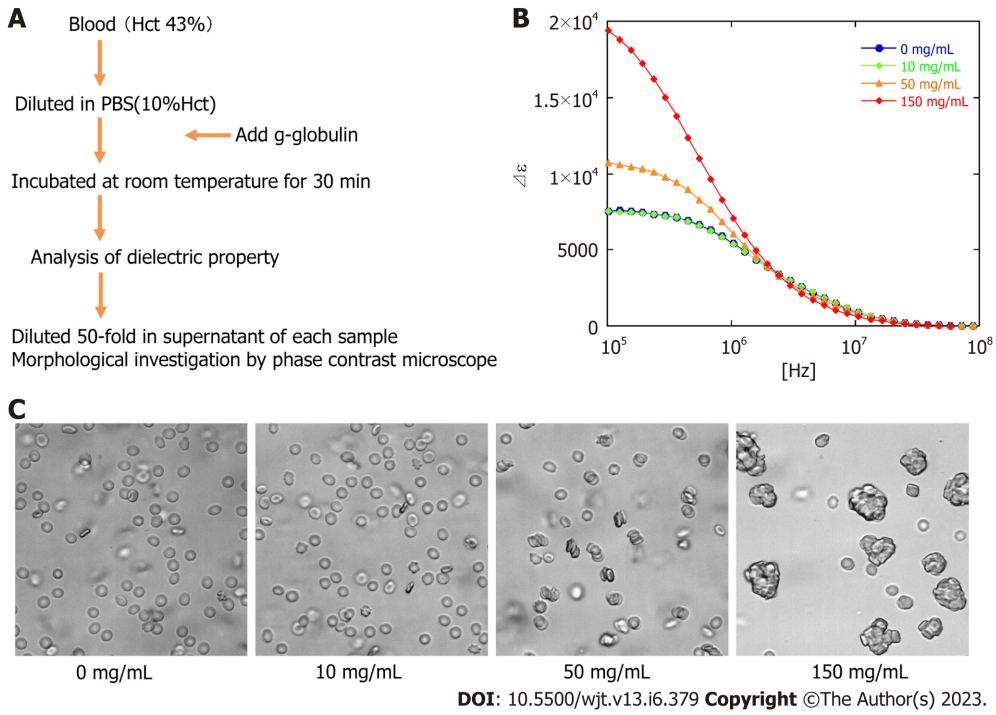

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Figure 6 The observed rate of change in the dielectric constant. A: Blood sample from a healthy volunteer (Hct 43%) was diluted in PBS to 10% Hct, and g-globulin was added to a final concentration of 0 mg/dL, 10 mg/dL, 50 mg/dL, and 150 mg/dL; B: After incubation for 30 min, dielectric properties of each sample were measured; C: Morphology of erythrocytes was investigated by phase-contrast microscopy after further dilution by 50-fold. PBS: Phosphate buffer saline.

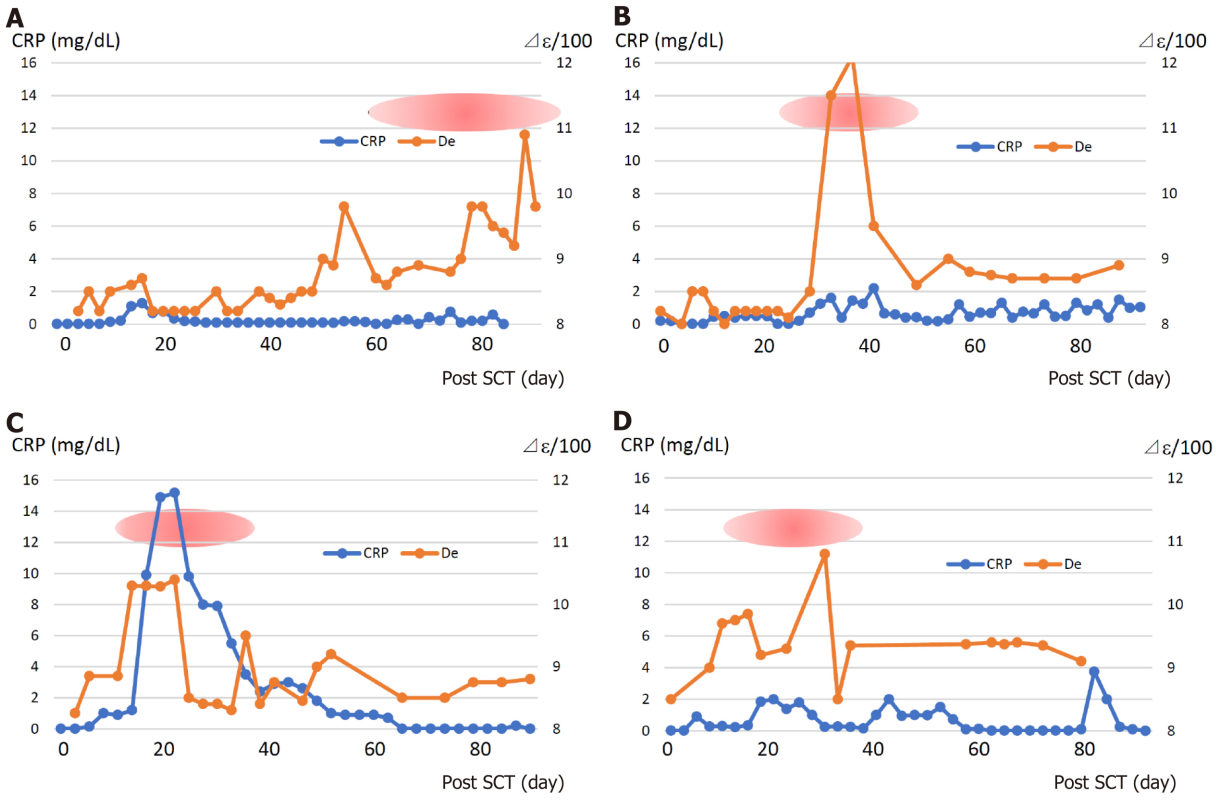

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Figure 7 Transition and relationship between dielectric relaxation strength and C-reactive protein in patients who developed graft-versus-host disease grade 3 after transplantation. A: Patient 3; B: Patient 4; C: Patient 12; D: Patient 13. Red oval marks indicate the period of grade 3 and more acute graft-versus-host disease. CRP: C-reactive protein; SCT: Stem cell transplantation.

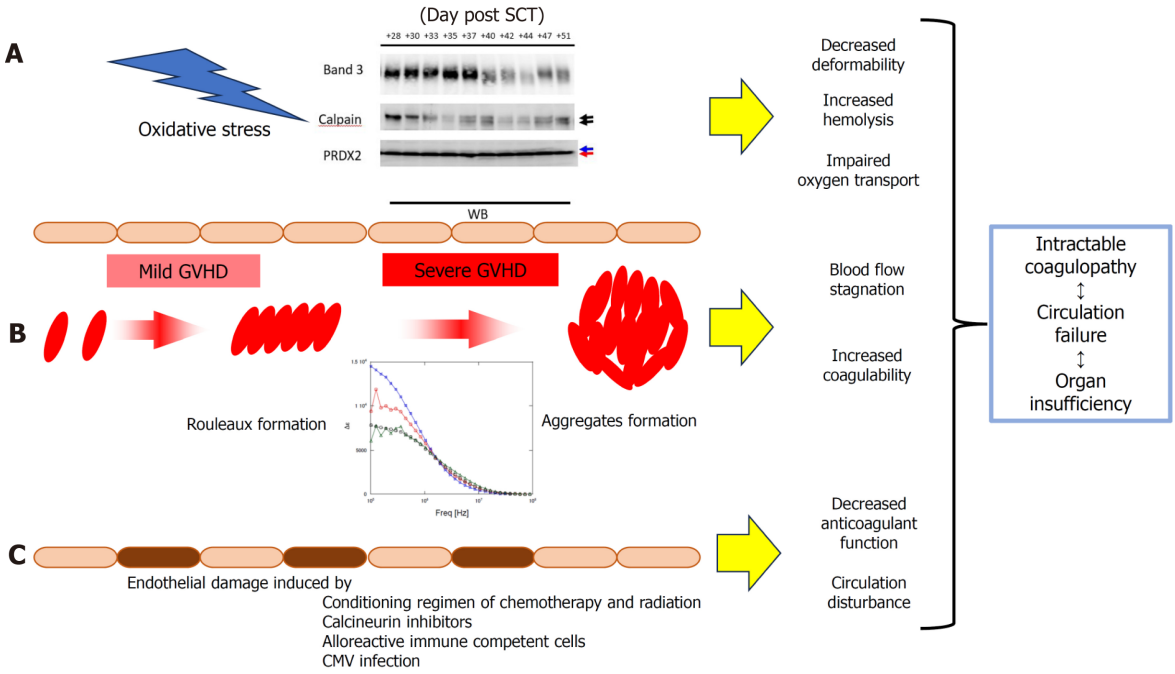

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Figure 8 Schematic illustration of graft-versus-host disease from the perspective of dielectric analysis of whole blood cells. A: In graft-versus-host disease (GVHD) of grade 3 or higher, oxidative stress-activated calpains lead to degradation of band3 and truncation of the C-terminus of PRDX2, leading to decreased erythrocyte plasticity, increased fragility, and impaired oxygen transport; B: Changes in plasma contents due to complex inflammation such as GVHD cause rouleaux formation and aggregation of erythrocytes, causing stasis in blood circulation and becoming more susceptible to coagulation activation; C: Vascular endothelial cell damage by pretreatment or calcineurin inhibitors is further prolonged and increased by alloimmune reactions and reactivation of cytomegalovirus virus, and the anticoagulant function of vascular endothelial cells is reduced. These phenomena are compounded and lead to refractory coagulopathy and subsequent organ circulatory failure and dysfunction. CMV: Cytomegalovirus; CRP: C-reactive protein; GVHD: Graft-versus-host disease; SCT: Stem cell transplantation.

The differences between rouleaux formation, which is indicated by changes in dielectric relaxation strength, and the erythrocyte sedimentation rate (ESR), which has been classically used as a biomarker of inflammation, could not be examined in detail in this study. Moreover, ESR requires 30–60 min or more for measurement, whereas dielectric properties can be measured within 10 s, and it is believed that the condition of erythrocytes can be accurately depicted with minimal external influences during the assay. Furthermore, ESR is considered to be affected by many factors other than changes in the dielectric relaxation strength and does not necessarily reflect the same factors. Additionally, ESR is also affected by anemia and polycythemia. In this regard, our previous study on Hct and dielectric relaxation strength identified an increase in dielectric relaxation strength with increasing Hct[45]. An increase in Hct results in a decrease in ESR, which indicates that the phenomena of an increase in the dielectric relaxation strength and enhancement of ESR are not necessarily the same.

As demonstrated in Figure 3, when the dielectric relaxation strength was higher than a certain level, a decrease in band 3 of red blood cells and fragmentation of the C-terminus of PRDX2 were observed, possibly due to oxidative stress-induced calpain activation[37]. In a study of 16 transplant patients, these changes were observed only in cases of severe GVHD with grade 3 or higher, and not in grade 1–2 GVHD[37]. This suggests that changes in the intensity of the total blood dielectric relaxation after HSCT reflect the severity of GVHD and oxidative stress. By comprehensively considering the results of this study and the principle of dielectric coagulation measurement, a conclusion can be reached that the dielectric relaxation strength sensitively reflects the distribution pattern of blood cells in the solvent and their shape change[14]. Based on clinical data and *in vitro* experiments, the stage from rouleux to aggregate formation may be an indicator of serious illness. From this perspective, the experimental results displayed in Figure 6 provide important information for the quantitative interpretation of the dielectric relaxation strength. Figure 8 displays a schematic illustration of the mechanism of circulatory and coagulopathy development in GVHD after HSCT, as proposed in this study, of changes in the dielectric relaxation strength and membrane protein changes in erythrocytes.

In this study, we could not compare various previously reported GVHD biomarkers with changes in the dielectric properties of whole blood. This report does not indicate that changes in the dielectric properties of blood are superior to those in previously reported GVHD biomarkers[46]. The changes in the dielectric properties of blood are not specific to GVHD. A modification in the dielectric properties of blood is believed to arise due to conditions that induce alterations in plasma composition, fostering the development of erythrocyte aggregations. One of these includes classically recognized acute inflammatory alterations, and as previously mentioned, the parameter known as ESR is believed to indirectly mirror similar phenomena. The pathway by which severe GVHD leads to irreversible organ failure is not necessarily limited to direct organ damage caused by the GVH reaction through the alloimmune response; secondary and latent circulatory disorders are also involved. From this perspective, we believe that the changes in the dielectric properties of

blood examined in this study provide a new perspective for understanding and managing GVHD.

CONCLUSION

The pathological significance of the dynamic changes in blood dielectric relaxation strength in acute GVHD identified in this study requires further investigation. In the future, we believe that more detailed quantitative analysis of dielectric relaxation strength and consideration of its relationship with other clinical parameters will be necessary. Furthermore, the clinical usefulness of dielectric relaxation strength as an interesting and unique biomarker as well as a target for therapeutic intervention should be duly considered.

ARTICLE HIGHLIGHTS

Research background

We previously presented the discovery of a principle and the development of a novel instrument for measuring whole blood coagulation. This was achieved by assessing the variations in the dielectric properties of whole blood.

Research motivation

This assay of dielectric properties of whole blood may be useful for evaluation of coagulation abnormalities observed in graft-versus-host disease (GVHD).

Research objectives

To investigate how GVHD affects the changes of dielectric properties of whole blood in patients with hematopoietic stem cell transplantation (HSCT) and pathological significance of dielectric properties of whole blood in GVHD.

Research methods

We examined the changes of dielectric properties of whole blood and erythrocyte proteins by sodium dodecyl sulfate-polyacrylamide electrophoresis sequentially in patients with HSCT and compared it with clinical symptoms and inflammatory parameters of GVHD.

Research results

During severe GVHD, the dielectric relaxation strength markedly increased and expression of band3 decreased. The dielectric relaxation strength normalized with the improvement of GVHD. *In vitro* analysis confirmed that the increase of relaxation strength was associated with severe erythrocyte aggregates, but not with decreased expression of band3.

Research conclusions

Severe erythrocyte aggregates observed in GVHD may cause coagulation abnormalities and circulatory failure, which, together with the irreversible erythrocyte dysfunction we recently reported, could lead to organ failure.

Research perspectives

The pathological significance of the dynamic changes in blood dielectric relaxation strength in acute GVHD identified in this study requires further investigation. Furthermore, the clinical usefulness of dielectric relaxation strength as an interesting and unique biomarker as well as a target for therapeutic intervention should be duly considered.

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FOOTNOTES

Author contributions: Nagasawa M conceptualized and designed the study, supervised the study, drafted and revised the manuscript.

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Informed consent statement: Written informed consent was obtained from the guardians of each SCT patient.

Conflict-of-interest statement: All the authors have no conflicts of interest to declare.

Data sharing statement: Data available on request from the authors.

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Abstract

BACKGROUND

The United States has witnessed significant advancements in the field of organ transplantation over the course of the last five decades, as demonstrated by a notable increase in the quantity of academic research. The presence of a highly dynamic research environment necessitates continuous evaluations to maintain the integrity and progress of the field.

AIM

To evaluate the total output and thematic emphasis of transplant research conducted in the United States.

METHODS

On January 10, 2023, we conducted a bibliometric search of United States research output in transplantation journals from the Web of Science database's Science Citation Index Expanded. We excluded editorials, meeting abstracts, and other non-article types. We analyzed annual trends, authors, institutions, articles, keywords, and countries collaborating with the United States, using VOSviewer 1.6.18 to create figures and tables.

RESULTS

The United States published 25956 papers (3078 reviews and 22878 articles) representing 37.7% of the world's scientific output. Canada emerged as the top collaborator with the United States, co-authoring 1263 articles. Leading institutions in United States transplantation research were the University of Pittsburgh (1749 articles), Mayo Clinic (1605 articles), Harvard Medical School (1549 articles), and Johns Hopkins University (1280 articles). The top three keywords with over 2000 occurrences were "recipients," "survival," and

"outcomes," indicating a focus on graft and recipient outcome markers by United States researchers.

CONCLUSION

Our findings demonstrate the United States leadership in organ transplantation research, contributing significantly to the global scientific output in this field. However, opportunities exist for fostering expansive partnerships, particularly with developing countries. This study provides valuable insights into the transplantation research landscape in the United States, emphasizing the importance of ongoing evaluations to maintain and propel advancements in this critical medical discipline. The results may facilitate future collaborations, knowledge exchange, and the pursuit of innovative solutions in the realm of organ transplantation.

Key Words: Scientometrics; Bibliometrics; Research output; Organ transplantation; United States

Core Tip: This manuscript presents a compelling bibliometric analysis exploring the United States's pioneering productivity in the field of organ transplantation research. The study delves into 50 years of academic publications, providing valuable insights into annual trends, key authors, institutions, top keywords, and international collaborations. With 25956 papers published, representing 37.7% of the world's output, this research highlights the United States dominant position. The findings emphasize the significance of ongoing evaluations in sustaining advancements and fostering potential collaborations. Reviewers will appreciate the comprehensive approach and the potential to shape future research directions in transplantation.

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INTRODUCTION

Organ transplantation in the United States has a long and storied history[1-3]. Since the early twenty century, American researchers, along with others from around the world, have made incredible strides in understanding the fundamentals of transplantation[1,4]. However, the first successful organ transplant did not occur until the middle of the twentieth century, when the Murray team in Boston performed the first kidney transplantation in the globe[5]. The field of transplantation in the United States has advanced significantly in the last 50 years; this advancement in the field has resulted in a large number of publications.

The total number of scientific papers and citations is a marker in international scientific rankings and a measure of the academic productivity of the field for countries, organizations, and even researchers. Continuous evaluation of research publications is crucial to the growth and maintenance of the research enterprise. The SCImago Journal & Country Rank shows that in 2021, the United States published 726552 scientific papers, up from 603364 in 2010 and 371642 in 2000. According to the SCImago report, the number of medical publications published in 2021 was 296782, up from 190470 in 2010 and 134443 in 2000[6]. However, the progress in academic performance of organ transplantation research in the United States has rarely been studied.

Bibliometric analysis is frequently employed to study patterns in scholarly publications and the relative significance of articles on a particular subject[7-9]. Numerous medical specialties, including surgical oncology, anesthesia, cosmetic and reconstructive surgery, and others, have increasingly used bibliometric analysis to evaluate the output of national research in recent years[10-13]. However, to our knowledge, bibliometric studies of articles written in the United States about organ transplantation have not been done before.

In this study, we aimed to evaluate the research output of the United States in the field of organ transplantation and the contributions of United States transplant centers and researchers from 1998 to 2022. We also aimed to assess the development of the research status of organ transplantation in the United States by evaluating research interests and hot topics over time.

MATERIALS AND METHODS

Data collection and retrieval methods

On January 10, 2023, a bibliographic search was undertaken to identify the publications published in transplantation journals in the United States. The journals were collected from the transplantation category of the Science Citation Index Expanded provided by Clarivate in the Web of Science database. The Web of Science database has a vast collection of

academic journals, providing a comprehensive historical view of academic publications. The utilization of databases such as Scopus and PubMed provide valuable insights. However, because of its extensive breadth and historical coverage, Web of Science emerged as the most suitable option for our specific research investigation[14]. We chose the Science Citation Index Expanded over the Emerging Sources Citation Index explicitly. The selection was made of the well-established reputation of the Expanded Index in the field of bibliometric studies. Within the transplantation category, every material from the Emerging Sources Citation Index was represented in the Expanded section, Notably, the sole exception was the Journal of Transplantation, which was listed under Emerging Sources Citation Index only and comprised only eight articles. Given the limited content from this journal and the comprehensive coverage offered by Expanded section, we deemed the latter more appropriate for our study rendering it unnecessary to reference both categories concurrently.

We have queried the Web of Science database using the publication titles to search all documents published in transplantation journals in the field "publication titles", listed in the supplement file. Articles published after 1998 were available only on the Web of Science Data and were included. We only included articles or reviews; we did not include abstracts of meetings, letters, notes, editorials, or errata. All the included articles were in English, but one was in Russian. We did not include journals related to bone marrow transplant and artificial organs, which were: Bone Marrow transplantation, Stem Cells and Development, Biology of Blood and Marrow Transplantation, Artificial Organs, International Journal of Artificial Organs, Journal of Artificial Organs, and Asaio Journal. A supplement file with the exact algorithm is attached.

Data analysis

We have analyzed annual trends, authors, institutions, journals, articles, keywords, and countries that collaborated with the United States. Figures and tables were generated using VOSviewer version 1.6.18 and MS Excel from Office 365. We have used a flow chart to elaborate on our included and excluded results. The categorization of articles was conducted by utilizing the institutional affiliations provided within the articles. In particular, an article was designated as a "United States article with international affiliation" if at least one of its authors was affiliated with an institution located outside of the United States. The primary objective of this classification system was to offer a comprehensive perspective on global cooperation, with a foundation based on simplicity. It is worth noting that in cases when authors have dual affiliations, an item was classified under the category of "United States article with international affiliation" if any of the affiliations indicated were non-American. In the keyword analysis, we have manually removed words that implied the study design such as "clinical trial" or "retrospective study", and redundant words such as "human", "disease", "male", "female", "adult", *etc.* we also removed the words that are related to the search like "transplant" and "transplantation" and "US" or "United states".

VOSviewer network visualization interpretation

The terms in the network visualization are circles whose size depends on their weight. An item's color depends on its cluster. Linkages are shown by lines. The visualization's distance between circles roughly indicates the co-citation relationship between the terms represented by the circles. The closer two circles are, the more linked they are.

RESULTS

Included studies

The number of publications in transplant journals was 241864. After considering only the articles and review articles, we have included 69110 total publications. There was a total of 25956 papers published in the United States only, representing 37.7% of the world's research output, of which 3078 were reviews, and 22878 were articles (Figure 1). The United States research on transplantation got 776262 citations.

Annual trends

Figure 2 shows annual publications since 1998. United States published 434 articles in 1998. That number rose to 837 in 2003 and climbed to 1192 in 2013. In 2016, the number of publications declined to 1078, but they rose again to 1724 in 2021. However, the number of publications in 2022 displayed a significant decline, with just 1222 articles.

Institutions

In terms of the total number of publications, there were 111 United States institutions that published at least 100 articles, 55 that published at least 200 articles, and 41 that published at least 300 articles. 22 institutions published at least 500 articles. However, only six institutes published at least 1000 articles. The top contributing institutions were the University of Pittsburgh with 1749, Mayo Clinic with 1605; Harvard Medical School with 1549; and Johns Hopkins University with 1280 publications.

In terms of the number of citations, again, the University of Pittsburgh ranked first with 68810 citations, followed by Harvard Medical School with 54838 citations, the University of Michigan with 49111 citations, and the University of California, Los Angeles with 45440 citations (Table 1).

Authors

Seven hundred and thirty-seven authors published at least 20 documents, 81 authors published at least 50 articles, and

Table 1 Top-contributing institutions in United States transplantation research according to the number of citations

Organization	Citations	Total link strength
University of Pittsburgh	68810	23409
Harvard Medical School	54838	13446
University of Michigan	49111	17482
University of California Los Angeles	45440	16112
University of Minnesota	43839	16069
Mayo Clinic	42903	14975
University Calif San Francisco	41100	15648
University of Pennsylvania	31353	14566
Washington University	31142	12641
Stanford University	30728	12815
Johns Hopkins University	30682	14709
Columbia University	25437	11562
University Washington	24740	8223
Northwestern University	24273	12338
Massachusetts Gen Hosp	22332	9733
Emory University	21746	10037
Duke University	21557	10097
Cleveland Clinic	20612	10384
University of Miami	19751	5763
University of Alabama Birmingham	19735	10386

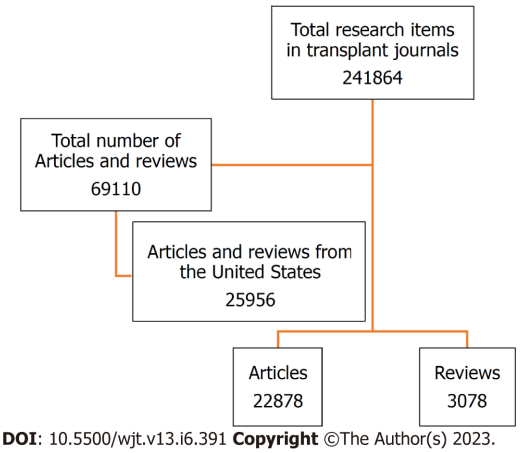

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Figure 1 Flow chart of the United States transplantation research output and exclusion criteria.

eight authors published at least 100 articles. In terms of citations, 138 authors got at least 2000 citations, 57 got at least 3000 citations, 36 got at least 4000 citations, and at least 23 authors got at least 5000 citations. The top 20 most cited authors are listed in Table 2. The top 5 authors, in order of citations on their transplant publications, were Dory Segev with 12490, James Kirklin with 10323, David Cooper with 10307, Merion, Robert M. with 9580, and Leah Edward with 9547.

Journals

The list under the category "transplantation" has 32 journals; after the exclusion, we searched 25 journals. Most publications were published in Transplantation (n = 4795) and the American Journal of Transplantation (n = 3954). Journal of

Table 2 Top 20 most cited authors in the transplantation research from the United States

Author	Research field	Citations	Documents
Segev Dorry L	Transplant epidemiology	12490	378
Kirklin James K	Heart transplantation	10323	145
Cooper David	Heart transplantation xenotransplantation	10307	228
Merion Robert M	Liver transplantation	9580	104
Edwards Leah B	Heart transplantation	9547	71
Stehlik Josef	Heart transplantation	9315	113
Naftel David C	Heart transplantation	8179	80
Kasiske Bertram I	Kidney transplantation	8144	179
Pagani Francis D	Heart transplantation	8039	52
Sachs David H	Transplant immunology	7942	207
Israni Ajay K	Kidney transplantation	7328	157
Kormos Robert I	Heart transplantation	7111	36
Snyder Jon J	Transplant epidemiology	6738	137
Kuchervavava Anna V	Research analyst/heart and lung	6248	35
Meier-Kriesche H-U	Kidney transplantation	5630	59
Yusen Roger D	Lung transplantation	5442	34
Wiesner Russel	Liver transplant	5338	40
Christie Jason D	Lung transplantation	5259	47
Skeans Melissa A	Kidney transplantation	5247	71
Smith Judi M	Kidney transplantation	4945	54

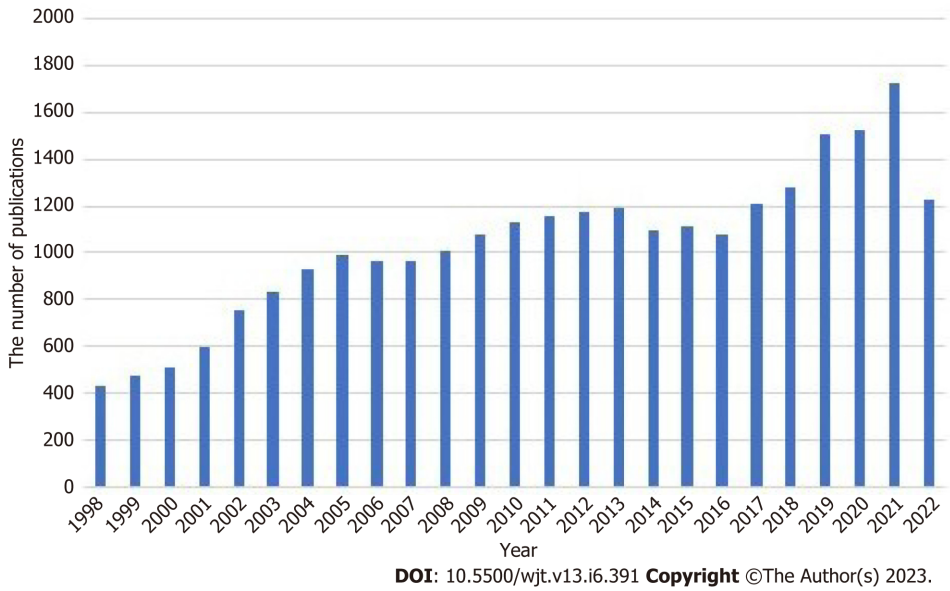

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Figure 2 Publication trends in the United States in the field of transplantation, as documented in transplantation journals from 1998 to 2022.

heart and lung transplantation (n = 2388). The American Journal of Transplantation has received the highest number of citations (n = 194905), followed by Transplantation (n = 185248) and the Journal of Heart and Lung Transplantation (n = 96827) on papers addressing transplantation. The Indian Journal of Transplantation, Transplant Research, Risk Management, and International Journal of Organ Transplantation Medicine have the least number of articles published in the United States, with 9, 7, and 7 documents, respectively. The lowest United States publications were found in the Russian Journal of Transplantology and Artificial Organs, with 1 document. Table 3 lists the transplant journals and the number of United States articles and citations found.

Countries collaborations

We found that American researchers were collaborating with individuals in 114 different nations. Fifty-five countries had at least ten articles in collaboration, 35 had at least 50, 22 had at least 100, 8 had at least 500, and 2 had at least 1000. The country with the highest number of articles collaborated with the United States with the highest number of articles was Canada, with 1263 articles, followed by Germany with 1012, England with 807, and Italy and France with 719 and 598, respectively. The collaboration also extends to Asia with Japan and China, which collaborated with 594 and 579 articles. Middle eastern countries also collaborated with Saudi Arabia with 75, United Arab Emirates and Jordan with 28 and 19, respectively. Table 4 and Figure 3 show the list of countries with the most publications on collaboration with the United States and a visualization of each country's collaboration and interconnections.

Keywords

The top 20 most frequently occurring keywords in this topic are shown in Table 5. There are 3 of them that have occurred more than 2000 times, including "recipients", "survival", and "outcomes". Figure 4 shows the most occurring keywords and their interconnection across the years.

DISCUSSION

This study provides a comprehensive review of scientific publications on organ transplantation in the United States during the past 25 years. The significant increase in publications demonstrates the growing commitment of the United States to advancing transplantation knowledge. This has been sparked by a number of factors, including increased organ donation and transplantation[15], the growth of transplant centers, innovations in machine perfusion and xenograft, and increased research funding[1,16].

Nevertheless, the decline in publications in the year 2022 necessitates a crucial reconsideration. Does the decrease in publication numbers suggest a diminished emphasis on transplantation-specific journals, or does it signify broader changes in research interests? The drop that has been seen highlights the importance of employing a diverse methodological approach in the assessment of research outputs. This may involve considering the inclusion of interdisciplinary journals in future evaluations.

The volume of research conducted on kidney transplantation corresponds with the frequency of kidney transplant surgeries in clinical practice[15]. However, this observation may also imply that there is an excessive focus on studies in specific areas, highlighting the necessity for a more diverse research strategy that encompasses less-explored organs or alternative transplantation approaches (Figure 5).

In 2022, American authors most frequently collaborated with authors from Canada, England, and Germany. In 2000, the United States collaborated most with Japan, Canada, and England. Canada, Germany, England, and Italy are the countries with the highest number of collaborative papers with the United States all over the years; nonetheless, it is noteworthy that Canada's collaboration with the United States increased steadily until its first peak in 2010 with 62 articles, after which it stabilized until a new spike in 2018 with 96 articles, double the number of articles published in 2014; this spike continued until its second peak in 2021 with 111 articles. On the other hand, cooperation with Germany has grown steadily since 2000, reaching a record high of 61 articles in 2011. However, the number of collaborative publications decreased from 2011 to 2015 before beginning to rise again, although not to the level of collaboration with Canada; the second peak was in 2021, with only 62 articles. With a peak of 81 articles in 2021, the number of articles written in collaboration with England has increased steadily and consistently since 2000.

Until 2004, only one article was published by Chinese and American authors. Since then, it has progressively increased, peaking at 34 papers in 2012 and 59 articles in 2019, before declining significantly to 29 by 2022. African countries and institutes collaborated poorly. Egypt and South Africa collaborated the most, with 86 and 44 articles, respectively. Zambia, Nigeria, and Morocco collaborated in a few articles. South American collaboration is represented mainly by Brazil with 238 articles, Argentina with 91, Mexico with 78, and Peru with nine articles. Egypt, India, Saudi Arabia, and the Philippines were the countries that collaborated most recently, more so since 2016, compared to Germany, Italy, Switzerland, and Japan, which showed that the collaboration extends to 2010 and before, as shown in Figure 3B.

Since there is an imbalance between organ transplantation demand and availability in the United States, Global Paired Exchange could be an excellent option to improve the immunologic diversity of donors by including donor and recipient pairs from countries with low healthcare resources for end-stage renal disease. Alongside all precautions, We believe that collaborative research between the United States and other countries can enhance this global paired exchanges program [17-19].

Interestingly, the majority of the top 20 highly referenced authors were in the fields of heart and lung transplantation, with seven authors receiving citations for their papers on cardiac transplantations and three authors receiving the majority of citations for their papers on lung transplantations. Five authors on the list were cited for their research in the

Table 3 Number of publications and citations in transplant journals for transplantation-related research performed in the United States

Source	Citations	Documents	Total link strength
American Journal of Transplantation	194905	3954	41343
Transplantation	185248	4795	36949
Journal Of Heart and Lung Transplantation	96827	2388	11098
Liver Transplantation	74441	1815	11807
Nephrology Dialysis Transplantation	67526	1863	1779
Clinical Transplantation	32154	2082	15473
Cell Transplantation	25588	891	1668
Pediatric Transplantation	20700	1539	7404
Current Opinion in Organ Transplantation	13792	1029	10805
Transplant Infectious Disease	13246	932	3799
Transplantation Proceedings	12859	1166	4596
Transplant International	11987	624	5674
Xenotransplantation	11935	512	3930
Transplant Immunology	7017	342	2345
Progress In Transplantation	4622	550	2382
Transplantation Reviews	3010	153	2258
Experimental And Clinical Transplantation	1229	275	1272
Transplantation Direct	1183	341	2574
Annals of Transplantation	979	142	764
Transplantation And Cellular Therapy	902	375	166
Current Transplantation Reports	344	126	1366
International Journal of Organ Transplantation Medicine	35	7	35
Transplant Research and Risk Management	9	7	37
Indian Journal of Transplantation	0	9	52
Russian Journal of Transplantology and Artificial Organs	0	1	0

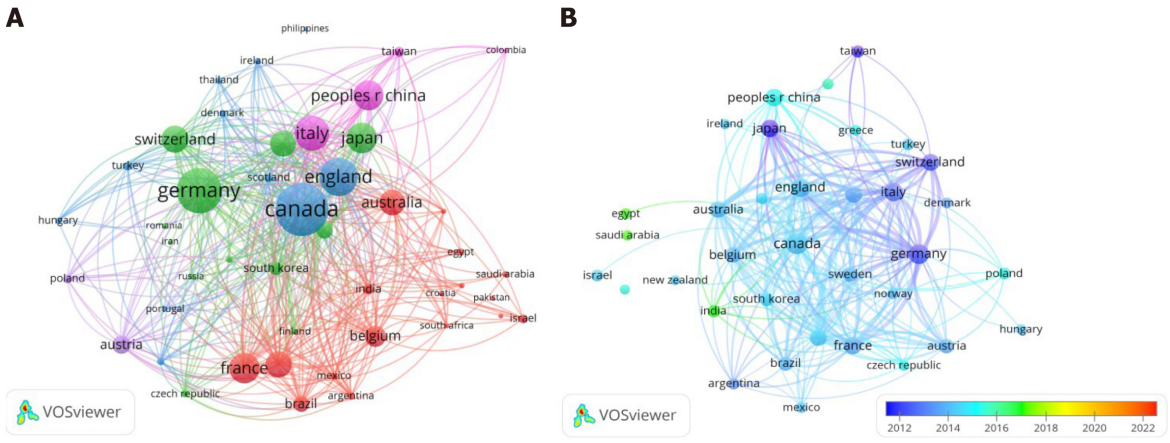

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Figure 3 Network visualization showing most cited countries contributed with the United States in the field of transplantation research. A and B: The size of the circles represents the weight of citations.

Table 4 Publications and citations of the top-collaborating countries with the United States

Country	Documents	Citations	Total link strength
Canada	1263	55702	28468
Germany	1012	44713	19065
England	807	36042	17916
Italy	719	33311	14855
France	598	32373	14968
Japan	594	21229	8395
China	579	13450	6755
Switzerland	506	25882	12413
Netherlands	479	18867	9232
Spain	478	24101	12506
Australia	473	24439	13353
Belgium	357	23002	9931
Austria	290	12571	7877
Sweden	266	11197	4958
Brazil	238	10420	6457
South Korea	197	6195	3019
India	138	4197	3039
Taiwan	129	4398	1949
Israel	116	2539	1121

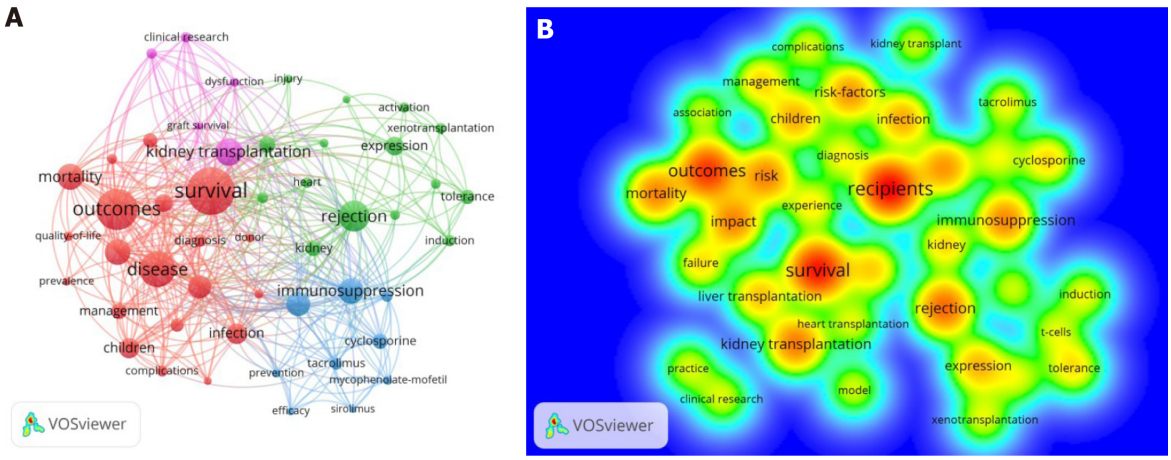

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Figure 4 Cluster map of the most occurring keywords and their interconnection across the years. A: Each circle represents a keyword, and the size of the circles represents the frequency of occurrence. Larger circles indicate that the keyword appears more frequently. Keywords included in the same cluster are displayed in the same color. The distance between the 2 circles shows the degree of the relationship; B: Overlay map of keyword occurrence over time for papers published on the field of transplantation from the United States. The red color represents the key words that have been discussed more frequently.

field of kidney transplantation, while two were cited for their work in liver transplantation. One author is a research analyst, and the majority of citations received are for the work on registries, which may not provide any insight into the scientific work. Two authors published in the field of transplant epidemiology, including the most cited author, Dorry Segev, who published 378 documents that received 12490 citations in transplant journals.

There is no doubt that solid organ transplantation is the area of medicine in the United States that has paid the most attention to the process of evaluating recipient and graft outcomes. The results of this study confirmed this assertion, as "survival" and "outcome" were the most frequently used keywords in connection to graft and recipient outcome measurements. The United Network for Organ Sharing and Organ Procurement and Transplantation Network in the

Table 5 Most occurring keywords in the research on transplantation from the United States

Keyword	Occurrences	Total link strength
Recipients	3045	13158
Survival	2779	11573
Outcomes	2399	10308
Rejection	1792	7691
Kidney transplantation	1605	7823
Mortality	1501	6280
Risk	1497	6105
Impact	1490	6486
Immunosuppression	1484	7371
Renal-transplantation	1403	6297
Therapy	1347	4974
Risk-factors	1340	5482
Infection	1195	4580
Children	1154	4366
Expression	1083	3647
Kidney-transplantation	1080	4763
Liver transplantation	1020	3976
Lung transplantation	931	3809

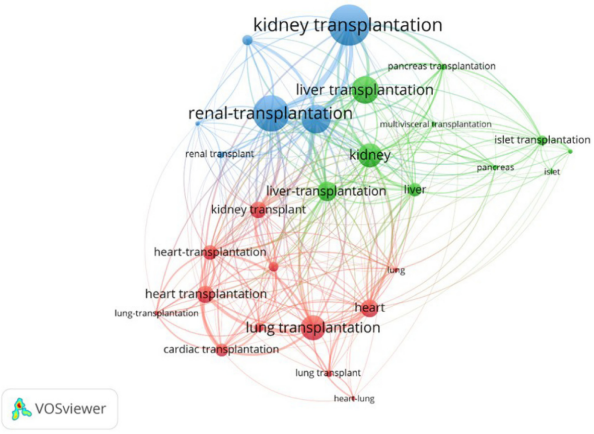

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Figure 5 Network visualization of the most common keyword after restricting the search to organs transplanted name. Larger circles indicate that the keyword appears more frequently. Keywords included in the same cluster are displayed in the same color. The distance between the 2 circles shows the degree of the relationship.

United States are responsible for monitoring the outcomes of organ transplantation centers and patients[20,21]. Outcome management aims to ensure that transplant recipients receive the best possible care and that the transplant successfully improves the recipient's quality of life. This is achieved by using the data and information collected during the transplant process to make decisions about the recipient's care and treatment.

Although the United States has made significant advances in transplantation over the past few decades, readers should keep in mind that counts of publications and citations conceal unmeasured characteristics like the density of knowledge in each article and data sets that accompany articles when assessing the data presented in this report. Publication numbers are one indicator of a nation's research output, but not the only one.

Some of these articles' limitations should be addressed. First, the primary emphasis of our analysis was on academic journals categorized under the "transplant" category within the Web of Science database. This approach was taken to ensure that our study included fundamental research in the field of transplantation. Although this approach is thorough,

it might overlook journals that are not primarily categorized under the field of 'transplantation' but occasionally publish articles that are pertinent to the topic. Second, we removed journals specific for bone marrow transplant and artificial organs journals; however, specific articles published in the journals included in our analysis may not be directly connected to solid organ transplant. Furthermore, articles pertinent to transplant may have been published in other journals not included in our analysis. Consequently, our investigation may offer a cautious approximation of transplantation studies. However, despite the presence of consistent criteria, our findings continue to provide significant insights.

CONCLUSION

The number of scientific research articles authored by American authors on the subject of organ transplantation has been steadily increasing over the past 25 years, which has paralleled the progression of the field as a whole. However, there has been a significant reduction in research production in 2022, which may demand observation and monitoring in the coming few years. The top three most frequent words in this study were "outcome," "survival," and "recipients," indicating that graft and recipient outcome measurements were of considerable significance to American researchers. The United States has made substantial contributions to the global advancement of organ transplantation practice. Nevertheless, there is room for substantial partnerships with other countries, particularly developing ones.

ARTICLE HIGHLIGHTS

Research background

Over the years, significant advancements have been made in the field of organ transplantation, saving innumerable lives and improving the quality of life for patients worldwide. The United States plays a pivotal role in advancing these advancements, contributing a substantial volume of research and clinical innovations. Given this position of leadership, ongoing assessments are essential for navigating the swiftly evolving landscape of transplantation research. These evaluations aid in identifying emerging trends, identifying areas of interest, and highlighting opportunities for international collaborations. Therefore, periodic evaluations, especially using techniques such as bibliometric analysis, are essential for maintaining the United States' cutting-edge contributions to this vital medical field and for guiding future research endeavors.

Research motivation

Bibliometric analysis is a useful method for systematically evaluating research output in order to navigate this ever-expanding landscape. This form of analysis provides a comprehensive overview of current research trends, influential publications, and key areas of concentration. It establishes a data-driven foundation for future research directions, ensuring that efforts are focused on areas with the greatest impact and need. Consequently, periodic bibliometric assessments are essential for maintaining the field's ongoing progress and sustaining the vitality of research efforts in this essential medical discipline.

Research objectives

This study's primary objective is to conduct a thorough analysis of the current status of organ transplantation research in the United States. Specific objectives include identifying the primary contributing authors and institutions, assessing the predominant research topics through keyword analysis, and determining the scope of international collaborations.

Research methods

In this first-of-its-kind study, we utilized the Web of Science database to perform a comprehensive bibliometric analysis. This resource was selected due to its extensive collection of academic journals. The software VOSviewer was used to visualize data, enabling the identification of key trends, such as top-contributing institutions and international collaborations. This bibliometric approach provided unprecedented insight into the prevalent research trends, major contributors, and key focus areas in United States organ transplantation research output.

Research results

This comprehensive analysis provides important insights into the current status of organ transplantation research in the United States. The University of Pittsburgh emerges as an important institution, indicating a concentration of expertise and potentially functioning as a hub for future cooperation. The American Journal of Transplantation's high number of citations demonstrates its reputation as a prominent venue for disseminating influential research, thereby influencing the field's practices and policies. Moreover, Canada's position as the United States' top international collaborator demonstrates the efficacy of global partnerships in advancing research. Despite these contributions, obstacles remain, such as the need for expanded international collaborations, particularly with developing nations, and the exploration of under researched areas in organ transplantation. These findings highlight the significance of ongoing evaluations in maintaining and advancing the field of study.

Research conclusions

In this study, a bibliometric analysis method was introduced to quantitatively evaluate the landscape of organ transplantation research authored by American researchers. Over the past 25 years, the number of scientific research articles written by American authors on the topic of organ transplantation has increased consistently, paralleling the overall development of the field. Nevertheless, a significant decline in research output was observed in 2022, necessitating close observation and monitoring in the years to come. Our study revealed that the three most frequently occurring keywords were outcome, survival and recipients, indicating the importance of graft and recipient outcome measurements to American researchers. Although the United States has made significant contributions to the global advancement of organ transplantation practice, there is still plenty of opportunity for collaboration with other nations, particularly developing countries.

Research perspectives

The study sheds new light on collaboration in organ transplantation research. By utilizing bibliometric methods, we were able to identify crucial collaboration patterns. Within the United States, the interdependence of prominent institutions is evident. An important international collaborator stands out. These insights suggest that future research could benefit from targeted collaborations that capitalize on the assets of prominent United States and international centers and authors. Particularly, unrealized potential exists for partnerships with developing nations to expand the global scope of organ transplantation research.

FOOTNOTES

Author contributions: Rawashdeh B and Alryalat SA contribute to planning and writing, VOSviewer analysis; Abuassi M contribute to literature review; Prasad R contribute to critical revision; Cooper M contribute to critical revision; all authors have read and approved the final manuscript.

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