Journal of
Clinical Medicine

Progress and Recent Advances in Solid Organ Transplantation

Edited by
Charat Thongprayoon, Wisit Cheungpasitporn and Wisit Kaewput

Printed Edition of the Special Issue Published in
Journal of Clinical Medicine

www.mdpi.com/journal/jcm

MDPI

Progress and Recent Advances in Solid Organ Transplantation

Progress and Recent Advances in Solid Organ Transplantation

Editors

Charat Thongprayoon
Wisit Cheungpasitporn
Wisit Kaewput

MDPI • Basel • Beijing • Wuhan • Barcelona • Belgrade • Manchester • Tokyo • Cluj • Tianjin

Editors

Charat Thongprayoon
Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic
USA

Wisit Cheungpasitporn
Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic
USA

Wisit Kaewput
Phramongkutklao College of Medicine
Thailand

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Journal of Clinical Medicine* (ISSN 2077-0383) (available at: https://www.mdpi.com/journal/jcm/special_issues/Solid_Organ_Transplantation).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-4007-8 (Hbk)
ISBN 978-3-0365-4008-5 (PDF)

Contents

About the Editors

Charat Thongprayoon is affiliated with the Mayo Clinic Hospital Rochester. Their research interests include nephrology, electrolytes, acute kidney injury, renal replacement therapy, epidemiology, and outcome studies.

Wisit Cheungpasitporn is American-board-certified in nephrology and internal medicine. He completed his nephrology fellowship training at the Mayo Clinic, Rochester, Minnesota. Dr. Cheungpasitporn also completed his additional training at Mayo and has become an expert on kidney transplantation. He also enrolled and completed his postdoctoral diploma in the clinical and translational science (CCaTS) program in 2015. Dr. Cheungpasitporn received the 2016 Donald C. Balfour Research Award, given in recognition of outstanding research as a junior scientist whose primary training is in a clinical field at the Mayo Clinic, Rochester, Minnesota, as well as the 2016 William H. J. Summerskill Award, given in recognition of outstanding achievement in research for a clinical fellow at the Mayo Clinic, Rochester, Minnesota. Dr. Cheungpasitporn has been part of Division of Nephrology and Hypertension at the Mayo Clinic, Rochester, MN, since 2020.

Wisit Kaewput is affiliated with the Phramongkutklao College of Medicine, Bangkok, Thailand. Their research interests include acute kidney injury, observational studies, statistical analysis, and epidemiology.

Journal of
Clinical Medicine

MDPI

Editorial

Progress and Recent Advances in Solid Organ Transplantation

Charat Thongprayoon [1], Wisit Kaewput [2], Pattharawin Pattharanitima [3] and Wisit Cheungpasitporn [1,*]

[1] Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN 55905, USA; charat.thongprayoon@gmail.com
[2] Department of Military and Community Medicine, Phramongkutklao College of Medicine, Bangkok 10400, Thailand; wisitnephro@gmail.com
[3] Department of Internal Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand; pattharawin@hotmail.com
* Correspondence: wcheungpasitporn@gmail.com

Over the past decade, the number of organ transplants performed worldwide has significantly increased for patients with advanced organ failure [1–5]. In the United States, 41,354 organ transplants were performed in 2021, increasing by 5.9% compared to 2020 [6]. While there have been significant improvements in the short-term survival of solid organ transplant recipients due to advances in immunosuppression and transplant techniques [1,2,7], long-term graft and patient outcomes still lag behind and remain areas for improvement in solid organ transplantation [2].

In this Special Issue, "Progress and Recent Advances in Solid Organ Transplantation", researchers from different disciplines with different expertise and resources highlighted the novelty of their recent investigations in the field of organ transplantation, including issues related to donors, allografts, and patient survival [8–20]. While there have been significant advances in regional and national kidney paired-donation programs in matching incompatible pairs, data suggest that there may be a role for desensitization in select cases to facilitate organ transplantation [21]. In this Special Issue, Weinhard et al. summarized the roles of tocilizumab and desensitization in kidney transplant candidates [18]. In addition to progress in desensitization and preoperative monitoring of donor-specific antibodies, this Special Issue also provided insights into the monitoring and management of chronic active antibody-mediated rejection [17]. Furthermore, investigators also shed light on post-transplant complication research, including osteoporotic fractures [9], diarrhea [15], psychological changes [19], and recurrent primary disease [17].

Immunosuppression management is essential for patient and graft survival in transplant recipients [22–24], and studies have demonstrated the impacts of tacrolimus metabolism rates on outcomes after transplantation [25–27]. In this Special Issue, Kolonko et al. found the novel findings of influences of body composition parameters assessed by bioimpedance analysis on the tacrolimus metabolism, which may potentially be useful in optimizing initial tacrolimus dosing [10]. Additionally, while fast tacrolimus metabolism is associated with lower renal function after kidney transplantation [26,27], in this Special Issue, Thölking et al. found no significant impact of fast tacrolimus metabolism on dyslipidemia parameters [13].

Better understanding of subgroups of transplant recipients, such as older transplant recipients and Black transplant recipients, can help the transplant community to identify individualized strategies to improve outcomes among these vulnerable populations [11,14,28]. In this Special Issue, Zompolas et al. conducted a retrospective study to evaluate outcomes of 85 kidney transplant recipients aged $\geq$ 75 years in the Eurotransplant Senior Program from January 2010 to July 2018 at the Charité-Universitätsmedizin Berlin in Germany [11]. The investigators demonstrated comparable outcomes among older patients compared to their younger counterparts [11], confirming excellent outcomes, including in patient and graft survival, in carefully selected older kidney transplant recipients

Citation: Thongprayoon, C.; Kaewput, W.; Pattharanitima, P.; Cheungpasitporn, W. Progress and Recent Advances in Solid Organ Transplantation. *J. Clin. Med.* **2022**, *11*, 2112. https://doi.org/10.3390/jcm11082112

Received: 6 April 2022
Accepted: 8 April 2022
Published: 11 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

aged ≥ 75 years [29–31]. Lastly, in this Special Issue, we reported outcomes of kidney transplant recipients with sickle cell disease (SCD) from an analysis of the 2000–2019 United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Network Database [14]. In this study, we found that SCD was significantly associated with lower patient survival and death-censored graft survival compared to non-SCD recipients. The findings of our study suggest that urgent future studies are required to identify strategies to improve outcomes in SCD kidney recipients. Additionally, the assignment of risk adjustment for SCD patients should be considered.

In summary, the findings published in this Special Issue provide novelty and additional knowledge and may help the transplant community to ultimately improve the management and outcomes of patients with solid organ transplantation.

Author Contributions: C.T., W.K., P.P. and W.C. contributed to the outlines of the manuscript. W.C. and C.T. drafted the manuscript. All authors gave comments on the earlier versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lentine, K.L.; Smith, J.M.; Hart, A.; Miller, J.; Skeans, M.A.; Larkin, L.; Robinson, A.; Gauntt, K.; Israni, A.K.; Hirose, R.; et al. OPTN/SRTR 2020 Annual Data Report: Kidney. *Am. J. Transplant.* **2022**, *22*, 21–136. [CrossRef]
2. Thongprayoon, C.; Hansrivijit, P.; Leeaphorn, N.; Acharya, P.; Torres-Ortiz, A.; Kaewput, W.; Kovvuru, K.; Kanduri, S.R.; Bathini, T.; Cheungpasitporn, W. Recent Advances and Clinical Outcomes of Kidney Transplantation. *J. Clin. Med.* **2020**, *9*, 1193. [CrossRef]
3. Larpparisuth, N.; Cheungpasitporn, W.; Lumpaopong, A. Global Perspective on Kidney Transplantation: Thailand. *Kidney360* **2021**, *2*, 1163–1165. [CrossRef] [PubMed]
4. Divard, G.; Goutaudier, V. Global Perspective on Kidney Transplantation: France. *Kidney360* **2021**, *2*, 1637–1640. [CrossRef] [PubMed]
5. Zhang, Z.; Liu, Z.; Shi, B. Global Perspective on Kidney Transplantation: China. *Kidney360* **2021**, *2*, 364. [CrossRef] [PubMed]
6. All-Time Records Again Set in 2021 for Organ Transplants, Organ Donation from Deceased Donors. Available online: https://unos.org/news/2021-all-time-records-organ-transplants-deceased-donor-donation/#:~{}:text=In%202021%2C%2041%2C354%20organ%20transplants,and%20Transplantation%20Network%20under%20federal (accessed on 2 April 2022).
7. Thongprayoon, C.; Kaewput, W.; Kovvuru, K.; Hansrivijit, P.; Kanduri, S.R.; Bathini, T.; Chewcharat, A.; Leeaphorn, N.; Gonzalez-Suarez, M.L.; Cheungpasitporn, W. Promises of Big Data and Artificial Intelligence in Nephrology and Transplantation. *J. Clin. Med.* **2020**, *9*, 1107. [CrossRef]
8. El-Bandar, N.; Lerchbaumer, M.H.; Peters, R.; Maxeiner, A.; Kotsch, K.; Sattler, A.; Miller, K.; Schlomm, T.; Hamm, B.; Budde, K.; et al. Kidney Perfusion in Contrast-Enhanced Ultrasound (CEUS) Correlates with Renal Function in Living Kidney Donors. *J. Clin. Med.* **2022**, *11*, 791. [CrossRef]
9. Jehn, U.; Kortenhorn, A.; Schütte-Nütgen, K.; Thölking, G.; Westphal, F.; Strauss, M.; Wennmann, D.O.; Pavenstädt, H.; Suwelack, B.; Görlich, D.; et al. The Influence of Parathyroidectomy on Osteoporotic Fractures in Kidney Transplant Recipients: Results from a Retrospective Single-Center Trial. *J. Clin. Med.* **2022**, *11*, 654. [CrossRef]
10. Kolonko, A.; Pokora, P.; Słabiak-Błaż, N.; Czerwieńska, B.; Karkoszka, H.; Kuczera, P.; Piecha, G.; Więcek, A. The Relationship between Initial Tacrolimus Metabolism Rate and Recipients Body Composition in Kidney Transplantation. *J. Clin. Med.* **2021**, *10*, 5793. [CrossRef]
11. Zompolas, I.; Peters, R.; Liefeldt, L.; Lehner, L.J.; Budde, K.; Ralla, B.; Goranova, I.; Maxeiner, A.; Lerchbaumer, M.H.; Marticorena Garcia, S.R.; et al. Outcomes of Deceased Donor Kidney Transplantation in the Eurotransplant Senior Program with A Focus on Recipients ≥75 Years. *J. Clin. Med.* **2021**, *10*, 5633. [CrossRef]
12. Chang, K.C.; Hsieh, Y.P.; Chao, H.N.; Lin, C.M.; Lin, K.H.; Tsai, C.C.; Heish, C.E.; Lin, P.R.; Kor, C.T.; Chen, Y.L.; et al. Association of Hematuria with Renal Progression and Survival in Patients Who Underwent Living Donor Liver Transplant. *J. Clin. Med.* **2021**, *10*, 4345. [CrossRef] [PubMed]
13. Thölking, G.; Schulte, C.; Jehn, U.; Schütte-Nütgen, K.; Pavenstädt, H.; Suwelack, B.; Reuter, S. The Tacrolimus Metabolism Rate and Dyslipidemia after Kidney Transplantation. *J. Clin. Med.* **2021**, *10*, 3066. [CrossRef]
14. Leeaphorn, N.; Thongprayoon, C.; Vaitla, P.; Hansrivijit, P.; Jadlowiec, C.C.; Mao, S.A.; Chewcharat, A.; Katari, S.; Pattharanitima, P.; Boonpheng, B.; et al. Outcomes of Kidney Transplant Recipients with Sickle Cell Disease: An Analysis of the 2000-2019 UNOS/OPTN Database. *J. Clin. Med.* **2021**, *10*, 3063. [CrossRef]

15. Rodrigues, F.G.; Swarte, J.C.; Douwes, R.M.; Knobbe, T.J.; Sotomayor, C.G.; Blokzijl, H.; Weersma, R.K.; Heilberg, I.P.; Bakker, S.J.L.; de Borst, M.H.; et al. Exhaled Hydrogen as a Marker of Intestinal Fermentation Is Associated with Diarrhea in Kidney Transplant Recipients. *J. Clin. Med.* **2021**, *10*, 2854. [CrossRef] [PubMed]
16. Hau, H.M.; Jahn, N.; Rademacher, S.; Sucher, E.; Babel, J.; Mehdorn, M.; Lederer, A.; Seehofer, D.; Scheuermann, U.; Sucher, R. The Value of Graft Implantation Sequence in Simultaneous Pancreas-Kidney Transplantation on the Outcome and Graft Survival. *J. Clin. Med.* **2021**, *10*, 1632. [CrossRef] [PubMed]
17. Nakamura, T.; Shirouzu, T. Antibody-Mediated Rejection and Recurrent Primary Disease: Two Main Obstacles in Abdominal Kidney, Liver, and Pancreas Transplants. *J. Clin. Med.* **2021**, *10*, 5417. [CrossRef]
18. Weinhard, J.; Noble, J.; Jouve, T.; Malvezzi, P.; Rostaing, L. Tocilizumab and Desensitization in Kidney Transplant Candidates: Personal Experience and Literature Review. *J. Clin. Med.* **2021**, *10*, 4359. [CrossRef]
19. Battaglia, Y.; Zerbinati, L.; Belvederi Murri, M.; Provenzano, M.; Esposito, P.; Andreucci, M.; Storari, A.; Grassi, L. Exploring the Level of Post Traumatic Growth in Kidney Transplant Recipients via Network Analysis. *J. Clin. Med.* **2021**, *10*, 4747. [CrossRef]
20. Westenberg, L.B.; van Londen, M.; Sotomayor, C.G.; Moers, C.; Minnee, R.C.; Bakker, S.J.L.; Pol, R.A. The Association between Body Composition Measurements and Surgical Complications after Living Kidney Donation. *J. Clin. Med.* **2021**, *10*, 155. [CrossRef]
21. Kuppachi, S.; Axelrod, D.A. Desensitization strategies: Is it worth it? *Transpl. Int.* **2020**, *33*, 251–259. [CrossRef]
22. Cheungpasitporn, W.; Lentine, K.L.; Tan, J.C.; Kaufmann, M.; Caliskan, Y.; Bunnapradist, S.; Lam, N.N.; Schnitzler, M.; Axelrod, D.A. Immunosuppression Considerations for Older Kidney Transplant Recipients. *Curr. Transplant. Rep.* **2021**, *8*, 100–110. [CrossRef] [PubMed]
23. Axelrod, D.A.; Cheungpasitporn, W.; Bunnapradist, S.; Schnitzler, M.A.; Xiao, H.; McAdams-DeMarco, M.; Caliskan, Y.; Bae, S.; Ahn, J.B.; Segev, D.L.; et al. Posttransplant Diabetes Mellitus and Immunosuppression Selection in Older and Obese Kidney Recipients. *Kidney Med.* **2022**, *4*, 100377. [CrossRef] [PubMed]
24. Lentine, K.L.; Cheungpasitporn, W.; Xiao, H.; McAdams-DeMarco, M.; Lam, N.N.; Segev, D.L.; Bae, S.; Ahn, J.B.; Hess, G.P.; Caliskan, Y.; et al. Immunosuppression Regimen Use and Outcomes in Older and Younger Adult Kidney Transplant Recipients: A National Registry Analysis. *Transplantation* **2021**, *105*, 1840–1849. [CrossRef]
25. Thongprayoon, C.; Hansrivijit, P.; Kovvuru, K.; Kanduri, S.R.; Bathini, T.; Pivovarova, A.; Smith, J.R.; Cheungpasitporn, W. Impacts of High Intra- and Inter-Individual Variability in Tacrolimus Pharmacokinetics and Fast Tacrolimus Metabolism on Outcomes of Solid Organ Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 2193. [CrossRef] [PubMed]
26. Thölking, G.; Schütte-Nütgen, K.; Schmitz, J.; Rovas, A.; Dahmen, M.; Bautz, J.; Jehn, U.; Pavenstädt, H.; Heitplatz, B.; Van Marck, V.; et al. A Low Tacrolimus Concentration/Dose Ratio Increases the Risk for the Development of Acute Calcineurin Inhibitor-Induced Nephrotoxicity. *J. Clin. Med.* **2019**, *8*, 1586. [CrossRef]
27. Thölking, G.; Siats, L.; Fortmann, C.; Koch, R.; Hüsing, A.; Cicinnati, V.R.; Gerth, H.U.; Wolters, H.H.; Anthoni, C.; Pavenstädt, H.; et al. Tacrolimus Concentration/Dose Ratio is Associated with Renal Function After Liver Transplantation. *Ann. Transplant.* **2016**, *21*, 167–179. [CrossRef]
28. Thongprayoon, C.; Jadlowiec, C.C.; Leeaphorn, N.; Bruminhent, J.; Acharya, P.C.; Acharya, C.; Pattharanitima, P.; Kaewput, W.; Boonpheng, B.; Cheungpasitporn, W. Feature Importance of Acute Rejection among Black Kidney Transplant Recipients by Utilizing Random Forest Analysis: An Analysis of the UNOS Database. *Medicines* **2021**, *8*, 66. [CrossRef]
29. Lønning, K.; Midtvedt, K.; Leivestad, T.; Reisæter, A.V.; Line, P.D.; Hartmann, A.; Heldal, K. Are Octogenarians With End-Stage Renal Disease Candidates for Renal Transplantation? *Transplantation* **2016**, *100*, 2705–2709. [CrossRef]
30. Huang, E.; Poommipanit, N.; Sampaio, M.S.; Kuo, H.T.; Reddy, P.; Gritsch, H.A.; Pham, P.T.; Wilkinson, A.; Danovitch, G.; Bunnapradist, S. Intermediate-term outcomes associated with kidney transplantation in recipients 80 years and older: An analysis of the OPTN/UNOS database. *Transplantation* **2010**, *90*, 974–979. [CrossRef]
31. Ravichandran, B.R.; Sparkes, T.M.; Masters, B.M.; Thomas, B.; Demehin, M.; Bromberg, J.S.; Haririan, A. Survival benefit of renal transplantation in octogenarians. *Clin. Transplant.* **2020**, *34*, e14074. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

The Association between Body Composition Measurements and Surgical Complications after Living Kidney Donation

Lisa B. Westenberg [1,2], Marco van Londen [2], Camilo G. Sotomayor [2], Cyril Moers [1], Robert C. Minnee [3], Stephan J. L. Bakker [2] and Robert A. Pol [1,*]

[1] Department of Surgery, University Medical Center Groningen, University of Groningen, 9713 GZ Groningen, The Netherlands; l.b.westenberg@umcg.nl (L.B.W.); c.moers@umcg.nl (C.M.)
[2] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, 9713 GZ Groningen, The Netherlands; m.van.londen@umcg.nl (M.v.L.); c.g.sotomayor.campos@umcg.nl (C.G.S.); s.j.l.bakker@umcg.nl (S.J.L.B.)
[3] Department of Surgery, Erasmus University Medical Center, Erasmus University Rotterdam, 3015 CN Rotterdam, The Netherlands; r.minnee@erasmusmc.nl
* Correspondence: r.pol@umcg.nl; Tel.: +31-503613382

Abstract: Obesity is considered a risk factor for peri- and postoperative complications. Little is known about this risk in overweight living kidney donors. The aim of this study was to assess if anthropometric body measures and/or surgical determinants are associated with an increased incidence of peri- and postoperative complications after nephrectomy. We included 776 living kidney donors who donated between 2008 and 2018 at the University Medical Center Groningen. Prenephrectomy measures of body composition were body mass index (BMI), body surface area (BSA), waist circumference, weight, and waist–hip ratio. Incidence and severity of peri- and postoperative complications were assessed using the Comprehensive Complication Index. Mean donor age was 53 ± 11 years; 382 (49%) were male, and mean BMI at donor screening was 26.2 ± 3.41 kg/m^2. In total, 77 donors (10%) experienced peri- and postoperative complications following donor nephrectomy. Male sex was significantly associated with fewer surgical complications (OR 0.59, 0.37–0.96 95%CI, $p = 0.03$) in binomial logistic regression analyses. Older age (OR: 1.03, 1.01–1.05 95%CI, $p = 0.02$) and a longer duration of surgery (OR: 1.01, 1.00–1.01 95%CI, $p = 0.02$) were significantly associated with more surgical complications in binomial logistic regression analyses. Multinomial logistic regression analyses did not identify any prenephrectomy measure of body composition associated with a higher risk of surgical complications. This study shows that higher prenephrectomy BMI and other anthropometric measures of body composition are not significantly associated with peri- and postoperative complications following living donor nephrectomy.

Keywords: living donation; nephrectomy; hand-assisted laparoscopic nephrectomy; body composition; complications

Citation: Westenberg, L.B.; van Londen, M.; Sotomayor, C.G.; Moers, C.; Minnee, R.C.; Bakker, S.J.L.; Pol, R.A. The Association between Body Composition Measurements and Surgical Complications after Living Kidney Donation. *J. Clin. Med.* **2021**, *10*, 155. https://doi.org/10.3390/jcm10010155

Received: 6 December 2020
Accepted: 31 December 2020
Published: 5 January 2021

1. Introduction

Transplantation of kidneys from living donors has many advantages in comparison with transplantation of deceased donor grafts. For patients with end-stage renal disease that undergo transplantation, patient and graft survival is better when transplanted with a graft from a living donor [1]. Although these findings support a need for more living kidney donors, the total number of living donor transplantations in the United States has remained constant since 2011 [2].

During screening for living kidney donation, body mass index (BMI) plays an important role in the assessment of a potential donor. A BMI ≥ 35 kg/m^2 has been associated with an increased risk of peri- and postoperative complications such as surgical site infection, deep venous thrombosis development, and incisional hernia [3–5]. This risk of surgical complications was not significant in individuals with a BMI < 30 kg/m^2 [6]. Therefore,

many transplantation centres have decided that donors with a BMI $\geq$ 35 kg/m^2 are not accepted for donation, and those who are obese with a BMI between 30 and 35 kg/m^2 are advised to make lifestyle changes to reduce their weight [7,8]. Interestingly, studies on the effect of BMI as a risk factor for surgical complications in living donor nephrectomies report contradictory results [9,10], and especially, little is known about this risk in donors with a BMI between 30 and 35 kg/m^2, who constitute a relatively large part of the living kidney donor population. Confronted with a changing living kidney donor pool due to the increasing prevalence of obesity worldwide [11] and the lack of a consensus on the threshold of BMI for living kidney donation acceptance criteria, the aim of this study is to assess whether BMI and other anthropometric body measures that are easily obtained in clinics are associated with an increased incidence of peri- and postoperative complications after nephrectomy.

2. Materials and Methods

2.1. Study Design

A total of 776 living kidney donors were included in this longitudinal prospective cohort study. Donor nephrectomies took place between 2008 and 2018 in the University Medical Center Groningen (The Netherlands). Potential donors were screened by a team of medical experts consisting of nephrologists, surgeons, radiologists, psychologists, and social workers. The main inclusion criterion was age >18 years of age at the time of donation. The exclusion criteria for donation were in accordance with the Dutch Guidelines for Evaluation of Potential Donors for Living Donor Kidney Transplantation from 2008 (i.e., BMI > 35 kg/m^2, unable to provide informed consent, manifested Diabetes Mellitus, major cardiovascular risk factors, prior kidney disease or glomerular filtration rate (GFR) of <60 mL/min $\times$ 1.73 m^2, monokidney, pregnancy, recent or active malignancies, chronic/active infection (e.g., HIV, HCV, HTLV, HBV), hypertension with end organ damage, inadequately regulated hypertension, proteinuria (>0.5 g/24 h), microscopical haematuria, and bilateral nephrolithiasis on CT scan) [9]. Informed consent was obtained from all participants.

Every donor underwent hand-assisted endoscopic donor nephrectomy, either laparoscopic (hand-assisted laparoscopy (HALN)) or retroperitoneoscopic (hand-assisted retroperitoneal nephrectomy (HARN)). Our hospital's hand-assisted donor nephrectomy procedure has been described in detail in a previous publication [12]. All living kidney donors donated to recipients >18 years of age. The study was approved by the institutional ethical review board (METc 2014/077). All procedures were conducted in accordance with the Declarations of Helsinki.

2.2. Data Collection

Data were collected as part of the TransplantLines research project conducted at the UMCG [13]. During all visits, donors' weight and height were measured by trained nurses. These measurements were used to calculate BMI (kg/m^2) and body surface area (BSA); the latter was calculated using the Du Bois and Du Bois equation [14], a method most widely used in clinical practice. Waist and hip circumference were also measured at each visit of the donor as part of the TransplantLines study. Waist–hip ratio was calculated as the quotient of waist circumference and hip circumference.

Additional anthropometric, clinical, and laboratory measurements were extracted from the digital hospital registration system. Surgical complications were assessed using the Comprehensive Complication Index (CCI) [15], a continuous scale that measures surgical morbidity, considering all complications according to the Clavien–Dindo classification [16]. The CCI considers the incidence of each complication, using a specific calculation that results in a score between 0 and 100. Complications were prospectively recorded, and for this study, a full description of the reported complication was retrieved from the complication registry of the surgical department or otherwise extracted from the digital hospital registration system at our university hospital. As part of our follow-up

protocol, donors regularly visit the hospital (i.e., 3 months, 1 year, 5 years, and 10 years after donation).

2.3. Statistical Analysis

Data were analysed using SPSS version 23.0 (IBM, Armonk, NY, USA). Categorical variables are presented as numbers with percentages and were analysed using the χ^2 test or Fisher's exact test. Normally distributed variables are presented as mean (standard deviation) and skewed variables are displayed as median [IQR], with analysis by means of Student's *t*-test and the Mann–Whitney U test, respectively. We have performed these analyses for the total study population, complication vs. no complication, and BMI < 30 kg/m^2 vs. BMI $\geq$ 30 kg/m^2.

Since the Comprehensive Complication Index, a continuous score, was not distributed as a continuous variable in our study population, we performed logistic regression analyses. To determine which factors are associated with surgical complications, binomial and multinomial logistic regression analyses were performed. Each variable with a value of $p < 0.05$ with our outcome (i.e., a CCI score > 0) and variables known from the literature to be risk factors for perioperative complications were included in multinomial logistic regression models. Since living kidney donors need to be healthy individuals, most risk factors for perioperative complications (e.g., major comorbidities) do not apply. We have included the most important risk factors for perioperative complications pertaining to living kidney donors [17] in our analyses: longer duration of surgery has been associated with an increased risk of surgical complications such as surgical site infections, venous thromboembolism, and bleeding [18]. In addition, prior abdominal surgery shows strong evidence of association with an increased risk of intra-abdominal adhesions, complicating the procedure possibly leading to surgical complications [19]. Surgical technique could also affect the complication rate, since hand-assistance is associated with fewer surgical complications than an open laparoscopic procedure, and a retroperitoneoscopic approach might be associated with even fewer surgical complications [20].

Since BMI, the duration of surgery, and the occurrence of complications are interconnected, we have performed mediation analyses to investigate whether the duration of surgery might act as a mediator (Figures S1 and S2, Supplementary Materials). This analysis shows that there is an association between BMI and the duration of surgery, and between the duration of surgery and perioperative complications. However, since mediation requires the presence of a direct effect (in this case, an association between BMI and CCI > 0), which was not the case in our analyses, potential mediation of an association between BMI and CCI > 0 by the duration of surgery could not be assessed. Therefore, the duration of surgery could be included in our multinomial logistic regression models.

Independent variables in the multinomial logistic regression models were the body measures, age, sex, previous abdominal surgery, donor nephrectomy technique, and the duration of surgery. The dependent outcome variable was the category of CCI score. In all analyses, two-tailed values of $p < 0.05$ were seen as evidence for the presence of an association.

3. Results

We included 776 living kidney donors. Mean age at screening was 53 (SD: 11) years, and 49% were male. Mean BMI at donor screening was 26.2 (SD: 3.41) kg/m^2 (Table 1). Mean waiting time between screening and donation was 9.7 months (SD: 12.8). The majority of donors donated their left kidney (n = 551, 72%), and the preferred surgical technique was hand-assisted laparoscopic donor nephrectomy in 679 (92%) donors. Conversion to another procedure was necessary in 17 (2%) cases (i.e., HARN to HALN in 13, HALN to open in 3, and HALN to HARN in 1). Mean duration of surgery was 215 (SD: 50) min, median blood loss was 50 [IQR: 50–150] mL, and median hospital length of stay was 4 [IQR: 4–5] days (Table 1).

Table 1. Characteristics of the study population.

	Study Population	Donors without Complication	Donors with Complication	*p*
	n = 776	*n* = 699	*n* = 77	
Gender, *n* (%)				
Male	382 (49.2%)	353 (50.5%)	29 (37.7%)	0.04
Age at nephrectomy, years	54 ± 11	53.7 ± 11	56.8 ± 11	0.02
Weight, kg	80.5 ± 13.2	80.8 ± 13.3	78.2 ± 12.6	0.11
Length, cm	175.1 ± 9.6	175.3 ± 9.5	173.7 ± 10.6	0.15
BMI, kg/m^2	26.2 ± 3.41	26.2 ± 3.4	25.9 ± 3.4	0.43
BSA, m^2	1.96 ± 0.20	1.96 ± 0.19	1.92 ± 0.20	0.1
Hip size, cm	98.9 ± 7.6	98.9 ± 7.6	98.8 ± 7.4	0.93
Waist size, cm	91.1 ± 10.5	91.2 ± 10.6	91.0 ± 10.2	0.87
Waist–hip ratio	0.92 ± 0.11	0.92 ± 0.11	0.92 ± 0.09	0.84
Blood pressure				
Systolic, mmHg	126.4 ± 12.8	126.4 ± 12.9	126.6 ± 12.3	0.91
Diastolic, mmHg	75.7 ± 8.99	75.7 ± 9.0	75.9 ± 9.2	0.89
mGFR, mL/min × 1.73 m^2	112.8 ± 22.4	113.1 ± 22.7	109.6 ± 19.4	0.19
Side nephrectomy				
Left, *n* (%)	551 (72%)	495 (71.9%)	56 (72.7%)	0.99
Right, *n* (%)	214 (28%)	193 (28.1%)	21 (27.3%)	
Previous abdominal surgery, *n* (%)	26 (3.4%)	25 (3.6%)	1 (1.3%)	0.47
Surgical technique				
HALN, *n* (%)	679 (92.3%)	605 (91.8%)	74 (96.1%)	0.27
HARN, *n* (%)	55 (7.5%)	54 (8.2%)	1 (1.3%)	0.05
Open, *n* (%)	2 (0.3%)	0 (0%)	2 (2.6%)	0.003
Duration of surgery, min	215 ± 50	213 ± 51	228 ± 47	0.02
Blood loss, mL	50.0 (50.0–150.0)	50.0 (50.0–100.0)	125.0 (50.0–462.5)	<0.001
HLOS, days	4.0 (4.0–5.0)	4.0 (4.0–5.0)	5.0 (5.0–8.0)	<0.001
Conversion rate, *n* (%)				
No, primary HALN	666 (90.5%)	593 (90.0%)	73 (94.8%)	0.25
No, primary HARN	53 (7.2%)	52 (7.9%)	1 (1.3%)	0.06
No, primary open	0 (0%)	0 (0%)	0 (0%)	
Conversion HARN to HALN	13 (1.8%)	12 (1.8%)	1 (1.3%)	1
Conversion HARN to open	0 (0%)	0 (0%)	0 (0%)	
Conversion HALN to open	3 (0.4%)	1 (0.2%)	2 (2.6%)	0.03
Conversion HALN to HARN	1 (0.1%)	1 (0.2%)	0 (0%)	1

Values of variables are given as mean ± standard deviation, median [interquartile range], or *n* (%); BMI, body mass index (kg/m^2); BSA, body surface area (m^2); mGFR, measured glomerular filtration rate (mL/min × 1.73 m^2); HALN, hand-assisted laparoscopy; HARN, hand-assisted retroperitoneal nephrectomy; HLOS, hospital length of stay.

The results of our subanalysis with a BMI < 30 kg/m^2 vs. BMI ≥ 30 kg/m^2 (Table S1, Supplement) show that donors with a BMI ≥ 30 kg/m^2 had a higher measured GFR before donation (120.0 (SD: 24.0) vs. 111.5 (SD: 21.9) mL/min × 1.73 m^2). Donors with a BMI ≥ 30 kg/m^2 also had a longer duration of surgery (224.9 (SD: 50.1) vs. 213.0 (SD: 50.4) min) a and longer hospital length of stay (five [IQR: 4–5] vs. four [IQR: 4–5] days).

3.1. Surgical Determinants and Complications after Donor Nephrectomy

A total of 77 donors (10%) experienced peri- or postoperative complications following donor nephrectomy (Table 1). The most frequent complications were perioperative bleeding (19 donors, 22%), iatrogenic spleen lesion (13 donors, 15%), urinary retention (7 donors, 8%), or iatrogenic colon lesion (5 donors, 6%). The distribution of CCI scores for all complications is shown in Table 2. An overview of all complications is displayed in

Supplementary Table S2. Forty-three donors experienced complications that required secondary surgical interventions. More female donors experienced complications compared to male donors (48 (12%) versus 29 (8%) male donors; $p = 0.04$). Donors that were older (mean: 57 (SD: 11) versus 54 (SD: 11) years; $p = 0.02$), those with a longer duration of surgery (228 (SD: 47) versus 213 (SD: 51) min; $p = 0.02$), those experiencing more intraoperative blood loss (125 [IQR: 50–463] versus 50 [IQR: 50–100] mL; $p < 0.001$), and those who had a longer hospital length of stay (five [IQR: 5–8] versus four [IQR: 4–5] days; $p < 0.001$) more frequently experienced a complication. All donors who underwent open donor nephrectomy experienced conversion from HALN to open due to a complication.

Table 2. Distribution of Comprehensive Complication Index scores within the study population.

CCI Score	Number of Donors *n* (%)
0	699 (90)
8.7	20 (3)
12.2	1 (0.1)
20.9	11(1.4)
22.6	1 (0.1)
29.6	1 (0.1)
33.7	41 (5)
35.9	1 (0.1)
44.9	1 (0.1)

3.2. Determinants of Surgical Complications Following the Comprehensive Complication Index

Binomial logistic regression analyses with different body measures showed no significant association between BMI and surgical complications (OR for CCI > 0 vs. CCI = 0; 0.97, 0.91–1.04 95%CI, $p = 0.43$) (Table 3). Male gender was significantly associated with fewer surgical complications in binomial logistic regression analysis (OR: 0.59, 0.37–0.96 95%CI, $p = 0.03$). Older age (OR: 1.03, 1.01–1.05 95%CI, $p = 0.02$) and a longer duration of surgery (OR: 1.01, 1.00–1.01 95%CI, $p = 0.02$) were also associated with more surgical complications in binomial logistic regression analysis. In subanalyses among donors with a BMI between 30 and 35 kg/m^2, we found no significant association with peri- and postoperative complications following nephrectomy (Table 3). Following multinomial logistic regression analysis with correction for possible confounders, no measure for body composition was a significant determinant of surgical complications (Table 4).

Table 3. Binomial logistic regression analysis of the association of anthropometrics with surgical complications (Comprehensive Complication Index score).

	Odds Ratio for CCI > 0 vs. CCI = 0		
	OR	**95% CI**	***p*-Value**
Gender			
Female	1	-	-
Male	0.59	0.37–0.96	0.03
Age, years	1.03	1.01–1.05	0.02
Previous abdominal surgery			
No	1	-	-
Yes	2.82	0.38–21.1	0.31
Surgical technique			
HALN	1	-	-
HARN	6.61	0.90–48.4	0.06
Duration surgery, min	1.01	1.00–1.01	0.02
Weight, kg	0.99	0.97–1.00	0.11
BMI, kg/m^2	0.97	0.91–1.04	0.43
BMI 30–34.99 kg/m^2 [a]	1.32	0.64–2.73	0.45
BSA, m^2	0.35	0.10–1.21	0.1
Waist circumference, cm	1	0.97–1.02	0.87
Waist–hip ratio	0.78	0.07–8.91	0.84

The reference category is CCI = 0 (i.e., no complication). BMI, body mass index (kg/m^2); BSA, body surface area (m^2); HALN, hand-assisted laparoscopy; HARN, hand-assisted retroperitoneal nephrectomy. a. A total of nine (8%) of donors with a BMI of 30–34.99 kg/m^2 experienced one or more surgical complications.

Table 4. Multinomial logistic regression analysis of the association of anthropometrics with surgical complications (with Comprehensive Complication Index score classified into categories).

	CCI 0.1–20.0		CCI 20.1–30.0		CCI > 30.0	
	n = 21		*n* = 13		*n* = 43	
	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Weight, kg						
Model 1	0.99 (0.95–1.03)	0.59	1.01 (0.97–1.06)	0.61	0.99 (0.96–1.02)	0.56
Model 2	0.99 (0.95–1.03)	0.51	1.02 (0.97–1.07)	0.46	0.98 (0.95–1.01)	0.27
BMI, kg/m^2						
Model 1	0.92 (0.81–1.06)	0.24	0.93 (0.79–1.10)	0.42	1.00 (0.92–1.10)	0.92
Model 2	0.91 (0.79–1.04)	0.18	0.96 (0.82–1.14)	0.66	0.96 (0.86–1.06)	0.39
BSA, m^2						
Model 1	0.77 (0.04–15.4)	0.87	13.4 (0.36–490.0)	0.16	0.40 (0.05–3.44)	0.4
Model 2	0.71 (0.04–14.2)	0.82	14.2 (0.35–566.9)	0.16	0.31 (0.03–3.46)	0.34
Waist circumference, cm						
Model 1	1.00 (0.95–1.05)	0.89	0.99 (0.94–1.06)	0.82	1.01 (0.98–1.04)	0.57
Model 2	0.99 (0.95–1.04)	0.74	1.01 (0.95–1.07)	0.69	0.99 (0.96–1.03)	0.72
Waist–hip ratio						
Model 1	1.38 (0.01–188.6)	0.9	0.03 (0.00–58.5)	0.37	3.73 (0.18–76.3)	0.39
Model 2	0.88 (0.01–119.9)	0.96	0.36 (0.00–324.1)	0.77	1.98 (0.05–75.5)	0.71

The Comprehensive Complication Index (CCI) [15] score is classified into categories (CCI 0.1–20.0; CCI 20.1–30.0; CCI > 30.0) and compared to the reference category of CCI = 0 (e.g., no complication). BMI, body mass index (kg/m^2); BSA, body surface area (m^2). Model 1 is age- and sex-adjusted. Model 2 is adjusted for age, sex, previous abdominal surgery, donor nephrectomy technique, and duration of surgery.

4. Discussion

This study showed that in our cohort of living donors, there was no significant association between BMI or other anthropometric body measures and peri- and postoperative complications.

Higher BMI has previously been associated with an increased risk of peri- and postoperative complications in different study populations. Incidence of surgical site infection increases with increasing BMI in general surgery patients [3], possibly due to low regional perfusion and oxygen tension resulting from excessive subcutaneous fat tissue impairing wound healing. Duration of surgery is also often prolonged in obese individuals [21], adding to the risk of surgical site infection [22]. Obese individuals were at increased risk of major postoperative complications following surgery for gastrointestinal malignancy and renal cancer [23,24]. Due to a larger BSA and more complex fluid management, risk of intraoperative hypothermia is increased in obese individuals, predisposing them to surgical and thromboembolic complications [5,23]. In overweight and obese donors, a larger extraction incision is usually necessary due to the thicker layer of adipose tissue, leading to a higher risk for abdominal wall complications (e.g., incisional hernia and wound infections) [6,25].

Different from the aforementioned studies, our results, from one of the largest prospective cohorts, suggest no significant deleterious effect of high fat mass on peri- and postoperative complications following donation. A possible explanation might be that living kidney donors differ significantly from other surgical populations in which they have little to no comorbidities at the time of surgery [18]. Although the donor population in our study did not allow an analysis of donors with a BMI $\geq$ 35 kg/m^2, we found no evidence of an association between BMI and surgical complications following donation in subanalyses of obese donors with a BMI between 30 and 35 kg/m^2.

Whether BMI is the best way to measure obesity remains unclear. Various other anthropometric measures, such as BSA, waist circumference, and waist–hip ratio, are also frequently used in clinical settings but with varying results with respect to each other. Cross-sectional surveys evaluating the predictive power of BMI and waist circumference have shown waist circumference to be a better predictor for obesity-related comorbidities

than solely BMI [26,27]. In assessing obesity-related renal effects, waist–hip ratio appears to be superior to both BMI and waist circumference [28].

We detected strong evidence of an association between longer duration of surgery and peri- and postoperative complications following donor nephrectomy, which are, of course, interconnected. Although the occurrence of a surgical complication might result in a prolonged duration of surgery, the likelihood of surgical complications such as surgical site infections, venous thromboembolism, bleeding, hematoma formation, and necrosis also increases with prolonged duration of surgery [29]. A recent systematic review and meta-analysis demonstrated that the risk of complications approximately doubled with prolonged operative duration and the risk of surgical complications increased by 14% for every 30 min of additional operating time [29]. Although the underlying mechanisms are not yet fully understood, a prolonged microbial exposure [30] and a diminishing efficacy of antimicrobial prophylaxis over time [31] appear to be contributing factors. Venous thromboembolism formation is more likely to occur with prolonged surgical procedures due to an increased risk of blood stasis, coagulation activation, and endothelial damage, also known as Virchow's triad [32]. Obesity prolongs the duration of surgery and can therefore also lead to a higher risk of aforementioned complications [22].

Living kidney donors are a unique group of surgical patients, given that a low comorbidity burden is required to be eligible for donation. In current donor screening guidelines, BMI is a widely applied measure for assessing obesity [9]. It is, however, a poor estimate of fat mass distribution. Muscular individuals or those with more subcutaneous fat can have a similar BMI to individuals with more visceral fat, but these different types of high BMI are associated with different disease risks [33]. Contradictory to what is generally known about obesity and its effect on disease and mortality risk, some studies show a protective effect of high BMI in patients [34]. This apparent protective effect is often referred to as the "obesity paradox" and also underlines that BMI poorly reflects the actual balance or imbalance in fat mass distribution and muscle volume. Therefore, we need to incorporate more reliable tools to measure body composition when defining obesity and determining its effect on postsurgical outcome. Bioelectrical impedance analysis (BIA) is a tool for assessing body composition by measuring the resistance of the body as a conductor to a very small alternating electrical current. This technique might provide a more detailed and reliable analysis of fat and muscle mass, enabling assessment of the association between these two determinants of body composition and peri- and postoperative complications after surgery. This method, however, is not yet sufficiently validated among living kidney donors. Another promising technique to assess the risk of surgical complications following donor nephrectomy is a volumetric measurement of perirenal fat mass based on CT-scans, which shows a stronger correlation with outcome measures of laparoscopic donor nephrectomy than BMI alone [35]. Future studies, also by our group, should investigate other parameters defining the outcome of donation based on body composition and BMI, such as slow or delayed graft function, long-term renal function, and development of comorbidity. There is also a call for more studies assessing obesity in living kidney donors with a variety of ethnicities, especially since donors with an African background seem to more commonly be obese and develop conditions such as chronic kidney disease, proteinuria, and nephrotic syndrome [36,37]. In addition, data on lifetime risk of chronic kidney disease and mortality in young living kidney donors are sparse and should be a focus of future studies.

Our study has a few limitations that need to be addressed. Although our study consists of a large cohort of living kidney donors and missing data for predonation body measures was limited in this study, the exclusion criteria that were applied in donor screening [9] (e.g., BMI > 35 kg/m^2, manifested Diabetes Mellitus, major cardiovascular risk factors, proteinuria > 0.5 g/24 h) affected our results. Especially the exclusion of potential donors with a BMI > 35 kg/m^2 resulted in a narrow range of BMI, making our study population a selection of the total group of living kidney donors, which might not be representative to other kidney donor populations where this criterion is not applied in donor screening guidelines. The incidence of complications in the subgroup with a BMI of 30–34.99 was low.

Future studies with the inclusion of a larger number of donors with a BMI in this range are required to further investigate the effect of living kidney donation on the development of complications in this group. Although our study shows a similar complication rate to observations in the United States [22], complications might have been underreported, especially following procedures performed in the early years of our data collection, which might have influenced the results. Furthermore, living kidney donors are part of a highly selected population. Therefore, our results cannot automatically be extrapolated to other kinds of surgery and to other populations. We have used literature on living kidney donors when available but referred to other kinds of surgery or other study populations when this was lacking.

In conclusion, this study shows no strong evidence of an association between BMI and other anthropometric body measures and peri- and postoperative complications following donor nephrectomy and should therefore be no reason to refrain from surgery.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10010155/s1, Figure S1: Standardized Regression Coefficients and Odds Ratios for the Relationship Between Body Mass Index and Comprehensive Complications Index score above zero as Mediated by Duration of Surgery, Figure S2: Standardized Regression Coefficients and Odds Ratios for the Relationship Between Duration of Surgery and Comprehensive Complications Index score above zero as Mediated by Body Mass Index, Table S1: Characteristics of the study population by BMI category, Table S2: Overview of complications.

Author Contributions: Conceptualization, L.B.W., M.v.L., S.J.L.B., and R.A.P.; data curation, L.B.W.; formal analysis, L.B.W.; methodology, L.B.W.; supervision, M.v.L., S.J.L.B., and R.A.P.; writing—original draft, L.B.W.; writing—review and editing, M.v.L., C.G.S., C.M., R.C.M., S.J.L.B., and R.A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of University Medical Center Groningen (METc 2014/077).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Axelrod, D.; McCullough, K.; Brewer, E.; Becker, B.; Segev, D.; Rao, P. Kidney and Pancreas Transplantation in the United States, 1999-2008: The Changing Face of Living Donation. *Am. J. Transplant.* **2010**, *10*, 987–1002. [CrossRef] [PubMed]
2. Hart, A.; Smith, J.; Skeans, M.; Gustafson, S.; Wilk, A.; Robinson, A. OPTN/SRTR 2016 Annual Data Report: Kidney. *Am. J. Transplant.* **2018**, *18*, 18–113. [CrossRef] [PubMed]
3. Tjeertes, E.; Hoeks, S.; Beks, S.; Valentijn, T.; Hoofwijk, A.; Stolker, R. Obesity—A Risk Factor for Postoperative Complications in General Surgery? *BMC Anesthesiol.* **2015**, *15*, 112. [CrossRef] [PubMed]
4. Klovaite, J.; Benn, M.; Nordestgaard, B. Obesity as a causal risk factor for deep venous thrombosis: A Mendelian randomization study. *J. Intern. Med.* **2014**, *277*, 573–584. [CrossRef] [PubMed]
5. Arfi, N.; Baldini, A.; Decaussin-Petrucci, M.; Ecochard, R.; Ruffion, A.; Paparel, P. Impact of Obesity on Complications of Laparoscopic Simple or Radical Nephrectomy. *Curr. Urol.* **2014**, *8*, 149–155. [CrossRef]
6. Flancbaum, L.; Chobab, P.S. Surgical implications of obesity. *Ann. Rev. Med.* **1998**, *49*, 215–234. [CrossRef]
7. Mandelbrot, D.; Pavlakis, M.; Danovitch, G.; Johnson, S.R.; Karp, S.J.; Khwaja, K.; Hanto, D.W.; Rodrigue, J.R. The Medical Evaluation of Living Kidney Donors: A Survey of US Transplant Centers. *Am. J. Transplant.* **2007**, *7*, 2333–2343. [CrossRef]
8. Landelijk Overleg Niertransplantatie; van der Heide, J.H. *Nederlandse Richtlijn: Evaluatie Van Potentiële Donoren Voor Levende Donor Niertransplantatie*; NTV: Haarlem, The Netherlands, 2008; pp. 23–27.
9. Heimbach, J.; Taler, S.; Prieto, M.; Cosio, F.G.; Textor, S.C.; Kudva, Y.C.; Chow, G.K.; Ishitani, M.B.; Larson, T.S.; Stegall, M.D. Obesity in Living Kidney Donors: Clinical Characteristics and Outcomes in the Era of Laparoscopic Donor Nephrectomy. *Am. J. Transplant.* **2005**, *5*, 1057–1064. [CrossRef]

10. Unger, L.; Feka, J.; Sabler, P.; Rasoul-Rockenshaub, S.; Gyori, G.; Holfmann, M.; Schwarz, C.; Soliman, T.; Bohmig, G.; Kains, A. High BMI and male sex as risk factor for increased short-term renal impairment in living kidney donors—Retrospective analysis of 289 consecutive cases. *IJS* **2017**, *46*, 172–177. [CrossRef]
11. Taler, S.J.; Messersmith, E.E.; Leichtman, A.B.; Gillespie, B.W.; Kew, C.E.; Stegall, M.D.; Merion, R.M.; Matas, A.J.; Ibrahim, H.N. Demographic, Metabolic, and Blood Pressure Characteristics of Living Kidney Donors Spanning Five Decades. *Am. J. Transplant.* **2013**, *13*, 390–398. [CrossRef]
12. Zorgdrager, M.; van Londen, M.; Westenberg, L.; Nieuwenhuijs-Moeke, G.J.; Lange, J.F.M.; Borst, M.H.; Bakker, S.J.L.; Leu-venink, H.G.D.; Pol, R.A. Chronic pain after hand-assisted laparoscopic donor nephrectomy. *BJS* **2019**, *106*, 711–719. [CrossRef] [PubMed]
13. Eisenga, M.; Gomes-Neto, A.; van Londen, M.; Ziengs, A.; Douwes, R.M.; Stam, S.P.; Osté, M.C.J.; Knobbe, T.J.; Hessels, N.R.; Buunk, A.M. Rationale and design of TransplantLines: A prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* **2018**, *8*, e024502. [CrossRef]
14. Du Bois, D.; Du Bois, E. A formula to estimate the approximate surface area if height and weight be known. *Nutrition* **1989**, *5*, 303–311. [PubMed]
15. Slankamenac, K.; Graf, R.; Barkun, J.; Puhan, M.A.; Clavien, P.-A. The Comprehensive Complication Index. *Ann. Surg.* **2013**, *258*, 1–7. [CrossRef] [PubMed]
16. Dindo, D.; Demartines, N.; Clavien, P. Classification of Surgical Complications. *Ann. Surg.* **2004**, *240*, 205–213. [CrossRef]
17. Alberts, V.; Idu, M.M.; Minnee, R.C. Risk factors for perioperative complications in hand-assisted laparoscopic donor nephrectomy. *Prog. Transplant.* **2014**, *24*, 192–198. [CrossRef]
18. Patel, S.; Cassuto, J.; Orloff, M.; Tsoulfas, G.; Zand, M.; Kashyap, R.; Jain, A.; Bozorgzadeh, A.; Abt, P. Minimizing Morbidity of Organ Donation: Analysis of Factors for Perioperative Complications After Living-Donor Nephrectomy in the United States. *Transplantation* **2008**, *85*, 561–565. [CrossRef]
19. Menzies, D. Peritoneal adhesions. Incidence, cause, and prevention. *Surg. Annu.* **1992**, *24*, 27–45.
20. Özdemir-van Brunschot, D.M.; Koning, G.G.; van Laarhoven, K.C.; Ergün, M.; van Horne, S.B.; Rovers, M.M.; Warlé, M.C. A comparison of technique modifications in laparoscopic donor nephrectomy: A systematic review and meta-analysis. *PLoS ONE* **2015**, *10*, e0121131. [CrossRef]
21. Ri, M.; Aikou, S.; Seto, Y. Obesity as a surgical risk factor. *Ann. Gastroenterol. Surg.* **2017**, *2*, 13–21. [CrossRef]
22. Kurmann, A.; Vorburger, S.; Candinas, D.; Beldi, G. Operation time and body mass index are significant risk factors for surgical site infection in laparoscopic sigmoid resection: A multicenter study. *Surg. Endosc.* **2011**, *25*, 3531–3534. [CrossRef] [PubMed]
23. STARSurg Collaborative; Bhangu, A.; Drake, T.; Nepogodiev, D. Multicentre prospective cohort study of body mass index and postoperative complications following gastrointestinal surgery. *BJS* **2016**, *103*, 1157–1172.
24. Kurzer, E.; Leveillee, R.; Bird, V. Obesity as a Risk Factor for Complications During Laparoscopic Surgery for Renal Cancer: Multivariate Analysis. *J. Endourol.* **2006**, *20*, 794–799. [CrossRef] [PubMed]
25. Barlas, I.; Aydogdu, I.; Sinangil, A.; Zuhal, A.U.; Yeney, K.; Tevfik, E.; Emin, B.A. Hand-Assisted Nephrectomy Predisposes Incisional Herniation in Obese Living Donors. *Transplant. Proc.* **2019**, *51*, 2210–2214. [CrossRef]
26. Janssen, I.; Katzmarzyk, P.T.; Ross, R. Waist circumference and not body mass index explains obesity-related health risk. *Am. J. Clin. Nutr.* **2004**, *79*, 379–384. [CrossRef]
27. Rexrode, K.M.; Carey, V.J.; Hennekens, C.H.; Walters, E.E.; Colditz, G.A.; Stampfer, M.J.; Willett, W.C.; Manson, J.E. Abdominal Adiposity and Coronary Heart Disease in Women. *JAMA* **1998**, *280*, 1843–1848. [CrossRef]
28. Stefansson, V.T.N.; Schei, J.; Jenssen, T.G.; Melsom, T.; Eriksen, B.O. Central obesity associates with renal hyperfiltration in the non-diabetic general population: A cross-sectional study. *BMC Nephrol.* **2016**, *17*, 1–10. [CrossRef]
29. Cheng, H.; Clymer, J.; Po-Han Chen, B.; Sadeghirad, B.; Ferko, N.C.; Cameron, C.G.; Hinoul, P. Prolonged operative duration is associated with complications: A systematic review and meta-analysis. *J. Sur. Res.* **2018**, *229*, 134–144. [CrossRef]
30. Korol, E.; Johnston, K.; Waser, N.; Sifakis, F.; Jafri, H.S.; Lo, M.; Kyaw, M.H. A Systematic Review of Risk Factors Associated with Surgical Site Infections among Surgical Patients. *PLoS ONE* **2013**, *8*, e83743. [CrossRef]
31. Campbell, D.; Henderson, W.; Englesbe, M.; Hall, B.L.; O'Reilly, M.; Bratzler, D.; Dellinger, E.P.; Neumayer, L.; Bass, B.L.; Hutter, M.M. Surgical Site Infection Prevention: The Importance of Operative Duration and Blood Transfusion—Results of the First American College of Surgeons–National Surgical Quality Improvement Pro-gram Best Practices Initiative. *J. Am. Coll. Surg.* **2008**, *207*, 810–820. [CrossRef]
32. Kim, J.; Khavanin, N.; Rambachan, A. Surgical Duration and Risk of Venous Thromboembolism. *JAMA Surg.* **2015**, *150*, 110. [CrossRef] [PubMed]
33. Forouzanfar, M.; Alexander, L.; Anderson, H.; GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: A systematic analysis for the Global Burden of Disease Study. *Lancet* **2015**, *386*, 2287–2323. [CrossRef]
34. Hainer, V.; Aldhoon-Hainerova, I. Obesity Paradox Does Exist. *Diabetes Care* **2013**, *36*, S276–S281. [CrossRef] [PubMed]
35. Lafranca, J.; Prens, L.; Moelker, A.; Koek, M.; Niessen, W.; IJzermans, J.; Dor, F. Perirenal and Intra-Abdominal Fat Mass is Superior to BMI in Predicting Outcome of Laparoscopic Donor Nephrectomy. *Transplantation* **2014**, *98*, 612. [CrossRef]
36. Lentine, K.L.; Schnitzler, M.A.; Garg, A.X.; Xiao, H.; Axelrod, D.; Tuttle-Newhall, J.E.; Brennan, D.C.; Segev, D.L.R. Relationship and Renal Diagnoses after Living Kidney Donation. *Transplantation* **2015**, *99*, 1723–1729. [CrossRef]

37. Locke, J.E.; Reed, R.D.; Massie, A.; MacLennan, P.A.; Sawinski, D.; Kumar, V.; Mehta, S.; Mannon, R.; Gaston, R.; Lewis, C.E. Obesity increases the risk of end-stage renal disease among living kidney donors. *Kidney Int.* **2017**, *99*, 699–703. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

The Value of Graft Implantation Sequence in Simultaneous Pancreas-Kidney Transplantation on the Outcome and Graft Survival

Hans-Michael Hau [1,2,*,†], Nora Jahn [3,†], Sebastian Rademacher [1], Elisabeth Sucher [4], Jonas Babel [1], Matthias Mehdorn [1], Andri Lederer [1], Daniel Seehofer [1], Uwe Scheuermann [1,‡] and Robert Sucher [1,‡]

1 Department of Visceral, Transplantation, Vascular and Thoracic Surgery, University Hospital of Leipzig, 04103 Leipzig, Germany; sebastian.rademacher@medizin.uni-leipzig.de (S.R.); jonas.babel@medizin.uni-leipzig.de (J.B.); matthias.mehdorn@medizin.uni-leipzig.de (M.M.); andri.lederer@medizin.uni-leipzig.de (A.L.); daniel.seehofer@medizin.uni-leipzig.de (D.S.); uwe.scheuermann@medizin.uni-leipzig.de (U.S.); robert.sucher@medizin.uni-leipzig.de (R.S.)
2 Department of Visceral, Thoracic and Vascular Surgery, University Hospital and Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany
3 Department of Anesthesiology and Intensive Medicine, University Hospital of Leipzig, 04103 Leipzig, Germany; nora.jahn@medizin.uni-leipzig.de
4 Department of Gastroenterology, Section of Hepatology, University Hospital of Leipzig, 04103 Leipzig, Germany; elisabeth.sucher@medizin.uni-leipzig.de
* Correspondence: hans-michael.hau@uniklinikum-dresden.de; Tel.: +49-(0)351/-458-18703; Fax: +49-(0)351/-458-4395
† Both authors contributed equally to this work and share first authorship.
‡ Both authors contributed equally to this work and share senior authorship.

Citation: Hau, H.-M.; Jahn, N.; Rademacher, S.; Sucher, E.; Babel, J.; Mehdorn, M.; Lederer, A.; Seehofer, D.; Scheuermann, U.; Sucher, R. The Value of Graft Implantation Sequence in Simultaneous Pancreas-Kidney Transplantation on the Outcome and Graft Survival. *J. Clin. Med.* **2021**, *10*, 1632. https://doi.org/10.3390/jcm10081632

Academic Editor: Charat Thongprayoon

Received: 2 March 2021
Accepted: 7 April 2021
Published: 12 April 2021

Abstract: Background/Objectives: The sequence of graft implantation in simultaneous pancreas-kidney transplantation (SPKT) warrants additional study and more targeted focus, since little is known about the short- and long-term effects on the outcome and graft survival after transplantation. Material and methods: 103 patients receiving SPKT in our department between 1999 and 2015 were included in the study. Patients were divided according to the sequence of graft implantation into pancreas-first (PF, $n = 61$) and kidney-first (KF, $n = 42$) groups. Clinicopathological characteristics, outcome and survival were reviewed retrospectively. Results: Donor and recipient characteristics were similar. Rates of post-operative complications and graft dysfunction were significantly higher in the PF group compared with the KF group (episodes of acute rejection within the first year after SPKT: 11 (18%) versus 2 (4.8%); graft pancreatitis: 18 (18%) versus 2 (4.8%), $p = 0.04$; vascular thrombosis of the pancreas: 9 (14.8%) versus 1 (2.4%), $p = 0.03$; and delayed graft function of the kidney: 12 (19.6%) versus 2 (4.8%), $p = 0.019$). The three-month pancreas graft survival was significantly higher in the KF group (PF: 77% versus KF: 92.1%; $p = 0.037$). No significant difference was observed in pancreas graft survival five years after transplantation (PF: 71.6% versus KF: 84.8%; $p = 0.104$). Kidney graft survival was similar between the two groups. Multivariate analysis revealed order of graft implantation as an independent prognostic factor for graft survival three months after SPKT (HR 2.6, 1.3–17.1, $p = 0.026$) and five years (HR 3.7, 2.1–23.4, $p = 0.040$). Conclusion: Our data indicates that implantation of the pancreas prior to the kidney during SPKT has an influence especially on the early-post-operative outcome and survival rate of pancreas grafts.

Keywords: simultaneous pancreas-kidney transplantation; immunosuppression; graft order; sequence; outcome; survival

1. Introduction

Simultaneous pancreas-kidney transplantation (SPKT) is an established therapy for patients with insulin-dependent diabetes mellitus, complicated by end-stage renal disease. Successful SPKT leads to euglycemia, which could slow the progression of diabetic microvascular and macrovascular complications and it improves survival rates and recipients' quality of life compared with patients on dialysis or patients after kidney transplantation alone [1–9].

However, its success depends on several factors such as the profiles of donors and recipients, methods of implantation techniques, and graft harvesting such as effects of ischemia-reperfusion injuries (IRI) on graft damages [6,9–11].

In this context, pancreas transplantation is associated with a high incidence of postoperative complications and up to 15% graft losses within the first year after SPKT [6,9–11].

Implantation of the pancreas before the kidney seems reasonable to avoid prolonged cold ischemic time and subsequent ischemic reperfusion injury of the pancreas graft, especially since kidney grafts can tolerate cold ischemia better than pancreas grafts [12]. However, there is currently no consensus on the best sequence of graft implantation during SPKT and in most cases the choice of the order is only made by the surgeon.

Therefore, the aim of this study was to determine the impact of graft implantation order on the outcome and survival after SPKT. We analyzed post-transplant outcome characteristics, survival rates, and risk factors for graft failures in SPKT depending on graft implantation order.

2. Material and Methods

2.1. Data Collection and Study Population

After approval by the local ethics committee [AZ-Nr: 111-16-14032016] medical data from all patients undergoing SPKT at the University Hospital of Leipzig between 1999 and 2015 were retrospectively analyzed from a prospectively collected electronic data base.

Patients were divided into two groups according to the order of graft implantation: (1) pancreas first (PF) and (2) kidney first (KF). The transplantation order was determined based on the ischemia times and implantation time points from the transplantation protocols.

2.2. Outcome Measures

Special emphasis was placed on patient and graft characteristics, postoperative complications, metabolic outcomes, renal function, and causes of graft failure depending on graft implantation order.

Characteristics included donor and recipient age, gender, and body mass index (BMI, weight in kg/height in m^2), cytomegalovirus (CMV)-status, donor cause of death, duration of insulin dependent diabetes mellitus, duration of dialysis, and time on the waiting list. Peri- and post-transplant data included information on cold ischemia time (CIT) and warm ischemia time (WIT) of the grafts, immunosuppressive therapy as well as organ graft function: Duration of operation, rates of re-operation, infectious complications, number of rejection episodes, and delayed graft function (DGF). CIT is defined as time the organ spent in cold preservation solution after removal from the donor. WIT is the time from cross-clamping until cold perfusion, plus the time of implantation (organ out of ice until reperfusion). Surgical complications were defined as the need for relaparotomy within the first three months after transplantation.

Acute rejection episodes were suspected if there was an abrupt increase in serum amylase/lipase and/or serum glucose levels, together with a significant drop in serum C-peptide level and/or increased serum creatinine levels and missing diuresis as well as abdominal pain associated with sonographic swelling of the graft. If possible, the diagnosis was confirmed from endoscopic biopsies of the duodenal segment of the graft. Biopsies of the kidney graft were performed to confirm rejection. Pancreatic biopsies were not performed. Treatment of acute cellular rejection consisted of pulsed steroids

(500 mg methylprednisolone on three consecutive days) or administration of 8 mg per kg bodyweight anti-thymocyte globulin (ATG) in parallel with increased baseline immunosuppression.

DGF of the kidney was defined as the requirement of dialysis in the first week following transplantation [13].

Pancreas graft failure was defined as resumed insulin therapy, removed pancreas, re-transplantation, or patient death.

Kidney graft failure was defined as the need for dialysis, removed kidney, re-transplantation, or patient death.

Postoperative mortality was considered as in-hospital mortality in all cases.

Laboratory parameters of ischemia-reperfusion-injury: Peak of C-reactive protein (CRP, mg/L) and serum lipase (mmol/L) within the first three days; endocrine function: low-density lipoprotein (LDL)-cholesterol/high density lipoprotein (HDL)-cholesterol ratio, HbA1C (%), C-peptide (ng/mL), and renal function: Creatinine (mmol/L) and urea (mmol/L) were analyzed up to five years after transplantation.

2.3. Organ Procurement and Transplantation

The procurement and transplantation of pancreas and kidney allografts were performed according to international standards and guidelines as described previously [6,10,14–18].

In short, the pancreas was transplanted into the right iliac fossa using a standard technique with an intraperitoneal location in the right iliac fossa. The Y-graft was anastomosed to the recipient's common iliac artery, the portal vein was connected to the inferior vena cava of the recipient. Exocrine drainage was carried out with a hand-sutured side-to-side duodenojejunostomy 40 cm beyond the flexure of Treitz [10,18]. The exocrine drainage was always accomplished immediately after reperfusion, to decrease the risk of donor duodenum distension and trigger of consecutive graft pancreatitis. The main reason why the kidney transplant was performed before the pancreas transplant was the possibility of working in two teams. One was responsible for the back-table preparation, one team was responsible for the recipient operation and transplant procedure. Since the back-table preparation of the kidney is less time consuming, the preparation of the kidney was always performed first. Once completed the kidney was immediately handed over to the implant surgeons for transplant.

2.4. Immunosuppression

Immunosuppressive therapy comprised an induction therapy with the interleukin-2 receptor antagonist basiliximab or antithymocyte globulin, followed by a triple maintenance immunosuppression consisting of calcineurin inhibitors (tacrolimus or cyclosporine), and/or antimetabolites (mycofenolate mofetil or sirolimus) and tapered steroids (prednisolone).

2.5. Statistical Analysis

Baseline data are presented as mean values with the standard deviation (SD) such as the proportion percentage (%). For comparison between the two groups, the appropriate statistical significance test including the Student's *t*-test, $\chi 2$, analysis of variance (ANOVA), Kruskal–Wallis, and Wilcoxon–Mann–Whitney test was used. Survival rates were calculated using the Kaplan–Meier analysis and the log-rank test was applied to test statistical significance. Graft survival was calculated as the time from initial transplant to graft failure, censoring for death with a functioning graft, and grafts still functioning at time of analysis. Patient survival is defined as time from transplant to patient death, censoring for patients still alive at time of analysis. If a recipient was alive or lost to follow-up at time of last contact, then survival time was censored at time of last contact. Multivariate analysis was performed with logistic regression analysis. Variables to be entered into the multiple logistic regression analysis were chosen on the basis of the results of univariate analysis.

p values < 0.05 were regarded as significant. All statistical analyses were performed by using IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics

The overall study population included 103 patients receiving SPKT in our department between 1999 and 2015. In 61 patients (59.2%), the pancreas was implanted before the kidney (PF), and in 42 patients (40.8%) the kidney was implanted first (KF). The mean follow-up period was 9.1 ± 1.2 years (PF: 9.1 ± 1.6 years versus KF: 9.2 ± 0.8 years, $p = 0.949$). Donor, recipient, and graft characteristics according to the different implantation order are summarized in Table 1. The two groups were similar in most of their transplant characteristics.

Table 1. Donor, recipient, and transplant characteristics.

Variables		PF ($n = 61$)	KF ($n = 42$)	p-Value
Donor				
Age, years		22 ± 1.7	23 ± 1.5	0.928
Gender, male/female		33/28	28/14	0.202
BMI, kg/m^2		22.2 ± 0.5	23.1 ± 0.4	0.416
Cause of death (head trauma, SAH, stroke, anoxia, infection, other, unknown)		28, 14, 4, 8, 1, 2, 4	17, 11, 3, 5, 2, 2, 2	0.964
Recipient				
Age, years		43 ± 1.1	42 ± 1.5	0.787
Gender, male/female		33/28	24/18	0.76
BMI, kg/m^2		24.7 ± 0.5	25.0 ± 0.7	0.842
Duration of Diabetes, years		27 ± 1.2	26.5 ± 1.2	0.589
Previous Dialysis		46	34	0.518
Duration of dialysis, months		34.9 ± 5.9	30.1 ± 3.8	0.521
Waiting time, months		8.7 ± 1.5	8.8 ± 1.1	0.970
Transplant				
Era, 1998–2006/2007–2015		34/29	28/14	0.309
CMV D+/R−		16	7	0.252
Cold ischemia time, hours				
	Pancreas	10.5 ± 0.3	11.5 ± 0.4	0.08
	Kidney	12.8 ± 0.4	10.1 ± 0.3	0.001
Warm ischemia time, minutes				
	Pancreas	39.1 ± 1.6	36.5 ± 2.2	0.348
	Kidney	33.2 ± 2.3	31.0 ± 1.9	0.785
Operating time, hours		5.1 ± 0.9	5.2 ± 0.8	0.789
Immunosuppression				
	Induction therapy (ATG/ IL-2 RA/ none)	38/16/7	29/9/4	0.779
	CNI (Tacrolimus/CsA)	56/5	39/3	0.843
	AP drug (MMF/SRL/none)	51/9/1	36/5/1	0.890

Data are shown as mean ± SD. BMI, body mass index; AP drug, antimetabolite; ATG, anti-thymocyte globulin; CNI, calcineurin inhibitor; CMV, cytomegalovirus; CSA, cyclosporin; D+, donor positive; IL-2 RA, Interleukin-2 receptor antagonist; KF, kidney first; MMF, mycofenolate mofetil; PF, pancreas first; R+ recipient positive; SAH, subarachnoid hemorrhage; and SRL, sirolimus.

3.2. Outcome

The analysis of post-operative outcome parameters is shown in Table 2. In the overall study population, the most frequent complications were episodes of acute rejection and delayed kidney graft function. In a comparison of the two groups, delayed graft function of the kidney ($p = 0.030$), episodes of acute rejection ($p = 0.034$), rates of graft pancreatitis ($p = 0.04$), and total rate of vascular thrombosis of the pancreas ($p = 0.03$) were significantly higher in the PF group.

Table 2. Postoperative complications after simultaneous pancreas-kidney transplantation.

Variables	PF (n = 61)	KF (n = 42)	p-Value
Delayed graft function (%)			
Pancreas	3 (4.9)	2 (4.8)	0.978
Kidney	13 (21.3)	2 (4.8)	0.019
Acute rejection episodes, combinded (%)			
1st year	11 (18.0)	2 (4.8)	0.040
Total	20 (32.8)	6 (14.3)	0.034
Pancreatitis (%)	11 (18.0)	2 (4.8)	0.040
Vascular thrombosis (%)			
Pancreas, total	9 (14.8)	1 (2.4)	0.030
Artery	2 (3.3)	0 (0)	0.234
Vein	7 (14.5)	1 (2.4)	0.09
Anastomosic leakage (%)	1 (1.6)	0 (0)	0.400
Bleeding (%)	7 (11.5)	3 (7.1)	0.466
Re-operation (%)	25 (41.0)	13 (31.0)	0.300
CMV-infection (%)	19 (31.1)	14 (33.3)	0.815

In total, 1.9% (n = 2) of the patients developed arterial thrombosis and 7.8% (n = 8) of the patients developed venous thrombosis. The majority of thrombosis (n = 8) occurred within four weeks after SPKT. All arterial thrombosis occurred in the PF group (n = 2). Thereby, in one patient the graft could be preserved with re-laparotomy and thrombectomy.

During the first year after SPKT acute rejection occurred in eleven patients (10.7%) in the PF group and in two patients (1.9%) in the KF group (p = 0.04). In total, acute rejection occurred in 26 patients (25%) during the complete follow-up period (PF: n = 20 versus KF: N = 6, p = 0.034). In 13 patients (50%) acute rejection could be confirmed histologically with renal biopsies. In one case (3.8%) the diagnosis was confirmed with endoscopic biopsies of the duodenal segment of the graft.

Within the first three days after transplantation, CRP peak—as an indicator of ischemia-reperfusion injury, such as peak of lipase—was also significantly higher in the PF group in comparison with the KF group (CRP, PF: 133.4 $\pm$ 7.9 mg/L versus KF: 104.1 $\pm$ 6.8 mg/L, p = 0.001; lipase, PF: 8.1 $\pm$ 4.1 mmol/L versus KF: 3.2 $\pm$ 3.5 mmol/L, p = 0.022).

Overall, in-hospital mortality was higher in the PF group (n = 5, 8.2%) compared with the KF group (n = 2, 4.8%) (p = 0.496). The causes of death included multiple organ failure (n = 2), septic shock (n = 2) and fatal heart attack (n = 1) in the PF group and septic shock (n = 1), and heart failure (n = 1) in the KF group, respectively.

3.3. Metabolic and Renal Function

With regard to renal function and LDL/HDL ratio, there were no significant differences between the two groups three months, one year, and five years after SPKT. Regarding the endocrine function of the pancreas, HbA1c levels tended lower for KF group by one and five years after SPKT but did not reach significance (p = 0.075 and p = 0.08, respectively) (Supplementary Table S1).

3.4. Short- and Long-Term Survival

Pancreas graft survival was significantly higher when the kidney was implanted first. During the first three months after SPKT, the percentage of pancreas graft loss was 23% in the PF group and 7.9% in the KF group (p = 0.034). The one-, three-, and five-year pancreas graft survival rates in patients after SPKT were 75.3%, 71.6%, and 71.6% in the PF group, respectively, and 90.4%, 87.7%, and 84.8% in the KF groups, respectively (p = 0.104) (Figure 1A). The one-, three-, and five-year kidney graft survival rates in patients after SPKT were 90.0%, 90%, and 84% in the PF group, respectively, and 92.8%, 90.2%, and 87.3% in the KF group, respectively (p = 0.499) (Figure 1B). Overall patient survival after one, three, and five years was 88.5%, 86.8%, and 84.9%, respectively, in the PF group, and 92.9%, 92.9%, and 90.2%, respectively, in the KF group (p = 0.419).

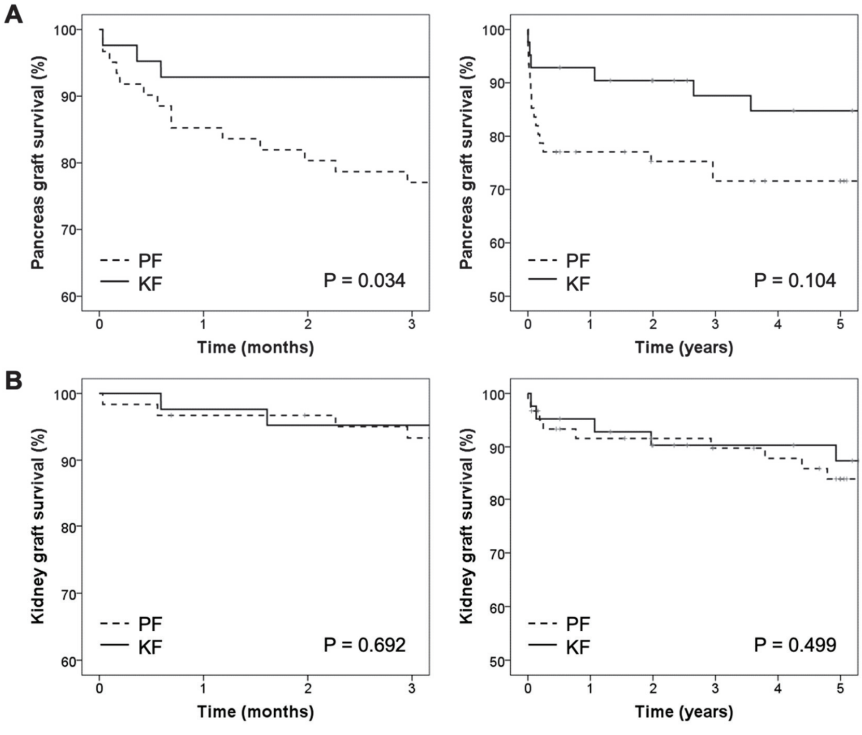


Figure 1. Graft and patient survival according to the graft implantation order. (**A**) pancreas graft survival, and (**B**) kidney graft survival three months and five years after simultaneous pancreas-kidney transplantation.

Multivariate Cox regression analysis of the total study population revealed that donor cause of death, donor recipient age and recipient BMI, duration of pancreas cold ischemia time and order of graft implantation are independent predictors of pancreas graft loss within three months and five years after SPKT. Era of transplantation and recipient gender showed a significant impact on pancreas graft survival at three months only, while they had no significant effect on 5-year graft survival (Table 3).

Table 3. Multivariate Cox regression analysis of predictors of pancreas graft loss three months and five years after simultaneous pancreas-kidney transplantation.

Variables	Time after SPKT											
	3 Months						5 Years					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p*-Value	HR	95 CI	*p*-Value	HR	95 CI	*p*-Value	HR	95 CI	*p*-Value
Donor												
Age	1.07	1.03–1.11	0.001	1.05	1.02–1.09	0.002	1.057	1.02–1.09	0.002	1.052	1.01–1.09	0.004
Gender (male versus female)	2.4	0.75–7.1	0.1				2.3	0.87–6.32	0.09			
BMI	1.21	1.03–1.41	0.015	1.247	1.052–1.478	0.011	1.20	1.05–1.38	0.01	1.180	1.023–1.361	0.023
Cause of death (Non-trauma versus trauma)	12.67	1.65–97.6	0.01	10.755	1.376–84.082	0.018	6.9	1.57–30.96	0.01	11.8	1.532–92.01	0.024
Recipient												
Age	1.08	1.02–1.14	0.008	1.126	1.050–1.208	0.001	1.053	1.005–1.104	0.003	1.093	1.035–1.154	0.001
Gender (male versus female)	0.32	0.10–0.91	0.030	0.302	0.105–0.866	0.026	0.34	0.11–0.99	0.04	0.426	0.180–1.088	0.052
BMI	1.13	1.03–1.23	0.006	1.117	1.041–1.331	0.010	1.21	1.10–1.32	<0.001	1.195	1.078–1.325	0.001
Transplant												
Era (1998–2006 versus 2007–2015)	9.49	1.26–71.6	0.029	8.1	1.1–61.5	0.04	2.83	0.96–8.34	0.05			
Warm ischemia time												
Pancreas	0.996	0.68–1.45	0.985				1.01	0.23–5.2	0.967			
Kidney	1.1	0.75–1.79	0.495				1.08	0.21–6.42	0.89			
CIT, hours												
Pancreas												
0–8	Ref.		0.005	Ref		0.007	Ref.		0.001	Ref.		0.005
8–12	0.58	0.04–9.36	0.71	0.56	0.03–8.93	0.681	0.88	0.14–5.29	0.889	1.03	0.17–62.3	0.601
>12	8.6	1.13–64.99	0.03	7.97	1.05–60.43	0.045	5.82	1.34–25.17	0.018	5.43	1.24–23.69	0.024
Kidney												
0–8	Ref.		0.06				Ref.		0.029	Ref.		0.027
8–12	0.56	0.05–6.29	0.65				0.21	0.03–1.23	0.08	0.19	0.03–1.17	0.074
>12	2.97	0.39–22.59	0.29				1.47	0.43–5.07	0.53	1.4	0.41–4.95	0.568
Graft implantation order (PF versus KF)	3.7	1.06–12.96	0.038	2.6	1.3–17.1	0.026	2.3	0.92–5.96	0.09	3.7	2.1–23.4	0.04
Immunosuppression												
Induction therapy												
None	Ref.		0.272				Ref.		0.342			
ATG	0.42	0.11–1.59	0.205				0.58	0.16–2.08	0.408			
IL–2 RA	0.89	0.22–3.56	0.870				1.1	0.29–4.18	0.880			
CNI (Tac versus CSA)	0.36	0.10–1.28	0.1				0.44	0.13–1.51	0.19			
AP drug												
None	Ref.		0.319				Ref.		0.292			
MMF	0.32	0.04–2.29	0.247				0.4	0.54–2.98	0.371			
SRL	0.11	0.01–1.9	0.133				0.18	0.01–1.78	0.121			

95CI, 95% confidence interval; HR, hazard ratio; and Tac, tacrolimus.

4. Discussion

The current study showed that the implantation of the kidney graft before the pancreas graft during SPKT is associated with reduced rates of post-operative complications and significantly better pancreas graft survival in the early post-operative course.

The most frequent postoperative complication after SPKT and subsequent pancreas graft loss remain pancreas graft thrombosis. In our study, the average pancreas graft thrombosis rate was 9.7% (1.9% arterial and 7.8% venous, respectively), which is comparable to published data [19,20]. There are a number of well-described donor and recipient risk factors associated with thrombosis in pancreas transplantation, such as donor age and obesity, cause of death [19]. However, none of these risk factors were different between the two groups (Table 1). Furthermore, in our cohort rates of acute rejection, delayed graft kidney function as well as overall graft survival are comparable with previous publications [6,21].

To our knowledge, only two other studies have previously examined the effect of graft implantation order on short- and long-term outcomes in SPKT. In a retrospective single-center analysis of 151 patients after SPKT, Salzedas–Netto et al. showed a significantly higher three-month pancreas graft survival when the kidney was implanted first (pancreas graft survival in three months, PF: 74.1 versus KF: 89.4%, $p = 0.022$) [22]. In accordance with our data, post-operative complications primarily occurred in the PF group and had a particular influence on the early post-operative outcome. However, a further comparison to our study is virtually impossible due to lack of detailed data.

In contrast, in a 2016 published register (Scientific Registry of Transplant Recipients) data analysis of 12,700 patients by Niclauss et al., the rate of pancreas graft loss within three months after SPKT was significantly lower in the PF group (PF: 9.4% versus KF: 10.8%, $p = 0.011$). Additionally, the frequency of technical graft failures was significantly lower in this group (PF: 5.6% versus KF: 6.9%, $p = 0.005$) [23]. Beyond three months, no significant differences were observed in graft survival between the two groups.

The reasons for an increased rate of complications and early graft loss in the PF group of our study remains speculative. One reason could be the mechanical stress after graft positioning and surgical retractor adjustment for the consecutive kidney transplant procedure. Salzedas–Netto et al. assumed that implantation of the kidney before the pancreas graft could reduce the risk of intra-operative damage from retractors to the pancreas and pancreatic edema. Unfortunately, no details on intraoperative findings and rector problems are available to us, that could support this thesis. Furthermore, different surgical access routes and procedures were used in the studies. In the study by Salzedas–Netto et al., organs were implanted intra-peritoneally (pancreas), as well as extra-peritoneally (kidney) by two surgical teams. In our analysis and the study by Salzedas–Netto et al., all patients underwent systemic drainage with side-to-side enteric anastomoses [22]. In contrast, in the study by Niclauss et al., exocrine pancreas secretion drainage was realized either enterically or into the bladder. However, influence of bladder drainage (44.7% of PF and 37.1% of KF) on graft survival and association with complication rates (especially vascular thrombosis) were not examined [23]. We recently introduced an intraoperative no touch real time monitoring technique for kidney and pancreas graft parenchyma evaluation using hyperspectral imaging (HSI) [24,25]. We believe that this novel procedure might be a useful tool to investigate on our hypothesis that mechanical stress to the organ implanted first during transplantation of the second organ might affect graft performance. In this context, HSI is well suited to detect venous congestion which would predominantly be detected by decreased perfusion indices and increased organ hemoglobin indices of the affected organ.

Prolonged CIT has a negative impact on pancreas graft survival and frequency of post-operative complications [23,26–29]. Therefore, transplanting pancreas grafts first seems to be reasonable as pancreas grafts tolerate cold ischemia worse than kidneys [12,30]. The study by Niclauss et al. revealed total pancreas preservation times of 12.2 ± 0.1 in the pancreas first and 14.3 ± 0.1 in the kidney first group, which were significantly longer when compared to pancreas preservation times in our patient groups [23]. In our analysis, Cox

regression analysis showed that prolonged pancreas CIT (above 12 h) is an independent risk factor for pancreas graft survival at three months and at five years after SPKT, while cold ischemia time of the kidney graft had no significant impact on pancreas graft survival (Table 3). In our analysis, total pancreas CIT was relatively short and did not show a significant difference between the two groups (CIT pancreas KF: 11.5 h versus PF: 10.5 h, $p = 0.08$). Moreover, the time difference between the implantation of both organs was lower in the KF group (PF: 2.3 h versus KF: 1.4 h, Table 1). The study by Niclauss et al. could demonstrate that when kidney grafts are implanted first, prolonged surgery time between kidney and pancreas graft implantation mainly above two hours is associated with reduced pancreas graft survival in comparison with patients in the PF group [23]. Both time factors (relatively short pancreas CIT and time gap between organ implantation) may have contributed to the good outcome in the KF group in our cohort.

4.1. Remote Ischemic Preconditioning—Potential Pathophysiology Linked to Implantation Sequence

Remote ischemic preconditioning (RIP) is the phenomenon whereby brief episodes of ischemia and reperfusion applied in distant tissues like the lower extremity render organs subject to transplantation, more resistant to ischemia. The underlying mechanism of RIP are not fully understood, however, experimental studies suggest that a combination of circulating mediators and neuronal signaling might be responsible for conditioning of the targeted organ [31,32]. Further clinical studies in kidney and lung transplant recipients suggest that RIP prior transplantation may be beneficial or at least not harmful to the transplanted organ [33].

In the event of simultaneous pancreas-kidney transplantation the successive implantation of both organs requires a sequential (two-step) temporary clamping of external iliac vessels and by nature, imitate the procedure of RIP for the second organ to be transplanted. The assumption that pancreatic allografts may benefit from RIP if transplanted second to a kidney is highly speculative but spurs further research.

4.2. Limiting Factors

There are some limiting factors of this study. First, the low number of patients in each group and the retrospective non-randomized design should be mentioned. Second, the long investigation period and different surgical teams restricted data evaluation, thus making further controlled and prospective studies necessary.

5. Conclusions

In conclusion, our results suggest that the sequence of graft implantation during SPKT influences the early post-operative course. In our analysis, KF patients seem to have a slight advantage compared with patients receiving PF during SPKT. We would recommend a kidney first transplant if two parallel working teams are available, one doing the back-table preparation, one doing the transplant procedure. If one team is responsible for both back-table and the transplant procedure we would recommend a pancreas transplant first, since the pancreas is more sensitive to ischemia and one or two hours might matter. However, we also recommend a careful surgical retractor adjustment not to compromise the pancreas allograft. Further investigation is required in larger series to determine how the order of graft implantation correlates with post-transplant graft function. Our two main floated hypotheses, that (1) mechanical stress may harm the first and (2) remote ischemic preconditioning may have beneficial effects on the second organ subject to transplantation. Both assumptions may stimulate further investigation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10081632/s1, Supplementary Table S1. Metabolic outcome during the first 5 years after simultaneous pancreas-kidney transplantation.

Author Contributions: U.S., N.J., H.-M.H. and R.S. were responsible for the study conception and design; H.-M.H., N.J., S.R., E.S. and R.S. were responsible for data acquisition; U.S., H.-M.H., S.R., and R.S. analyzed and interpreted the data; U.S., H.-M.H., S.R. and R.S. drafted the manuscript; and N.J., E.S., J.B., M.M., A.L. and D.S. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Open Access Funding by the Publication Fund of the TU Dresden. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. There are no potential conflicts of interest arising from associations with commercial or corporate interests in connection with the work submitted.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local ethical commission board from the University of Leipzig (AZ EK: 111-16-14032016).

Informed Consent Statement: Written informed consent from any patient for data collection in a prospectively collected data base is available. However, written informed consent to the study was waived by the local Ethics Committee (Ethics Committee of the first affliated University Hospital of Leipzig University) in view of the retrospective design of the study, accordingly the national and local guidelines such as the fact that all clinical/ laboratory measurements and procedures were part of the routine care.

Data Availability Statement: Our database contains highly sensible data which may provide insight in clinical and personnel information about our patients and lead to identification of these patients. Therefore, according to organizational restrictions and regulations these data cannot be made publically available. However, the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Abbreviations

BMI	Body mass index
CIT	Cold ischemia time
CMV	Cytomegalovirus
DGF	Delayed graft function
KF	Kidney-first
MMF	Mycophenolate mofetil
PF	Pancreas-first
RIC	Remote ischemic conditioning
SD	Standard deviation
SPKT	Simultaneous pancreas-kidney transplantation
WIT	Warm ischemia time
HSI	Hyperspectral Imaging
RIP	Remote ischemic preconditioning

References

1. Dean, P.G.; Kudva, Y.C.; Stegall, M.D. Long-term benefits of pancreas transplantation. *Curr. Opin. Organ Transplant.* **2008**, *13*, 85–90. [CrossRef] [PubMed]
2. Pera, P.I.; Vasallo, J.M.; Rabasa, A.T.; Salinas, F.O.; Pérez, L.F.C.; Brulles, M.J.R. Quality of life in simultaneous pancreas-kidney transplant recipients. *Clin. Transplant.* **2009**, *23*, 600–605. [CrossRef] [PubMed]
3. Scheuermann, U.; Rademacher, S.; Jahn, N.; Sucher, E.; Seehofer, D.; Sucher, R.; Hau, H.-M. Impact of pre-transplant dialysis modality on the outcome and health-related quality of life of patients after simultaneous pancreas-kidney transplantation. *Health Qual. Life Outcomes* **2020**, *18*, 1–9. [CrossRef]
4. Khairoun, M.; De Koning, E.J.P.; Berg, B.M.V.D.; Lievers, E.; De Boer, H.; Schaapherder, A.F.M.; Mallat, M.J.K.; Rotmans, J.I.; Van Der Boog, P.J.M.; Van Zonneveld, A.J.; et al. Microvascular Damage in Type 1 Diabetic Patients Is Reversed in the First Year After Simultaneous Pancreas-Kidney Transplantation. *Arab. Archaeol. Epigr.* **2013**, *13*, 1272–1281. [CrossRef]
5. Rajkumar, T.; Mazid, S.; Vucak-Dzumhur, M.; Sykes, T.M.; Elder, G.J. Health-related quality of life following kidney and simultaneous pancreas kidney transplantation. *Nephrology* **2018**, *24*, 975–982. [CrossRef]

6. Sollinger, H.W.; Odorico, J.S.; Becker, Y.T.; D'Alessandro, A.M.; Pirsch, J.D. One Thousand Simultaneous Pancreas-Kidney Transplants at a Single Center With 22-Year Follow-Up. *Ann. Surg.* **2009**, *250*, 618–630. [CrossRef]
7. White, S.A.; Shaw, J.A.; Sutherland, D.E. Pancreas transplantation. *Lancet* **2009**, *373*, 1808–1817. [CrossRef]
8. Jenssen, T.; Hartmann, A.; Birkeland, K.I. Long-term diabetes complications after pancreas transplantation. *Curr. Opin. Organ Transplant.* **2017**, *22*, 382–388. [CrossRef] [PubMed]
9. Mohan, P.; Safi, K.; Little, D.M.; Donohoe, J.; Conlon, P.; Walshe, J.J.; O'Kelly, P.; Thompson, C.J.; Hickey, D.P. Improved patient survival in recipients of simultaneous pancreas–kidney transplant compared with kidney transplant alone in patients with type 1 diabetes mellitus and end-stage renal disease. *BJS* **2003**, *90*, 1137–1141. [CrossRef] [PubMed]
10. Sollinger, H.W.; Odorico, J.S.; Knechtle, S.J.; D'Alessandro, A.M.; Kalayoglu, M.; Pirsch, J.D. Experience With 500 Simultaneous Pancreas-Kidney Transplants. *Ann. Surg.* **1998**, *228*, 284–296. [CrossRef]
11. Lindahl, J.P.; Hartmann, A.; Horneland, R.; Holdaas, H.; Reisæter, A.V.; Midtvedt, K.; Leivestad, T.; Øyen, O.; Jenssen, T. Improved patient survival with simultaneous pancreas and kidney transplantation in recipients with diabetic end-stage renal disease. *Diabetology* **2013**, *56*, 1364–1371. [CrossRef]
12. Krezdorn, N.; Tasigiorgos, S.; Wo, L.; Turk, M.; Lopdrup, R.; Kiwanuka, H.; Win, T.-S.; Bueno, E.; Pomahac, B. Tissue conservation for transplantation. *Innov. Surg. Sci.* **2017**, *2*, 171–187. [CrossRef] [PubMed]
13. Siedlecki, A.; Irish, W.; Brennan, D.C. Delayed Graft Function in the Kidney Transplant. *Arab. Archaeol. Epigr.* **2011**, *11*, 2279–2296. [CrossRef] [PubMed]
14. Eurotransplant. Chapter 9: The Donor. In *Eurotransplant Manual*; Eurotransplant: Leiden, The Netherlands, 2017.
15. Ladurner, R.; Steurer, W. Technik der Multiorganentnahme. *Viszeralchirurgie* **2004**, *39*, 439–442. [CrossRef]
16. Sucher, R.; Rademacher, S.; Jahn, N.; Brunotte, M.; Wagner, T.; Alvanos, A.; Sucher, E.; Seehofer, D.; Scheuermann, U.; Hau, H.-M. Effects of simultaneous pancreas-kidney transplantation and kidney transplantation alone on the outcome of peripheral vascular diseases. *BMC Nephrol.* **2019**, *20*, 1–10. [CrossRef]
17. Hau, H.-M.; Jahn, N.; Brunotte, M.; Lederer, A.A.; Sucher, E.; Rasche, F.M.; Seehofer, D.; Sucher, R. Short and long-term metabolic outcomes in patients with type 1 and type 2 diabetes receiving a simultaneous pancreas kidney allograft. *BMC Endocr. Disord.* **2020**, *20*, 1–13. [CrossRef] [PubMed]
18. Dholakia, S.; Mittal, S.; Quiroga, I.; Gilbert, J.; Sharples, E.J.; Ploeg, R.J.; Friend, P.J. Pancreas Transplantation: Past, Present, Future. *Am. J. Med.* **2016**, *129*, 667–673. [CrossRef] [PubMed]
19. Daga, J.A.P.; Rodriguez, R.P.; Santoyo, J. Immediate post-operative complications (I): Post-operative bleeding; vascular origin: Thrombosis pancreatitis. *World J. Transplant.* **2020**, *10*, 415–421. [CrossRef]
20. Schenker, P.; Vonend, O.; Ertas, N.; Wunsch, A.; Schaeffer, M.; Rump, L.-C.; Viebahn, R. Incidence of pancreas graft thrombosis using low-molecular-weight heparin. *Clin. Transplant.* **2009**, *23*, 407–414. [CrossRef]
21. Israni, A.K.; Feldman, H.I.; Propert, K.J.; Leonard, M.; Mange, K.C. Impact of Simultaneous Kidney-Pancreas Transplant and Timing of Transplant on Kidney Allograft Survival. *Arab. Archaeol. Epigr.* **2005**, *5*, 374–382. [CrossRef]
22. Salzedas-Netto, A.; Linhares, M.; Lopes-Filho, G.; Melaragno, C.; De Sa, J.; Rangel, E.; Goncalves, M.; Pestana, J.; Gonzalez, A. Simultaneous Pancreas-Kidney Transplantation: Which Organ Should Be Transplanted First? *Transplant. Proc.* **2010**, *42*, 2647–2649. [CrossRef] [PubMed]
23. Niclauss, N.; Bédat, B.; Morel, P.; Andres, A.; Toso, C.; Berney, T. Impact of graft implantation order on graft survival in simultaneous pancreas-kidney transplantation. *Transpl. Int.* **2016**, *29*, 627–635. [CrossRef] [PubMed]
24. Sucher, R.; Wagner, T.; Köhler, H.; Sucher, E.; Guice, H.; Recknagel, S.; Lederer, A.; Hau, H.M.; Rademacher, S.; Schneeberger, S.; et al. Hyperspectral Imaging (HSI) of Human Kidney Allografts. *Ann. Surg.* **2020**. [CrossRef]
25. Sucher, R.; Scheuermann, U.; Rademacher, S.; Lederer, A.; Sucher, E.; Hau, H.M.; Brandacher, G.; Schneeberger, S.; Gockel, I.S.D. Intraoperative reperfusion assessment of human pancreas allografts using hyperspectral imaging (HSI). *Hepatobiliary Surg. Nutr.* **2021**. [CrossRef]
26. Gruessner, A.C. 2011 Update on Pancreas Transplantation: Comprehensive Trend Analysis of 25,000 Cases Followed Up Over the Course of Twenty-Four Years at the International Pancreas Transplant Registry (IPTR). *Rev. Diabet. Stud.* **2011**, *8*, 6–16. [CrossRef] [PubMed]
27. Humar, A.; Kandaswamy, R.; Drangstveit, M.B.; Parr, E.; Gruessner, A.G.; Sutherland, D.E. Prolonged preservation increases surgical complications after pancreas transplants. *Surgery* **2000**, *127*, 545–551. [CrossRef] [PubMed]
28. Humar, A.; Ramcharan, T.; Kandaswamy, R.; Gruessner, R.W.; Gruessner, A.C.; Sutherland, D.E. Technical Failures after Pancreas Transplants: Why Grafts Fail and the Risk Factors—A Multivariate Analysis. *Transplantation* **2004**, *78*, 1188–1192. [CrossRef] [PubMed]
29. Finger, E.B.; Radosevich, D.M.; Dunn, T.B.; Chinnakotla, S.; Sutherland, D.E.R.; Matas, A.J.; Pruett, T.L.; Kandaswamy, R. A Composite Risk Model for Predicting Technical Failure in Pancreas Transplantation. *Arab. Archaeol. Epigr.* **2013**, *13*, 1840–1849. [CrossRef]
30. Rudolph, E.N.; Dunn, T.B.; Kandaswamy, R.; Finger, E.B.; Sutherland, D.E. Optimizing outcomes in pancreas transplantation: Impact of organ preservation time. *Clin. Transplant.* **2017**, *31*, e13035. [CrossRef] [PubMed]
31. Heusch, G.; Bøtker, H.E.; Przyklenk, K.; Redington, A.; Yellon, D. Remote Ischemic Conditioning. *J. Am. Coll. Cardiol.* **2015**, *65*, 177–195. [CrossRef]

32. Hausenloy, D.J.; Yellon, D.M. Remote ischaemic preconditioning: Underlying mechanisms and clinical application. *Cardiovasc. Res.* **2008**, *79*, 377–386. [CrossRef] [PubMed]
33. Farooqui, W.; Pommergaard, H.C.; Rasmussen, A. Remote ischemic preconditioning of transplant recipients to reduce graft ischemia and reperfusion injuries: A systematic review. *Transplant. Rev.* **2018**, *32*, 10–15. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Exhaled Hydrogen as a Marker of Intestinal Fermentation Is Associated with Diarrhea in Kidney Transplant Recipients

Fernanda Guedes Rodrigues [1,2,*], J. Casper Swarte [1,3], Rianne M. Douwes [1], Tim J. Knobbe [1], Camilo G. Sotomayor [1], Hans Blokzijl [3], Rinse K. Weersma [3], Ita P. Heilberg [2,4], Stephan J. L. Bakker [1], Martin H. de Borst [1] and TransplantLines Investigators [†]

[1] Department of Nephrology, University Medical Center Groningen, University of Groningen, 9700 RB Groningen, The Netherlands; j.c.swarte@umcg.nl (J.C.S.); r.m.douwes@umcg.nl (R.M.D.); t.j.knobbe@umcg.nl (T.J.K.); c.g.sotomayor.campos@umcg.nl (C.G.S.); s.j.l.bakker@umcg.nl (S.J.L.B.); m.h.de.borst@umcg.nl (M.H.d.B.)
[2] Nutrition Post Graduation Program, Universidade Federal de São Paulo, São Paulo 04023-062, Brazil; ita.heilberg@gmail.com
[3] Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, 9700 RB Groningen, The Netherlands; H.blokzijl@umcg.nl (H.B.); r.k.weersma@umcg.nl (R.K.W.)
[4] Division of Nephrology, Universidade Federal de São Paulo, São Paulo 04023-062, Brazil
* Correspondence: f.guedes.rodrigues@umcg.nl
† TransplantLines Biobank and Cohort Study, UMC Groningen Transplant Center, University of Groningen, 9700 RB Groningen, The Netherlands.

Citation: Rodrigues, F.G.; Swarte, J.C.; Douwes, R.M.; Knobbe, T.J.; Sotomayor, C.G.; Blokzijl, H.; Weersma, R.K.; Heilberg, I.P.; Bakker, S.J.L.; de Borst, M.H.; et al. Exhaled Hydrogen as a Marker of Intestinal Fermentation Is Associated with Diarrhea in Kidney Transplant Recipients. *J. Clin. Med.* **2021**, *10*, 2854. https://doi.org/10.3390/jcm10132854

Academic Editors: Wisit Cheungpasitporn, Charat Thongprayoon and Wisit Kaewput

Received: 19 May 2021
Accepted: 24 June 2021
Published: 28 June 2021

Abstract: Background: Diarrhea is common among kidney transplant recipients (KTR). Exhaled hydrogen (H_2) is a surrogate marker of small bowel dysbiosis, which may drive diarrhea. We studied the relationship between exhaled H_2 and diarrhea in KTR, and explored potential clinical and dietary determinants. Methods: Clinical, laboratory, and dietary data were analyzed from 424 KTR participating in the TransplantLines Biobank and Cohort Study (NCT03272841). Fasting exhaled H_2 concentration was measured using a model DP Quintron Gas Chromatograph. Diarrhea was defined as fast transit time (types 6 and 7 according to the Bristol Stool Form Scale, BSFS) of 3 or more episodes per day. We studied the association between exhaled H_2 and diarrhea with multivariable logistic regression analysis, and explored potential determinants using linear regression. Results: KTR (55.4 ± 13.2 years, 60.8% male, mean eGFR 49.8 ± 19.1 mL/min/1.73 m^2) had a median exhaled H_2 of 11 (5.0–25.0) ppm. Signs of small intestinal bacterial overgrowth (exhaled $H_2 \geq 20$ ppm) were present in 31.6% of the KTR, and 33.0% had diarrhea. Exhaled H_2 was associated with an increased risk of diarrhea (odds ratio 1.51, 95% confidence interval 1.07–2.14 per $\log_2$ ppm, $p = 0.02$). Polysaccharide intake was independently associated with higher H_2 (std. β 0.24, $p = 0.01$), and a trend for an association with proton-pump inhibitor use was observed (std. β 0.16 $p = 0.05$). Conclusion: Higher exhaled H_2 is associated with an increased risk of diarrhea in KTR. Our findings set the stage for further studies investigating the relationship between dietary factors, small bowel dysbiosis, and diarrhea after kidney transplantation.

Keywords: kidney transplantation; hydrogen; diarrhea; small intestinal bacterial overgrowth

1. Introduction

Kidney transplantation is the preferred treatment for end-stage kidney disease (ESKD) [1]. Given the advances in surgical techniques and immunosuppressive therapy in parallel with prophylaxis and treatment of infectious complications in the past decades, patient and graft short-term outcomes have considerably improved [2,3]. However, the quality of life of many outpatient kidney transplant recipients (KTR) is adversely affected by late complications, including diabetes, malignancies, risk of opportunistic infections due to maintenance immunosuppressive therapy, and gastrointestinal (GI) complaints [4,5]. GI

complaints affect 30 to 40% of these patients, with chronic diarrhea impacting the quality of life of 20% of otherwise stable KTR during the first year after kidney transplantation [6].

It has recently been shown that KTR are commonly affected by an unbalanced gut microbiome, i.e., gut dysbiosis, characterized by a diminished microbial diversity [7,8]. Emerging evidence indicates that changes in gut microbiota following kidney transplantation may play a key role in the development of GI symptoms and diarrhea [7,9]. Small intestinal bacterial overgrowth is a form of gut dysbiosis characterized by an excessive number of coliform bacteria in the upper part of the small bowel, which has been implicated in driving GI complaints such as diarrhea, abdominal pain, and bloating [10]. In the presence of small bowel dysbiosis, the conversion of substrates into short-chain fatty acids (SCFA) is shifted to a higher production of intestinal gases such as hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4) [11]. Exhaled hydrogen (H_2) can be used as a non-invasive surrogate marker for small intestinal bacterial overgrowth [12]. Whether exhaled H_2 is associated with the risk of diarrhea in KTR is currently unknown, and the role of post-kidney transplant medication regimens (e.g., maintenance immunosuppressive therapy and proton-pump inhibitors) and diet composition as potential determinants of exhaled H_2 have not been investigated.

Therefore, in the current study we assessed exhaled H_2 in a large KTR cohort, to study its relationship with diarrhea and to investigate its potential clinical and dietary determinants.

2. Materials and Methods

This is a cross-sectional study based on data from the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov identifier: NCT03272841), conducted at the outpatient clinic of the University Medical Centre Groningen (Groningen, The Netherlands), which investigates all different types of solid organ transplant recipients [1]. A detailed description of the study design, inclusion and exclusion criteria has been described previously [1]. The study protocol has been approved by the Institutional Review Board (METc 2014/077) (METc UMCG), adheres to the local UMCG Biobank Regulations, and is in accordance with the WMA Declaration of Helsinki and the Declaration of Istanbul [2]. KTR with available breath test data were included in the present study and all participants signed an informed consent prior to their TransplantLines visit. A flowchart diagram is presented in Figure 1.

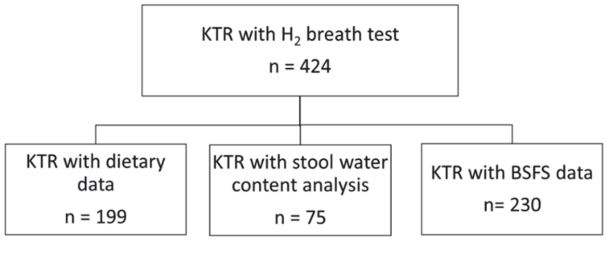


Figure 1. Flowchart diagram. Abbreviations: H_2, hydrogen; KTR, kidney transplant recipients; BSFS, Bristol Stool Form Scale.

2.1. Clinical Data

Clinical data were collected according to a detailed protocol, as described elsewhere [1]. Patients were recruited between January 2017 and June 2019. Patients taking antihypertensive drugs were classified as having hypertension [3]. Diabetes mellitus was defined according to the guidelines of the American Diabetes Association [4]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Body surface area (BSA) was calculated using the formula of Du Bois and Du Bois [5]. Estimated glomerular filtration rate (eGFR) was calculated using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation [6]. Body composition was determined using

a multifrequency bioelectrical impedance device (BIA, Quadscan 4000, Bodystat, Douglas, British Isles) at 5, 50, 100, and 200 Hz, which allows us to distinguish between lean mass and fat mass (expressed as body fat percentage) taking into account differences in volume status [7].

2.2. Breath H_2 Measurement

Breath samples were collected in a 50 cc syringe with a hole of 6 mm at approximately 40 cc with a 3-way-stopcock. Patients were fasting (and therefore did not ingest any carbohydrates) for at least eight hours and were not allowed to smoke for at least one hour before the sample collection [12]. The study subject inhaled normally and exhaled maximally in this syringe with the stamper set at 50 cc and the 3-way stopcock open. After full expiration, the hole was immediately closed by the study participant, the stamper was set to 30 cc, and the 3-way stopcock was closed. Breath samples were analyzed within 12 h after sample collection using a model DP Quintron Gas Chromatograph (Quintron Instrument Company, Milwaukee, WI, USA). H_2 results were automatically corrected for CO_2 in order to reduce the chance of dilution by environmental air. A fasting basal (i.e., without the ingestion of test sugar) exhaled H_2 concentration above 20 parts per million (ppm) was considered suggestively positive for small intestinal bacterial overgrowth (SIBO) as suggested elsewhere [8–11].

2.3. Stool Water Content Measurement

One day before the study visit, participants had collected a stool sample at home with a FaecesCatcher (TAG Hemi VOF, Zeijen, The Netherlands) [1]. The sample was collected in a tube and immediately frozen. At arrival, the sample was immediately stored at −80 °C (−112 °F) until further use. For analysis, samples were defrosted up to ~0 °C and homogenized. Then, a minimum of 1 g and preferably 5 g of every sample was put in a 15 mL tube for stool water content measurement. Prior to filling, two holes were pierced in the lid to allow water sublimation during freeze-drying. Subsequently, samples were freeze-dried for 48 h under 0.5 bar at −50 °C [13]. The samples were weighed before and after freeze-drying to calculate the dry weight as shown in the equation below [14].

Equation: Percentage of dry matter stool samples.

$$Dry\ matter\ \% = \frac{(Dry\ filled\ tube - empty\ tube)}{(Wet\ filled\ tube - empty\ tube)} \times 100\%$$

2.4. Diarrhea Classification

The stool form and consistency were graded using the Bristol Stool Form Scale (BSFS) [15]. The scale is structured from 1 to 7 according to form and consistency, from the hardest (type 1) to the most fluid kind (type 7). KTR classified as slow transit time (type-1 and -2 feces in the Bristol scale) and normal transit time (types 3, 4, and 5) were clustered as having no diarrhea, and those with fast transit time (types 6 and 7) with 3 or more episodes per day, as having diarrhea.

2.5. Dietary Assessment

Dietary intake was assessed using a validated self-administered food frequency questionnaire (FFQ) [16]. A trained researcher checked the FFQ for completeness on the day of the visit to the outpatient clinic. The FFQ inquired about consumption of 177 food items during the past month, taking seasonal variations into account, and included 7 fruit items and 18 vegetable items. Frequency was recorded in times per day, week, or month, and servings were expressed as natural units or household measures. The FFQ was linked to the Dutch Food Composition Table (NEVO) in order to calculate total energy intake and nutrients [17]. Adjustment for total energy intake according to the residual method was performed to calculate nutrients intake [18].

2.6. Statistical Analyses

Statistical analyses were performed using IBM SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). In all analyses, $p < 0.05$ was considered significant. Variable distribution was evaluated by Kolmogorov–Smirnov test. Categorical variables are presented as n (%), normally distributed variables as mean ± standard deviation (SD), and non-normally distributed variables as median (interquartile range). We divided patients into three groups according to exhaled hydrogen. The highest group was defined as >20 ppm (suggestive for SIBO [8–11]). We divided the remaining patients (exhaled H2 < 20 ppm) into two groups using the rank tool in SPSS software. Comparison of categorical variables was performed using a Chi-square test. Differences in groups of exhaled H_2 were tested through analysis of variance (ANOVA) with Bonferroni post hoc tests for normally distributed variables and the Kruskal–Wallis test for non-normal distribution. Possible determinants of exhaled H_2 were studied using univariable linear regression. Since we aimed to explore the potential relevance of any clinical or nutritional factor as potential determinant of exhaled H_2, we tested all available variables in individual univariable regression analysis. Subsequently, all variables with a $p < 0.05$ were included in a multivariable linear regression model to identify independent determinants of exhaled H_2 production. Residuals were checked for normality and variables were natural log-transformed when appropriate. Multivariable logistic regression was performed to determine the potential relationship between exhaled H_2 and diarrhea. Variables of known clinical importance for diarrhea in KTR, such as age, sex, eGFR, transplant vintage, immunosuppressive use [19,20], and H_2 determinants, such as use of PPI and polysaccharides intake, were used in the model.

3. Results

3.1. Clinical Parameters

A total of 424 KTR (55.4 ± 13.2 years, 258 (60.8%) male) were included in the study. The median transplant vintage was 1.8 (1.0–7.2) years, and 16.3% had a history of allograft rejection. With respect to comorbidities, 19.3% and 73.3% of patients had diabetes and hypertension, respectively. The mean eGFR was 49.8 ± 19.1 mL/min/1.73 m^2. According to the BSFS, 33% of KTR had diarrhea. Further clinical and laboratory characteristics are shown in Table 1. KTR were divided into three groups according to exhaled H_2 concentration (G1, 1.0–6.9 ppm, n = 151; G2, 7.0–19.9 ppm, n = 139; G3, ≥20.0 ppm, n = 134). One-hundred thirty-four (134) out of 424 patients (31.6%) were considered positive for small intestinal bacterial overgrowth ($H_2 \geq 20$ ppm). KTR in the highest H_2 group had lower BMI (26.8 ± 3.9 kg/m^2 vs. 28.2 ± 5.6 kg/m^2, $p = 0.02$) and body fat percentage (29.3 ± 9.0% vs. 32.3 ± 10.3%, $p = 0.02$) when compared to the lowest H_2 group. Patients in the highest H_2 group also had lower waist circumference when compared to both the lowest and intermediate group (96.9 ± 12.1 cm vs. 100.8 ± 13.6 cm and 100.8 ± 13.4 cm, $p = 0.03$).

Table 1. Baseline characteristics of KTR according to groups of exhaled H_2.

Baseline Characteristics	Total (n = 424)	Exhaled H_2, per Group		
		1.0–6.9 ppm (n = 151)	7.0–19.9 ppm (n = 139)	≥20.0 ppm (n = 134)
Fermentation Parameter				
H_2, ppm	11 (5.0–25.0)	4.0 (2.0–5.0)	11.0 (8.0–15.0) [b]	33.5 (26.0–49.0) [ab]
Demographics				
Age, years	55.4 ± 13.2	55.4 ± 13.7	57.2 ± 12.1	53.7 ± 13.5
Sex (male), *n* (%)	258 (60.8)	86 (57.0)	84 (62.7)	88 (63.3)
Transplant vintage, years	1.8 (1.0–7.1)	2.0 (1.0–8.1)	1.0 (0.6–5.0)	1.1 (0.8–7.8)
History of allograft rejection, *n* (%)	69 (16.3)	27 (17.8)	19 (14.2)	23 (16.5)
Body Composition				
Body mass index, kg/m^2	27.7 ± 4.8	28.2 ± 5.6	28.1 ± 4.6	26.8 ± 3.9 [a]
Waist circumference, cm	99.5 ± 13.2	100.8 ± 13.6	100.8 ± 13.4	96.9 ± 12.1 [ab]
Body fat percentage, %	31.1 ± 9.8	32.3 ± 10.3	31.6 ± 9.8	29.3 ± 9.0 [a]
Immunosuppressive Drug Use				
MMF, *n* (%)	311 (73.3)	113 (74.8)	100 (74.6)	98 (70.5)
Tacrolimus, *n* (%)	333 (78.5)	111 (73.5)	112 (83.5)	110 (79.1)
Cyclosporine, *n* (%)	38 (9.0)	17 (11.3)	12 (8.9)	9 (6.5)
Everolimus, *n* (%)	10 (2.4)	5 (3.3)	3 (2.2)	2 (1.4)
Prednisolone, *n* (%)	396 (93.4)	141 (93.4)	124 (92.5)	131 (94.2)
Azathioprine, *n* (%)	24 (5.7)	8 (5.3)	6 (4.5)	10 (7.2)
Immunosuppressive Drug Trough Levels				
MMF, ug/L	2.3 ± 1.6	2.2 ± 1.7	2.2 ±1.4	2.5 ± 1.8
Tacrolimus, ug/L	6.3 ± 2.5	5.9 ± 2.3	6.1 ± 2.2	6.8 ± 2.4 [a]
Lifestyle				
Current smoker, *n* (%)	13 (3.1)	7 (4.6)	4 (3.0)	2 (1.4)
Alcohol consumption, g/day	1.5 (0.0–7.9)	1.7 (0.0–7.3)	1.0 (0.2–9.2)	1.6 (0.0–7.6)
Glucose Homeostasis				
Diabetes mellitus, *n* (%)	82 (19.3)	28 (18.5)	32 (23.0)	22 (16.4)
Plasma glucose, mmol/L	6.1 ± 1.7	6.2 ± 1.6	6.1 ± 1.8	6.0 ± 1.8
HbA1c, %	6.0 ± 1.7	6.0 ± 0.9	6.0 ± 0.8	5.9 ± 0.9
Lipids				
Total cholesterol, mmol/L	4.6 ± 1.0	4.7 ± 1.0	4.7 ± 1.0	4.4 ± 1.1
HDL-cholesterol, mmol/L	1.3 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.4
LDL-cholesterol, mmol/L	2.9 ± 0.9	2.9 ± 0.9	3.0 ± 0.9	2.7 ± 0.9
Triglycerides, mmol/L	1.8 ± 0.8	1.9 ± 0.9	1.9 ± 0.8	1.8 ± 0.9
Cardiovascular				
SBP, mmHg	137.4 ± 16.9	137.9 ± 16.2	137.2 ± 16.6	137.1 ± 16.6
DBP, mmHg	79.9 ± 11.1	79.8 ± 10.7	80.2 ± 11.1	79.6 ± 11.4
Kidney Function				
eGFR, mL/min/1.73 m^2	49.8 ± 19.1	49.3 ± 18.9	49.5 ± 18.2	50.4 ± 5.9
Creatinine, µmol/L	155.3 ± 124.3	149.9 ± 92.2	152.4 ± 110.9	163.9 ± 161.6
Urinary protein excretion, g/24 h	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.2
Medication				
Proton pump inhibitors, *n* (%)	306 (72.2)	96 (63.5)	105 (78.4) [a]	105 (75.5) [a]
Statins, *n* (%)	220 (51.9)	80 (53.0)	72 (53.7)	68 (48.9)
Antihypertensive, *n* (%)	311 (73.3)	106 (70.2)	105 (78.4)	100 (71.9)
Diarrhea according to BSFS, *n* (%) *	76 (33.0)	24 (27.9)	25 (36.2)	27 (36.0)
Evacuation episodes, n/day *	2.1 ± 1.3	2.2 ± 1.3	2.2 ± 1.3	2.1 ± 1.3
Stool water content, % **	75.4 ± 6.3	73.8 ± 6.0	75.8 ± 7.0	77.7 ± 5.5 [c]

[a] $p < 0.05$ vs. G1 [b] $p < 0.05$ vs. G2 [c] $p = 0.08$ vs. G1; * $n = 230$; ** $n = 75$. Data are presented as mean ± standard deviation (SD), median with interquartile ranges (IQRs), or number with percentages (%). Abbreviations: H_2, hydrogen; MMF, mycophenolate mofetil; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; LDL, Low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; BSFS, Bristol Stool Form Scale.

The immunosuppressive regimen consisted mostly of triple therapy including prednisolone (in 93.4% of all patients) and mycophenolate mofetil (MMF) (73.3%) in combination with the calcineurin inhibitor tacrolimus (78.5%) or cyclosporine (9.0%). Alternatively, regimens could contain azathioprine (5.7%) or everolimus (2.4%). When comparing the

groups, we did not find statistical differences regarding immunosuppressive regimens. When comparing immunosuppressive trough blood concentrations, the highest H_2 group had significantly higher levels of tacrolimus when compared to the lowest H_2 group, and a trend when compared to the intermediate group (6.8 ± 2.4 ng/mL vs. 6.1 ± 2.2 ng/mL, $p = 0.07$). KTR in the intermediate (n = 105, 78.4%) and highest (n = 105, 75.5%) H_2 groups more commonly used proton-pump inhibitors when compared to the lowest group (n = 96, 63.5%), $p = 0.01$, while use of statins and antihypertensive drugs were similar among the groups. Nutritional data (Supplementary Table S1) showed no statistical differences regarding energy intake, macronutrients (protein, carbohydrates, and lipids), fiber, and micronutrients, including calcium, iron, zinc, and vitamins D, C, and E, among the three groups based on exhaled H_2. Patients in the highest H_2 group had lower intake of mono/disaccharides (113.6 ± 47.7 g vs. 126.2 ± 45.4 g and 124.9 ± 35.5 g, $p = 0.02$) and higher intake of polysaccharides (161.0 ± 36.3 g vs. 143.2 ± 38.7 g and 144.3 ± 28.3 g, $p = 0.004$) when compared to the lowest and intermediate groups, respectively.

3.2. Determinants of Exhaled H_2

We subsequently performed linear regression to investigate possible clinical, laboratory and dietary factors determinants of exhaled H_2. Associations were explored for all factors shown in Table 1; (borderline) significant results are presented in Table 2. Upon univariable analysis, we observed inverse associations between H_2 and mono/disaccharides (std. β −0.27, $p = 0.01$) and vitamin C intake (std. β −0.17, $p = 0.03$), as well as a positive association with polysaccharide intake (std. β 0.49, $p < 0.001$). Other possible influencing factors, such as waist circumference (WC), total cholesterol, low-density lipoprotein (LDL)-cholesterol, tacrolimus trough blood, and use of proton-pump inhibitors were also significant in univariable analyses. In multivariable linear regression analyses, only polysaccharide intake remained independently associated with exhaled H_2 (std. β 0.24, $p = 0.01$), while a trend for an association with proton-pump inhibitor use was observed (std. β 0.16, $p = 0.05$).

Table 2. Potential determinants of Log_2 exhaled H_2.

Potential Determinants	Univariate		Multivariate *	
	Std. β	*p*	Std. β	*p*
Polysaccharides intake, g	0.266	<0.001	0.243	0.01
Proton pump inhibitor use	0.160	<0.01	0.164	0.05
Mono and disaccharides intake, g	−0.188	0.01		
Tacrolimus trough levels, ug/L	0.133	0.02		
Vitamin C intake, mg	−0.162	0.02		
Total cholesterol, mmol/L	−0.106	0.03		
LDL-cholesterol, mmol/L	−0.101	0.04		
Waist circumference, cm	−0.098	0.05		

n = 196. Linear regression analysis with exhaled H_2 as dependent variable. * Run backwards. Std. β, standardized beta; LDL, low-density lipoprotein.

3.3. H_2 and Diarrhea

Table 3 summarizes the results of logistic regression, which revealed that exhaled H_2 and the use of MMF were significantly associated with diarrhea according to BSFS (OR = 1.51, 95% CI 1.07–2.14 per log_2 ppm, $p = 0.02$, and OR = 4.71 95% CI 1.24–17.77, $p = 0.02$, respectively), while adjusting for potential confounders including age, sex, eGFR, transplant vintage, tacrolimus use, and polysaccharides intake. Although individuals with higher stool water content also had a higher number of evacuation episodes (r = 0.45, $p = 0.01$), the number of evacuation episodes was not associated with H_2 (r = −0.02, $p = 0.75$).

Table 3. Multivariable association between exhaled H_2 and diarrhea.

Variable	OR (95% CI)	*p*
Log_2 exhaled H_2, ppm	1.51 (1.07–2.14)	0.02
Sex (male)	1.10 (0.41–2.99)	0.85
eGFR, mL/min/1.73 m^2	0.95 (0.92–0.99)	0.01
Transplant vintage, years	0.99 (0.99–1.01)	0.08
MMF use	4.71 (1.24–17.77)	0.02
Tacrolimus use	0.25 (0.05–1.22)	0.09
PPI, use	1.09 (0.37–3.30)	0.86
Polysaccharides intake, g	0.99 (0.98–1.01)	0.12

n = 196. Multivariable-adjusted binary logistic regression analysis. Abbreviations: OR, Odds ratio; CI, confidence interval; H_2, hydrogen, eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil; PPI, proton-pump inhibitors.

Feces samples of 75 KTR were available for the analysis of water content. Patients in the highest H_2 group displayed a trend towards higher percentage of water stool content when compared to the lowest group (77.7 ± 5.5% vs. 73.8 ± 6.0%, $p = 0.08$). Linear regression analysis disclosed a trend for an association between exhaled H_2 and stool water content (std. β 0.22, $p = 0.06$; data not shown in tables).

4. Discussion

Diarrhea is a common complication after kidney transplantation [21,22], with its etiology still under debate. In the current study, 33.0% of KTR had diarrhea according to the BSFS questionnaire, and 31.6% presented exhaled H_2 higher than 20 ppm. In multivariable-adjusted analyses, exhaled H_2 was associated with an increased risk of diarrhea in KTR. We identified polysaccharide intake as an independent dietary determinant of exhaled H_2. The present data suggest a relationship between small bowel dysbiosis, diarrhea, and diet after kidney transplantation.

As a frequent GI symptom after kidney transplantation, diarrhea may be related to infections, antibiotics, or immunosuppressive drugs [20]. In the present study, 33.0% of KTR had diarrhea, in line with the 35.0% described by Lee et al. [23], but lower than the 53.0% presented by Ekberg et al. [21]. This variable prevalence could be attributed to differences in diarrhea classification, immunosuppressive regimens, and sample size. Nevertheless, the prevalence of diarrhea in KTR far exceeded the prevalence observed in the general population (3.5 to 12.0%) [24,25].

We observed independent associations between both exhaled H_2 and MMF use with the presence of diarrhea. MMF use has consistently been implicated in posttransplant diarrhea [26]. Our finding regarding the relationship between exhaled H_2 and diarrhea is in line with previous data outside the transplant population [27]. In irritable bowel syndrome (IBS) patients with small intestinal bacterial overgrowth (SIBO), a type of gut microbiome dysbiosis, was present more often with diarrhea, higher stool frequency, and looser stool forms. Moreover, these symptoms were associated with higher bacterial count in upper gut aspirate and basal exhaled H_2 in both fasting state and following ingestion of a substrate [28].

Although our study did not reveal potential mechanisms underlying the association between exhaled H_2 and diarrhea, we hypothesize that lipopolysaccharide (LPS) present in Gram-negative bacteria, the most common microorganisms related to SIBO, may promote local inflammation, causing mucosal lesions and increasing intestinal permeability, disabsorption syndrome, and increased nutrient fermentation [29]. Because the production of H_2 in humans only occurs through microbial anaerobic fermentation of unabsorbed carbohydrates [30], higher exhaled H_2 is generally considered a marker for alterations in small bowel microbiota composition. Since higher levels of exhaled H_2 can be caused either by slow transit or by bacterial overgrowth with a delayed return of exhaled H_2 to baseline levels, Romagnuolo et al. [10] states that fasting exhaled H_2 levels ≥20 ppm can be representative of SIBO. In agreement, Corazza et al. [31] have demonstrated that, in

bacterial overgrowth patients, fasting exhaled H_2 values were significantly higher than in healthy volunteers (14.7 ± 14.0 ppm vs. 5.8 ± 3.1 ppm, $p < 0.001$) [31]. Perman et al. [32] observed a fasting exhaled H_2 of 2.0 ± 2.5 ppm after a dinner meal in healthy subjects, with no value exceeding 11.0 ppm, whereas exhaled H_2 after an identical meal in patients with bacterial overgrowth exceeded 48 ppm [32]. These studies support that elevated values of fasting H_2 can be considered suggestive of SIBO, potentially connecting higher exhaled H_2 with the observed increased risk of diarrhea.

In addition, patients with higher exhaled H_2 tended to have higher stool water content and the percentage of stool water content was positively associated with both exhaled H_2 and the number of evacuation episodes. An experimental study has disclosed that greater gastro-intestinal H_2 content shortened colonic transit time by 47% in the proximal colon, and by 10% in the distal colon [33]. These data suggest that there might be an association between an accelerated intestinal transit causing diarrhea and the H_2 production.

A recent meta-analysis suggested that the use of proton-pump inhibitors (PPI) can moderately increase the risk of SIBO [34]. Since gastric acid is an important barrier that prevents bacterial colonization of the stomach and small intestine, PPI therapy may promote small intestinal microbiota growth, through chronic acid suppression and subsequent hypochlorhydria [35]. In the current study, we observed a significantly higher use of PPI among individuals in the highest group of exhaled H_2, with a trend for an association with H_2 in multiple linear regression, which is in line with the notion that exhaled H_2 is associated with small intestinal microbiota overgrowth.

A complex interplay exists between diet, GI transit, and gut microbiota [36]. In the present study, we identified polysaccharide intake as an independent determinant of exhaled H_2. Polysaccharides are complex carbohydrates that can be divided into starch and non-starch [37]. Since there was no difference in fiber intake between the H_2 groups, we assume higher starch intake as the main cause of higher polysaccharide intake. Some starch, known as resistant starch (RS), escapes digestion in the small intestine and, upon reaching the large intestine, acts similarly to dietary fiber, fermenting and incorporating water [37]. Our findings are at least partly in line with a previous study suggesting that SIBO resulted from differences in fiber intake [38]. In the current cohort, the suggestive presence of SIBO could further promote starch malabsorption, which in turn could be a conceivable explanation for the higher water content in stool due to osmotic activity, subsequently causing diarrhea [39,40]. At the same time, there is limited evidence that SIBO is the primary driver of GI symptoms or that it is influenced by dietary factors.

RS and other types of starch that escape digestion in the small intestine may be quantitatively more important as substrates of fermentation than non-starch polysaccharides in the colon [41]. While the human genome does not encode adequate gastrointestinal enzymes that metabolize some polysaccharides, RS undergoes fermentation by members of the gut microbiota, resulting in the production of SCFAs, mainly butyrate [42,43]. Recently, our group has been able to show that the gut microbiome of KTR contained less butyrate-producing bacteria, more Proteobacteria, and fewer Actinobacteria [44]. Starch-utilizing bacteria in the gut include many members of the *Bacteroides* and various *Firmicutes* [45]. A study in KTR demonstrated that the phylum Bacteroidetes and its derivative Bacteroides, as well as *Ruminococcus*, *Coprococcus*, and *Dorea*, were significantly reduced in fecal specimens from patients with diarrhea [23,45]. These observations suggest that changes in starch-degrading bacteria in KTR could decrease carbohydrate fermentation in colonic lumen, promoting reduced nutrient absorption and watery stool.

Another important fact to be highlighted is that the elimination of the H_2 produced by bacterial fermentation depends significantly on methanogenic and sulfate-reducing bacteria that convert H_2 to methane and hydrogen sulfide [46]. We recently demonstrated that colonic presence of the methanogen *Methanobrevibacter smithii*, which plays an important role upon removing the end-product H_2 from bacterial fermentation, was reduced in KTR [47]. These findings may indicate that diminished abundance of methanogens after

kidney transplantation could lead to less H_2 metabolization, also contributing to a rise in the gas levels in the breath test.

To the best of our knowledge, this is the first study to evaluate the relationship between exhaled H_2 and diarrhea in a large-scale cohort of KTR. The breath test we used is widely available, safe, inexpensive, and noninvasive, advantages that make it ideal for daily clinical practice employment. Limitations of the present study include the cross-sectional nature, precluding conclusions on causality, the single H_2 measurement, which does not necessarily reflect the entire post-transplant period, and the lack of information regarding the course and duration of diarrhea (acute or chronic). Since our study population consisted almost entirely of Caucasians, our results cannot be extrapolated to different populations. The purpose of performing the breath test was to investigate exhaled H_2 in KTR under fasting basal conditions (i.e., without using substrates like glucose, lactulose, lactose, or fructose). Although some investigators suggest that the use of fasting hydrogen is insufficient for SIBO diagnosis [48], fasting hydrogen overproduction has been consistently found with bacterial overgrowth in several studies [8,10,32,33]. Finally, no direct tests such as duodenal aspirate cultures have been performed to detect changes in the gut microbiota colonization of small bowel.

In conclusion, a fasting exhaled H_2 higher than 20 ppm was present in 31.6% of KTR, which was associated with increased risk of diarrhea. Polysaccharide intake was an independent determinant of exhaled H_2. The present results suggest that diarrhea in KTR may reflect an altered small bowel gut microbial composition, at least partly under dietary control. These data encourage future studies to validate our findings; to further investigate the associations between the diet, small bowel dysbiosis, and post-kidney transplant diarrhea; and to explore whether lowering polysaccharide intake or correction of SIBO may reduce diarrhea in KTR. Characterizing small intestinal bacterial overgrowth in post-transplant patients with GI symptoms could support more focused antibacterial or dietary therapeutic approaches.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10132854/s1, Table S1: Nutritional Data; Table S2: Multivariate binary logistic regression results using exhaled H_2 ($\geq$ or <20 ppm) as dependent variable.

Author Contributions: F.G.R. conducted data analyses, research design and wrote the manuscript. J.C.S., R.M.D., T.J.K. conducted the study and data collection. C.G.S. conducted data analysis and reviewed the manuscript. H.B. and R.K.W. discussed and enriched the manuscript. I.P.H., S.J.L.B. and M.H.d.B. supervised and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Fernanda Guedes Rodrigues is supported by a doctoral study scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Institutional Review Board Statement: For this study we used data from the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov identifier: NCT03272841).The study protocol has been approved by the Institutional Review Board (METc 2014/077) (METc UMCG), adheres to the UMCG Biobank Regulation, and is in accordance with the WMA Declaration of Helsinki and the Declaration of Istanbul.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
CH_4	Methane
CKD	Chronic Kidney Disease
eGFR	Estimated glomerular filtration rate
FFQ	Food Frequency Questionnaire
GI	Gastrointestinal
H_2	Hydrogen
IBS	Irritable bowel syndrome
KTR	Kidney Transplant Recipients
MMF	Mycophenolate mofetil
PPI	Proton-pump inhibitors
RS	Resistant starch
SCFA	Short-chain Fatty Acids
SIBO	Small Intestinal Bacterial Overgrowth

References

1. Eisenga, M.F.; Gomes-Neto, A.W.; Van Londen, M.; Ziengs, A.L.; Douwes, R.M.; Stam, S.P.; Osté, M.C.J.; Knobbe, T.J.; Hessels, N.R.; Buunk, A.M.; et al. Rationale and design of TransplantLines: A prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* **2018**, *8*, e024502. [CrossRef] [PubMed]
2. U.S. National Library Medicine. TransplantLines: The Transplantation Biobank. 2015. Available online: https://clinicaltrials.gov/ct2/show?term=transplantlines&rank=1 (accessed on 20 April 2021).
3. Corpa, M.V.N.; Soares, V. Systemic hypertension in patients with glomerulonephritis. *Renal. Fail.* **2002**, *24*, 347–352. [CrossRef] [PubMed]
4. American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* **2003**, *26* (Suppl. 1), S5–S20. [CrossRef]
5. Du Bois, D.; Du Bois, E.F. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* **1989**, *5*, 303. [PubMed]
6. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.L.; Castro, A.F., 3rd; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T.; et al. A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* **2009**, *150*, 604–612. [CrossRef]
7. Kyle, U.G.; Bosaeus, I.; De Lorenzo, A.D.; Deurenberg, P.; Elia, M.; Gómez, J.M.; Heitmann, B.L.; Kent-Smith, L.; Melchior, J.-C.; Pirlich, M.; et al. Bioelectrical impedance analysis—part II: Utilization in clinical practice. *Clin. Nutr.* **2004**, *23*, 1430–1453. [CrossRef]
8. Sabaté, J.-M.; Jouët, P.; Harnois, F.; Mechler, C.; Msika, S.; Grossin, M.; Coffin, B. High Prevalence of Small Intestinal Bacterial Overgrowth in Patients with Morbid Obesity: A Contributor to Severe Hepatic Steatosis. *Obes. Surg.* **2008**, *18*, 371–377. [CrossRef]
9. Saad, R.J.; Chey, W.D. Breath Testing for Small Intestinal Bacterial Overgrowth: Maximizing Test Accuracy. *Clin. Gastroenterol. Hepatol.* **2014**, *12*, 1964–1972. [CrossRef]
10. Romagnuolo, J.; Schiller, D.; Bailey, R.J. Using breath tests wisely in a gastroenterology practice: An evidence-based review of indications and pitfalls in interpretation. *Am. J. Gastroenterol.* **2002**, *97*, 1113–1126. [CrossRef]
11. Kerckhoffs, A.P.M.; Visser, M.R.; Samsom, M.; Van Der Rest, M.E.; De Vogel, J.; Harmsen, W.; Akkermans, L.M.A. Critical Evaluation of Diagnosing Bacterial Overgrowth in the Proximal Small Intestine. *J. Clin. Gastroenterol.* **2008**, *42*, 1095–1102. [CrossRef]
12. Levitt, M.; Hirsh, P.; Fetzer, C.; Sheahan, M.; Levine, A. H2 excretion after ingestion of complex carbohydrates. *Gastroenterology* **1987**, *92*, 383–389. [CrossRef]
13. Ohno, H.; Murakami, H.; Tanisawa, K.; Konishi, K.; Miyachi, M. Validity of an observational assessment tool for multifaceted evaluation of faecal condition. *Sci. Rep.* **2019**, *9*, 3760. [CrossRef] [PubMed]
14. Hinnant, R.T.; Kothmann, M.M. Collecting, Drying, and Preserving Feces for Chemical and Microhistological Analysis. *J. Range Manag.* **1988**, *41*, 168. [CrossRef]
15. Heaton, K.W.; Radvan, J.; Cripps, H.; Mountford, R.A.; Braddon, F.E.; Hughes, A.O. Defecation frequency and timing, and stool form in the general population: A prospective study. *Gut* **1992**, *33*, 818–824. [CrossRef] [PubMed]
16. van den Berg, E.; Engberink, M.F.; Brink, E.J.; van Baak, M.; Gans, R.; Navis, G.; Bakker, S.J.L. Dietary protein, blood pressure and renal function in renal transplant recipients. *Br. J. Nutr.* **2012**, *109*, 1463–1470. [CrossRef] [PubMed]
17. Netherlands Nutrition Center. Dutch Food Composition Table 2006 NEVO-Tabel. 2006. Available online: https://www.rivm.nl/nederlands-voedingsstoffenbestand (accessed on 23 April 2021).
18. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.* **1997**, *65* (Suppl. 4), 1220S–1228S. [CrossRef] [PubMed]

19. Bunnapradist, S.; Neri, L.; Wong, W.; Lentine, K.L.; Burroughs, T.E.; Pinsky, B.W.; Takemoto, S.K.; Schnitzler, M.A. Incidence and Risk Factors for Diarrhea Following Kidney Transplantation and Association With Graft Loss and Mortality. *Am. J. Kidney Dis.* **2008**, *51*, 478–486. [CrossRef]
20. Aulagnon, F.; Scemla, A.; DeWolf, S.; Legendre, C.; Zuber, J. Diarrhea After Kidney Transplantation: A new look at a frequent symptom. *Transplantation* **2014**, *98*, 806–816. [CrossRef]
21. Ekberg, H.; Kyllonen, L.; Madsen, S.; Grave, G.; Solbu, D.; Holdaas, H. Increased prevalence of gastrointestinal symptoms associated with impaired quality of life in renal transplant recipients. *Transplantation* **2007**, *83*, 282–289. [CrossRef]
22. Shin, H.S.; Chandraker, A. Causes and management of postrenal transplant diarrhea: An underappreciated cause of transplant-associated morbidity. *Curr. Opin. Nephrol. Hypertens.* **2017**, *26*, 484–493. [CrossRef]
23. Lee, J.R.; Magruder, M.; Zhang, L.; Westblade, L.F.; Satlin, M.J.; Robertson, A.; Edusei, E.; Crawford, C.; Ling, L.; Taur, Y.; et al. Gut microbiota dysbiosis and diarrhea in kidney transplant recipients. *Am. J. Transplant.* **2019**, *19*, 488–500. [CrossRef]
24. Zinsmeister, A.R.; Herrick, L.M.; Saito Loftus, Y.A.; Schleck, C.D.; Talley, N.J. Identification and validation of functional gastrointestinal disorder subtypes using latent class analysis: A population-based study. *Scand. J. Gastroenterol.* **2017**, *53*, 549–558. [CrossRef]
25. Scallan, E.; Majowicz, S.; Hall, G.; Banerjee, A.; Bowman, C.; Daly, L.; Jones, T.F.; Kirk, M.D.; Fitzgerald, M.; Angulo, F.J. Prevalence of diarrhoea in the community in Australia, Canada, Ireland, and the United States. *Int. J. Epidemiol.* **2005**, *34*, 454–460. [CrossRef]
26. Knight, S.R.; Russell, N.K.; Barcena, L.; Morris, P.J. Mycophenolate Mofetil Decreases Acute Rejection and may Improve Graft Survival in Renal Transplant Recipients When Compared with Azathioprine: A Systematic Review. *Transplantation* **2009**, *87*, 785–794. [CrossRef]
27. Ghoshal, U.C. How to Interpret Hydrogen Breath Tests. *J. Neurogastroenterol. Motil.* **2011**, *17*, 312–317. [CrossRef]
28. Ghoshal, U.C.; Srivastava, D.; Ghoshal, U.; Misra, A. Breath tests in the diagnosis of small intestinal bacterial overgrowth in patients with irritable bowel syndrome in comparison with quantitative upper gut aspirate culture. *Eur. J. Gastroenterol. Hepatol.* **2014**, *26*, 753–760. [CrossRef] [PubMed]
29. Pyleris, E.; Giamarellos-Bourboulis, E.J.; Tzivras, D.; Koussoulas, V.; Barbatzas, C.; Pimentel, M. The Prevalence of Overgrowth by Aerobic Bacteria in the Small Intestine by Small Bowel Culture: Relationship with Irritable Bowel Syndrome. *Dig. Dis. Sci.* **2012**, *57*, 1321–1329. [CrossRef] [PubMed]
30. Sahakian, A.B.; Jee, S.-R.; Pimentel, M. Methane and the Gastrointestinal Tract. *Dig. Dis. Sci.* **2009**, *55*, 2135–2143. [CrossRef]
31. Corazza, G.R.; Strocchi, A.; Gasbarrini, G. Fasting breath hydrogen in celiac disease. *Gastroenterology* **1987**, *93*, 53–58. [CrossRef]
32. Perman, J.A.; Modler, S.; Barr, R.G.; Rosenthal, P. Fasting breath hydrogen concentration: Normal values and clinical application. *Gastroenterology* **1984**, *87*, 1358–1363. [CrossRef]
33. Jahng, J.; Jung, I.S.; Choi, E.J.; Conklin, J.L.; Park, H. The effects of methane and hydrogen gases produced by enteric bacteria on ileal motility and colonic transit time. *Neurogastroenterol. Motil.* **2011**, *24*, 185-e92. [CrossRef]
34. Su, T.; Lai, S.; Lee, A.; He, X.; Chen, S. Meta-analysis: Proton pump inhibitors moderately increase the risk of small intestinal bacterial overgrowth. *J. Gastroenterol.* **2017**, *53*, 27–36. [CrossRef] [PubMed]
35. Laine, L.; Ahnen, D.; McClain, C.; Solcia, E.; Walsh, J.H. Review article: Potential gastrointestinal effects of long-term acid suppression with proton pump inhibitors. *Aliment. Pharmacol. Ther.* **2000**, *14*, 651–668. [CrossRef] [PubMed]
36. Kashyap, P.C.; Marcobal, A.; Ursell, L.K.; Larauche, M.; Duboc, H.; Earle, K.A.; Sonnenburg, E.D.; Ferreyra, J.A.; Higginbottom, S.K.; Million, M.; et al. Complex Interactions Among Diet, Gastrointestinal Transit, and Gut Microbiota in Humanized Mice. *Gastroenterology* **2013**, *144*, 967–977. [CrossRef] [PubMed]
37. Slavin, J.L. Carbohydrates, Dietary Fiber, and Resistant Starch in White Vegetables: Links to Health Outcomes. *Adv. Nutr.* **2013**, *4*, 351S–355S. [CrossRef] [PubMed]
38. Saffouri, G.B.; Shields-Cutler, R.R.; Chen, J.; Yang, Y.; Lekatz, H.R.; Hale, V.L.; Cho, J.M.; Battaglioli, E.J.; Bhattarai, Y.; Thompson, K.J.; et al. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. *Nat. Commun.* **2019**, *10*, 1–11. [CrossRef] [PubMed]
39. Levitt, M.D. Malabsorption of starch: A normal phenomenon. *Gastroenterology* **1983**, *85*, 769–770. [CrossRef]
40. Hammer, H.F.; Hammer, J. Diarrhea Caused By Carbohydrate Malabsorption. *Gastroenterol. Clin. N. Am.* **2012**, *41*, 611–627. [CrossRef]
41. Bello-Perez, L.A.; Flores-Silva, P.C.; Agama-Acevedo, E.; Tovar, J. Starch digestibility: Past, present, and future. *J. Sci. Food Agric.* **2018**, *100*, 5009–5016. [CrossRef]
42. Ho Do, M.; Seo, Y.S.; Park, H.-Y. Polysaccharides: Bowel health and gut microbiota. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1212–1224. [CrossRef]
43. DeMartino, P.; Cockburn, D.W. Resistant starch: Impact on the gut microbiome and health. *Curr. Opin. Biotechnol.* **2020**, *61*, 66–71. [CrossRef] [PubMed]
44. Swarte, J.C.; Douwes, R.M.; Hu, S.; Vila, A.V.; Eisenga, M.F.; Van Londen, M.; Gomes-Neto, A.W.; Weersma, R.K.; Harmsen, H.J.; Bakker, S.J. Characteristics and Dysbiosis of the Gut Microbiome in Renal Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 386. [CrossRef]
45. Lee, J.R.; Muthukumar, T.; Dadhania, D.; Toussaint, N.C.; Ling, L.; Pamer, E.; Suthanthiran, M. Gut Microbial Community Structure and Complications After Kidney Transplantation. *Transplantation* **2014**, *98*, 697–705. [CrossRef] [PubMed]

46. Lin, H.C. Small intestinal bacterial overgrowth: A framework for understanding irritable bowel syndrome. *JAMA* **2004**, *292*, 852–858. [CrossRef] [PubMed]
47. Knobbe, T.J.; Douwes, R.M.; Kremer, D.; Swarte, J.C.; Eisenga, M.F.; Gomes-Neto, A.W.; Van Londen, M.; Peters, F.T.M.; Blokzijl, H.; Nolte, I.M.; et al. Altered Gut Microbial Fermentation and Colonization with Methanobrevibacter smithii in Renal Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 518. [CrossRef]
48. Rezaie, A.; Buresi, M.; Lembo, A.; Lin, H.; McCallum, R.; Rao, S.; Schmulson, M.; Valdovinos, M.; Zakko, S.; Pimentel, M. Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus. *Am. J. Gastroenterol.* **2017**, *112*, 775–784. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Outcomes of Kidney Transplant Recipients with Sickle Cell Disease: An Analysis of the 2000–2019 UNOS/OPTN Database

Napat Leeaphorn [1], Charat Thongprayoon [2,*], Pradeep Vaitla [3], Panupong Hansrivijit [4], Caroline C. Jadlowiec [5], Shennen A. Mao [6], Api Chewcharat [7], Sreelatha Katari [1], Pattharawin Pattharanitima [8,9,*], Boonphiphop Boonpheng [10], Wisit Kaewput [11], Michael A. Mao [12], Matthew Cooper [13] and Wisit Cheungpasitporn [2,*]

1 Renal Transplant Program, University of Missouri-Kansas City School of Medicine, Saint Luke's Health System, Kansas City, MO 64111, USA; napat.leeaphorn@gmail.com (N.L.); skatari@saint-lukes.org (S.K.)
2 Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN 59005, USA
3 Division of Nephrology, University of Mississippi Medical Center, Jackson, MS 39216, USA; pvaitla@umc.edu
4 Department of Internal Medicine, University of Pittsburgh Medical Center Pinnacle, Harrisburg, PA 17101, USA; hansrivijitp@upmc.edu
5 Division of Transplant Surgery, Mayo Clinic, Phoenix, AZ 85054, USA; Jadlowiec.Caroline@mayo.edu
6 Division of Transplant Surgery, Mayo Clinic, Jacksonville, FL 32224, USA; Mao.Shennen@mayo.edu
7 Department of Medicine, Mount Auburn Hospital, Harvard Medical School, Cambridge, MA 02138, USA; api.che@hotmail.com
8 Department of Internal Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand
9 Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
10 Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA; boonpipop.b@gmail.com
11 Department of Military and Community Medicine, Phramongkutklao College of Medicine, Bangkok 10400, Thailand; wisit_nephro@hotmail.com
12 Division of Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL 32224, USA; mao.michael@mayo.edu
13 Medstar Georgetown Transplant Institute, Georgetown University School of Medicine, Washington, DC 20007, USA; Matthew.Cooper@gunet.georgetown.edu
* Correspondence: charat.thongprayoon@gmail.com (C.T.); pattharawin@hotmail.com (P.P.); wcheungpasitporn@gmail.com (W.C.)

Citation: Leeaphorn, N.; Thongprayoon, C.; Vaitla, P.; Hansrivijit, P.; Jadlowiec, C.C.; Mao, S.A.; Chewcharat, A.; Katari, S.; Pattharanitima, P.; Boonpheng, B.; et al. Outcomes of Kidney Transplant Recipients with Sickle Cell Disease: An Analysis of the 2000–2019 UNOS/OPTN Database. *J. Clin. Med.* **2021**, *10*, 3063. https://doi.org/10.3390/jcm10143063

Academic Editor: Magdi Yaqoob

Received: 3 June 2021
Accepted: 9 July 2021
Published: 11 July 2021

Abstract: Background: Lower patient survival has been observed in sickle cell disease (SCD) patients who go on to receive a kidney transplant. This study aimed to assess the post-transplant outcomes of SCD kidney transplant recipients in the contemporary era. Methods: We used the OPTN/UNOS database to identify first-time kidney transplant recipients from 2010 through 2019. We compared patient and allograft survival between recipients with SCD (n = 105) vs. all other diagnoses (non-SCD, n = 146,325) as the reported cause of end-stage kidney disease. We examined whether post-transplant outcomes improved among SCD in the recent era (2010–2019), compared to the early era (2000–2009). Results: After adjusting for differences in baseline characteristics, SCD was significantly associated with lower patient survival (HR 2.87; 95% CI 1.75–4.68) and death-censored graft survival (HR 1.98; 95% CI 1.30–3.01), compared to non-SCD recipients. The lower patient survival and death-censored graft survival in SCD recipients were consistently observed in comparison to outcomes of recipients with diabetes, glomerular disease, and hypertension as the cause of end-stage kidney disease. There was no significant difference in death censored graft survival (HR 0.99; 95% CI 0.51–1.73, p = 0.98) and patient survival (HR 0.93; 95% CI 0.50–1.74, p = 0.82) of SCD recipients in the recent versus early era. Conclusions: Patient and allograft survival in SCD kidney recipients were worse than recipients with other diagnoses. Overall SCD patient and allograft outcomes in the recent era did not improve from the early era. The findings of our study should not discourage kidney transplantation for ESKD patients with SCD due to a known survival benefit of transplantation compared with remaining on dialysis. Urgent future studies are needed to identify strategies to improve patient and allograft survival in SCD kidney recipients. In addition, it may be reasonable to assign risk adjustment for SCD patients.

Keywords: sickle cell disease; sickle cell; kidney transplantation; transplantation; outcomes; big data

1. Introduction

Sickle cell disease (SCD) is the most common inherited blood disorder in the United States, affecting approximately 100,000 Americans [1–4]. SCD occurs in one out of every 365 African-American births and 1 out of every 16,300 Hispanic-American births [1–3]. Patients with SCD are at risk for progressive kidney disease, initially manifesting as microalbuminuria and urine-concentrating defects in childhood with subsequent progression to overt proteinuria and progressive decline in kidney function after age 20 [1–4]. Almost half of SCD patients with HbSS genotype develop chronic kidney disease; the prevalence of end stage kidney disease (ESKD) ranges from 5% up to 18% with a median age of 23 years [1,5–7]. Despite the prevalence of ESKD in SCD, few of these individuals receive a kidney transplant [8–10].

Ongoing unequal access to kidney transplantation for SCD is a reflection of the transplant community's concerns related to increased mortality risk for SCD patients compared to ESKD patients from other causes [11,12]. Nonetheless, despite having an increased risk, SCD ESKD patients receive a survival benefit following kidney transplantation similar to non-SCD ESKD patients [8,9]. Despite improvement in survival after transplantation in SCD patients [8,9], there are still concerns regarding worse outcomes among kidney transplant recipients with SCD compared to non-SCD recipients [8,10]. Data from prior to 2000 [8,10] including data from United States Renal Data System (USRDS) and the United Network for Organ Sharing (UNOS) databases demonstrated that kidney transplant recipients with SCD had reduced allograft and patient survival compared to non-SCD kidney transplant recipients [8,10]. Despite these concerns, a previous study using UNOS database demonstrated improved patient survival among SCD recipients over time (from years 1988–1999 to 2000–2011) [13].

Immunosuppression and kidney transplant care have significantly advanced in the past decades [14–17], and overall kidney transplantation outcomes have significant improved in the recent era [18,19]. In addition to emerging immunosuppressing agents, advances in human leukocyte antigen (HLA)/epitope matching, infection prevention, pre-transplant apolipoprotein L1 gene (APOL1), screening standardization of pre-transplant preparation for SCD patients, use of post-transplant SCD disease modifying therapy may impact transplant outcomes of patients with SCD [20–22]. The outcomes of kidney transplant for SCD ESKD recipients in the current transplant era remain underreported. We conducted this study using the UNOS database with the aim to (1) compare patient and allograft survival between recipients with SCD vs. non-SCD in the recent era (2010–2019), and to (2) examine outcomes of SCD recipients in the recent era (2010–2019), compared to the early era (2000–2009).

2. Methods

2.1. Data Source and Study Population

This study used the Organ Procurement and Transplantation Network (OPTN) /UNOS database. The OPTN/UNOS contains patient-level data of all United States transplant events. Institutional review board approval was waived due to publicly available nature of the de-identified OPTN/UNOS database.

The primary cohort includes pediatric and adult solitary first kidney transplant recipients occurring from 2010 to 2019. Patient and allograft survival between recipients with SCD ESKD were compared to recipients with all other diagnoses (non-SCD ESKD). In addition, we compared post-transplant outcomes of SCD recipients with the three most common causes of end-stage kidney disease: diabetes mellitus (DM), glomerular disease, and hypertension.

In the secondary cohort, pediatric and adult SCD ESKD patients receiving solitary first kidney transplants from 2000 to 2019 were identified. Post-transplant outcomes were compared between SCD recipients in two eras: an earlier era (Era 1: 2000–2009) versus recent (Era 2: 2010–2019)

2.2. Data Collection

The following variables were extracted: recipient age, sex, race, body mass index (BMI), transplant type, dialysis duration, causes of end-stage kidney disease, SCD status, history of diabetes mellitus, panel reactive antibody (PRA) level, donor age, kidney donor profile index (KDPI), induction and maintenance therapy. SCD as the cause of ESRD was reported by individual transplant center to OPTN/UNOS database. Missing data were not imputed.

2.3. Outcome Measures

The primary outcome was death-censored graft failure, defined as the need for dialysis or kidney re-transplant, with patients censored for death or at last follow-up date reported to the OPTN/UNOS database. The secondary outcome was all-cause mortality. Patients were followed until outcomes occurred, the end of study period (27 September 2019), or 5 years after kidney transplant, whichever was earlier.

2.4. Statistical Analysis

Baseline characteristics were summarized using medians with interquartile ranges for continuous variables or counts with percentage for categorical variables. Between-group comparison was performed using Wilcoxon rank-sum test for continuous variables and Chi-squared test for categorial variables. Kaplan–Meier plots were utilized to generate death-censored graft survival and patient survival curve. The log-rank test offered statistical comparison. Cox proportional hazard analysis was utilized to calculate hazard ratio (HR) and 95% confidence interval (95% CI) of death-censored graft failure and mortality. This association was adjusted for recipient, donor, and transplant-related factors that significantly differed between groups with $p < 0.05$. All p-values were two-tailed; p-values of < 0.05 were considered statistically significant. STATA version 14.1 (StataCorp, College Station, TX, USA) was utilized for all statistical analyses.

3. Results

3.1. Clinical Characteristics between SCD vs. Non-SCD Recipients in Recent Era

The primary cohort was comprised of a total of 146,430 patients who received a first kidney transplant from 2010 to 2019. There were 105 SCD and 146,325 non-SCD kidney transplant recipients. Of the non-SCD recipients, 40,362 (27.6%) had DM, 33,979 (23.2%) had glomerular disease, and 33,617 (23.0%) had hypertension as the reported cause of ESKD.

Table 1 compares baseline characteristics between SCD and non-SCD recipients. SCD recipients were younger, more likely to be female, African American, have a lower BMI, and less DM. They were more likely to have a higher PRA, be on dialysis at the time of transplant, and receive thymoglobulin as induction therapy. In addition, kidney donors for SCD recipients were younger, more African American, and more likely to have lower KDPI.

3.2. Post-Transplant Outcomes between SCD vs. Non-SCD Recipients in Recent Era

One-year death-censored graft survival in SCD was lower than in non-SCD recipients (94% vs. 98%; $p = 0.02$), whereas there was no significant difference in patient survival between SCD and non-SCD recipients (96% vs. 97%; $p = 0.36$). In adjusted analysis, SCD recipients were significantly associated with lower 1-year death-censored graft survival (HR 2.22; 95% CI 1.01–4.95). SCD recipients had lower 1-year patient survival than non-SCD recipients with HR of 2.24 although it did not reach statistical significance ($p = 0.11$) due to small number of deaths within 1 years after kidney transplant.

Table 1. Clinical characteristics between sickle cell disease and non-sickle cell disease kidney transplant recipients.

Characteristics	Sickle Cell Disease (n = 105)	Non-Sickle Cell Disease (n = 146,325)	p-Value
Recipient age (year), median (25th, 75th)	41 (33, 51)	53 (41, 63)	<0.001
Male, %	47.6	61.0	0.005
African American, %	93.3	26.9	<0.001
Recipient BMI (kg/m^2), median (25th, 75th)	22.7 (20.4, 27.1)	27.9 (24.0, 32.1)	<0.001
Living-donor kidney transplants, %	28.6	34.1	0.24
Dialysis duration (%)			
Preemptive	7.6	19.2	0.003
<1 years	13.3	14.2	0.80
1–3 years	20.0	23.6	0.39
>3 years	57.1	42.1	0.002
Missing	1.9	0.9	0.28
Diabetes, %	1.0	33.4	<0.001
PRA (%)			
<20	62.9	76.7	0.001
20–70	18.1	12.4	0.08
>70	15.2	9.3	0.035
Missing	3.8	1.6	0.07
ABO incompatible	1.0	1.2	0.84
HLA mismatches, median (25th, 75th)	4 (3, 5)	4 (3, 5)	0.44
Donor age (year), median (25th, 75th)	30 (24, 45)	41 (28, 52)	<0.001
Donor race, %			
White	54.3	68.4	0.002
Black	28.6	12.6	<0.001
Hispanic	14.3	14.2	0.98
Others	2.9	4.8	0.35
KDPI, median (25th, 75th)	39 (11, 60)	44 (23, 67)	0.008
Induction therapy, %			
Thymoglobulin	66.7	52.7	0.004
Alemtuzumab	8.6	15.8	0.04
Basiliximab	24.8	23.5	0.76
Other induction	3.8	1.9	0.15
No induction	8.6	9.6	0.72
Maintenance therapy, %			
Tacrolimus	90.5	91.6	0.68
Cyclosporine	1.9	2.0	0.96
Mycophenolate	94.3	92.9	0.57
Azathioprine	0	0.4	0.50
mTOR inhibitors	0	1.1	0.29
Steroids	70.5	65.8	0.31

Abbreviation: BMI, body mass index; KDPI, kidney donor profile index; mTOR inhibitors, the mammalian target of rapamycin inhibitors; PRA, panel reactive antibody.

Five-year death-censored graft survival in SCD was lower than in non-SCD recipients (71% vs. 89%; $p < 0.001$) (Figure 1), whereas five-year patient survival was comparable between the two groups (83% vs. 87%; $p = 0.12$) (Figure 2). In adjusted analysis, SCD recipients were significantly associated with lower death-censored graft survival (HR 1.98;

95% CI 1.30–3.01, $p = 0.001$) (Table 2 A) and patient survival (HR 2.87; 95% CI 1.75–4.68, $p < 0.001$), compared to non-SCD recipients (Table 2 B).

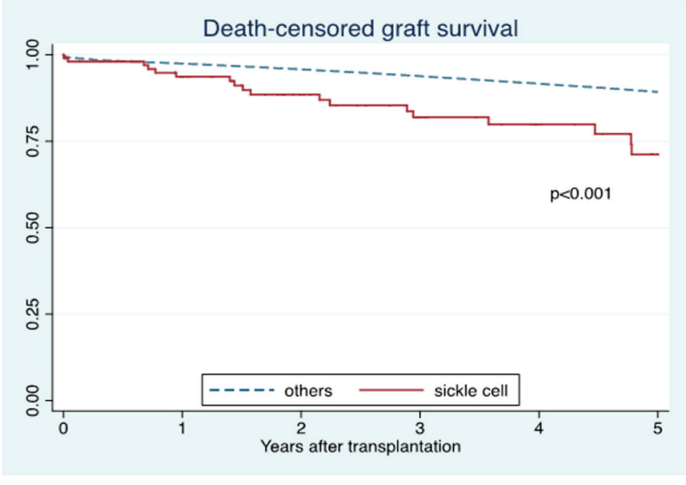


Figure 1. Death-censored graft survival of sickle cell disease compared to non-sickle cell disease recipients.

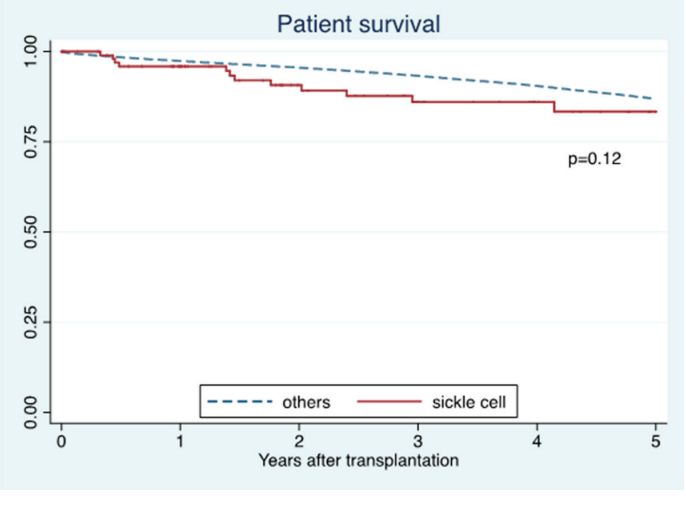


Figure 2. Patient survival of sickle cell disease compared to non-sickle disease recipients.

Post-transplant outcomes were compared between SCD and other more prevalent causes of end-stage kidney disease. Five-year death-censored graft survival was 89% for DM, 89% for glomerular disease, and 88% for hypertension (Figure 3). Five-year patient survival was 78% for DM, 93% for glomerular disease, and 86% for hypertension (Figure 4). In adjusted analysis, SCD was significantly associated with lower death-censored graft survival compared to DM (HR 2.32; 95% CI 1.52–3.56, $p < 0.001$), glomerular disease (HR 1.91; 95% CI 1.25–2.92), and hypertension (HR 1.91; 95% CI 1.25–2.92, $p = 0.003$) (Table 2A). In addition, SCD was significantly associated with lower patient survival compared to

DM (HR 1.96; 95% CI 1.20–3.22, $p = 0.007$), glomerular disease (HR 3.41; 95% CI 2.07–5.61, $p < 0.001$), and hypertension (HR 3.08; 95% CI 1.88–5.04, $p < 0.001$) (Table 2B).

Table 2. Post-transplant outcomes of sickle cell disease compared to non-sickle cell disease.

(A) Death-Censored Graft Failure				
	Univariate Model		Multivariate Model *	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Recent era (2010–2019)				
Sickle cell versus non-sickle cell	2.92 (1.92–4.44)	<0.001	1.98 (1.30–3.01)	0.001
Sickle cell versus diabetes	2.84 (1.87–4.32)	<0.001	2.32 (1.52–3.56)	<0.001
Sickle cell versus hypertension	2.53 (1.66–3.84)	<0.001	1.91 (1.25–2.92)	0.003
Sickle cell versus glomerular disease	2.80 (1.84–4.25)	<0.001	1.91 (1.25–2.92)	0.003
Sickle cell between 2000–2009 and 2010–2019	0.90 (0.54–1.50)	0.68	0.99 (0.57–1.73)	0.98
(B) Mortality				
	Univariate Model		Multivariate Model *	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Recent era (2010–2019)				
Sickle cell versus others	1.67 (1.03–2.73)	0.039	2.87 (1.75–4.68)	<0.001
Sickle cell versus diabetes	0.95 (0.58–1.54)	0.82	1.96 (1.20–3.22)	0.007
Sickle cell versus hypertension	1.61 (0.99–2.64)	0.056	3.08 (1.88–5.04)	<0.001
Sickle cell versus glomerular disease	3.18 (1.95–5.21)	<0.001	3.41 (2.07–5.61)	<0.001
Sickle cell between 2000–2009 and 2010–2019	0.89 (0.50–1.59)	0.70	0.93 (0.50–1.74)	0.82

* Adjusted for recipient age, sex, race, BMI, dialysis status, cPRA, donor age, donor race, KDPI, and induction therapy.

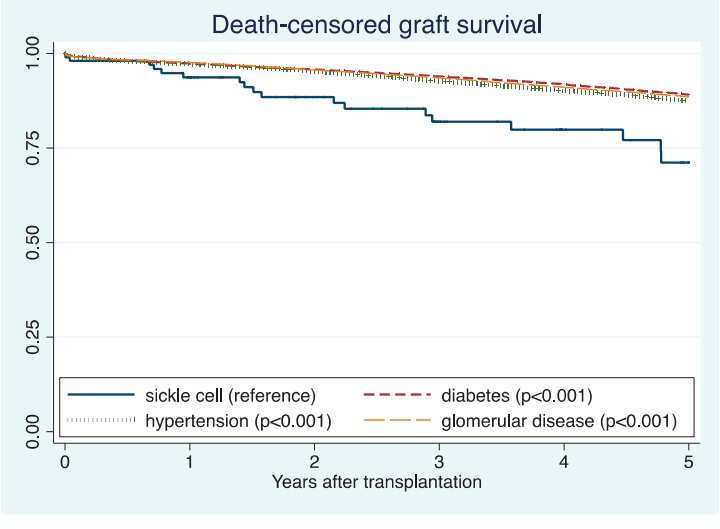


Figure 3. Death-censored graft survival according to the causes of end-stage kidney disease.

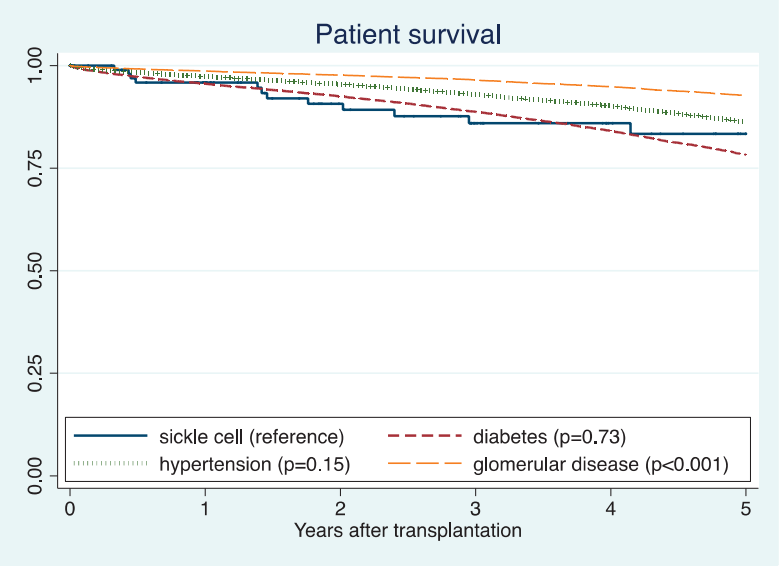


Figure 4. Patients survival according to the causes of end-stage kidney disease.

3.3. Clinical Characteristics and Post-Transplant Outcomes between SCD Recipients in an Early vs. Recent Era

In the secondary cohort, a total of 233 SCD ESKD kidney transplants were performed from 2000 to 2019. There were 128 SCD ESKD kidney recipients in 2000–2009 (early era) and 105 in 2010–2019 (recent era). Table 3 compares baseline characteristics of SCD recipients between the early and recent era.

Table 3. Clinical characteristics between sickle cell disease in 2010–2019 and 2000–2009.

Characteristics	Sickle Cell Disease in 2010–2019 (n = 105)	Sickle Cell Disease in 2000–2009 (n = 128)	p-Value
Recipient age (year), median (25th, 75th)	41 (33, 51)	35 (29, 45)	<0.001
male, %	47.6	58.6	0.10
African American, %	93.3	91.4	0.58
Recipient BMI (kg/m^2), median (25th, 75th)	22.7 (20.4, 27.1)	20.5 (18.1, 23.7)	<0.001
Living-donor kidney transplants, %	28.6	34.4	0.34
Dialysis duration (%)			
Preemptive	7.6	7.8	0.96
<1 years	13.3	18.0	0.34
1–3 years	20.0	25.8	0.30
>3 years	57.1	38.3	0.004
Missing	1.9	10.2	0.01
Diabetes, %	1.0	1.6	0.68
PRA (%)			
<20	62.9	57.8	0.43
20–70	18.1	15.6	0.62
>70	15.2	11.7	0.43
Missing	3.8	14.8	0.005

Table 3. *Cont.*

Characteristics	Sickle Cell Disease in 2010–2019 (n = 105)	Sickle Cell Disease in 2000–2009 (n = 128)	p-Value
ABO incompatible	1.0	0	0.27
HLA mismatches, median (25th, 75th)	4 (3, 5)	4 (3, 5)	0.33
Donor age (year), median (25th, 75th)	30 (24, 45)	39 (27, 47)	0.03
Donor race, %			
White	54.3	46.9	0.26
Black	28.6	37.5	0.15
Hispanic	14.3	12.5	0.69
Others	2.9	3.1	0.90
KDPI, median (25th, 75th)	39 (11, 60)	46 (30, 66)	0.02
Induction therapy, %			
Thymoglobulin	66.7	37.5	<0.001
Alemtuzumab	8.6	5.5	0.35
Basiliximab	24.8	19.5	0.34
Other induction	3.8	10.2	0.06
No induction	8.6	29.7	<0.001
Maintenance therapy, %			
Tacrolimus	90.5	71.1	<0.001
Cyclosporine	1.9	22.7	<0.001
Mycophenolate	94.3	81.3	0.003
Azathioprine	0	3.9	0.04
mTOR inhibitors	0	14.1	<0.001
Steroids	70.5	82.0	0.04

Death-censored graft survival (71% vs. 66%; p = 0.68) (Figure 5) and patient survival (83% vs. 78%; p = 0.69) (Figure 6) were comparable between SCD recipients in an early and recent era. There was no significant difference in death censored graft survival (HR 0.99; 95% CI 0.51–1.73, p = 0.98) and patient survival (HR 0.93; 95% CI 0.50–1.74, p = 0.82) between the two eras.

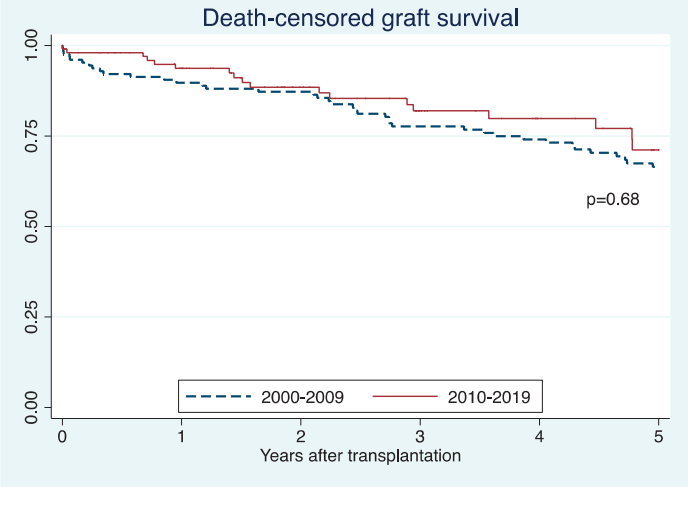


Figure 5. Death-censored graft survival of sickle cell disease recipients in 2010–2019 and 2000–2009.

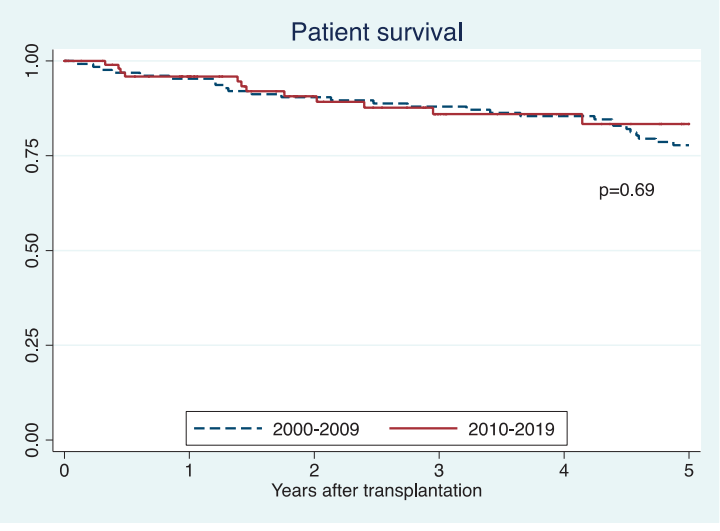


Figure 6. Patient survival of sickle cell disease recipients in 2010–2019 and 2000–2009.

4. Discussion

In this study utilizing the UNOS/OPTN database from 2000 to 2019, kidney transplant recipients with SCD had lower death censored graft survival and increased mortality compared to overall non-SCD recipients. There was no significant difference in the allograft and patient survival between the recent era (2010–2019) versus the early era (2000–2009).

The increased risk of allograft loss in SCD kidney transplant recipients is likely multifactorial. SCD is characterized by vaso-occlusion, especially in the kidneys [23], resulting in kidney infarction, papillary necrosis, hematuria, focal segmental glomerulosclerosis, and diabetes insipidus [23,24]. Successful kidney transplantation significantly improves kidney function among ESKD patients with SCD; however, unfortunately, the kidney allograft can still be affected by SCD [25], evidenced by iron/heme deposition in proximal renal tubules and related acute tubular injury in the kidney allograft biopsies [26,27]. In addition, chronic allograft injury and anemia may accelerate kidney function decline by limiting oxygen delivery [28]. Hypoxia may also lead to the formation of a reactive oxygen species that potentiates tissue inflammation [28]. Furthermore, allograft thrombosis during sickle cell crisis after kidney transplantation has been reported [27,29,30]. Despite improvement of overall transplant care and immunosuppression, our evaluation of the recent era (2010–2019) reveals persistent lower allograft survival among kidney transplant recipients with SCD compared to those with non-SCD, which is consistent with previously published studies before 2000 from the UNOS and USRDS databases [8,10]. Hydroxyurea and blood transfusion have been the primary methods used to treat SCD complications [20,21]. Although data on the effects of hydroxyurea on kidney transplant allograft are limited, the use of hydroxyurea has shown potential benefits in reduction of proteinuria, stabilization in kidney function, and reduced mortality risk among non-kidney transplant SCD patients [21,31–33]. While early post-transplant blood transfusion may increase immunological risk and increased risk of rejection, especially in under-immunosuppressed recipients [34], a recent study demonstrated that blood transfusion while on adequate immunosuppressive regimens within 1 week post-kidney transplantation is safe without significant association with de novo HLA-DSA development [35]. Among kidney transplant recipients with SCD, a well-conducted multicenter study on 34 SCD kidney transplant recipients between 1997 and 2017 across six London hospitals ensured the safety of long-term automated exchange blood transfusions (EBT) with im-

provement in allograft and patient outcomes and no increase in antibody formation or allograft rejection [20]. However, data on the use of hydroxyurea and blood transfusion are limited in the OPTN/UNOS database. Thus, future studies are needed to assess the impact of hydroxyurea, EBT, and perioperative management on outcomes of kidney transplant recipients with SCD. Furthermore, there are now new medications recently made available that are expected to be of significant benefit among SCD patients [36–39], which require future studies in kidney transplant recipients.

In our study of immunosuppression in the recent era, we also found a significantly reduced patient survival among kidney transplant recipients with SCD compared to non-SCD recipients. This is similar to the higher mortality found in SCD kidney recipients from published older era studies [8,10,13]. While a previous study utilizing the UNOS database demonstrated improved patient survival among SCD recipients from 2000–2011 compared with an earlier cohort of SCD recipients (1988–1999) [13], we did not find a significant improvement in patient survival and death-censored graft survival of SCD recipients in years 2010–2019 when compared to years 2000–2009. Furthermore, compared to other causes of ESKD, including diabetes, glomerular disease, and hypertension, our adjusted analysis showed that SCD was associated with the lower patient survival and death-censored graft survival. While patient survival in the prior era (2000–2011) was comparable among SCD recipients and diabetic recipients [13], our study shows better patient survival among diabetic recipients compared with SCD recipients. Given that there is no difference between patient survival among SCD in years 2010–2019 when compared to years 2000–2009, this finding likely reflects a significant improvement in diabetes care post kidney transplantation for the past decade [40–42]. Kidney transplant recipients with SCD still have complications of SCD post-transplant [20–22]. Since SCD is a systemic disease, it can result in extra-renal manifestations, such as pulmonary hypertension, thrombotic events, infections, cardiomyopathy and cirrhosis [43,44]. Unfortunately, the findings from our study suggest a lack in similar improvement in SCD care post-transplantation when compared to other causes of ESKD including diabetes. Future studies are required to identify effective strategies to improve care for kidney transplant recipients with SCD such as care in a multi-center system and long-term follow-up in tertiary care centers.

Kidney transplantation improves survival in patients with SCD after kidney transplant when compared to SCD patients on maintenance dialysis [9]. However, SCD patients have lower rates of waitlisting and transplantation rate after listing despite survival benefit with transplantation [9]. Thus, our study should not discourage kidney transplantation for ESKD patients with SCD. Rather, our findings highlight the urgent need for strategies to improve transplant outcomes among SCD kidney transplant recipients. Multidisciplinary subspecialty coordination likely offers the most benefit for management of kidney transplant recipients with SCD. Furthermore, SCD patients are less likely to be waitlisted or transplanted when compared to patients of similar age with other causes of ESKD [9]. This is potentially due to increased risk for graft loss and mortality after transplantation [8,10], or racial disparities in kidney transplantation [45,46], representing a potential "blind spot" regarding racial disparities in access and health outcomes [47]. Transplant centers are monitored for outcomes and may be sanctioned if transplant outcomes do not meet expected outcomes over a period of time. Expected outcomes are calculated based on patient population and comorbidities, which include primary diagnosis at transplant. Risk is adjusted for recipients with diabetes mellitus, hypertension and glomerular disease [48]; however, SCD is currently not considered for risk adjustment. Based on our data and previous reports [9,10], SCD recipients experience increased an risk of graft failure and mortality compared to the control group. SCD patients benefit from kidney transplant, despite increased morbidity and mortality, thus, it is reasonable to assign risk adjustment for SCD patients. This will reduce the disincentive for transplant centers and increase access to life-saving kidney transplantation in the SCD patient population [45,46].

Our study has some limitations. Firstly, information on non-renal SCD-related organ injuries/complications was not available to review in the OPTN/UNOS database, thus we

were unable to account for the effects of severity of SCD on transplant outcomes. Secondly, data on SCD genotype, comorbidity of SCD recipients, the frequency of vaso-occlusive crises, blood transfusion, the development of de novo HLA-DSA and RBC alloantibodies, treatment regimen for SCD, causes of death or kidney allograft failure in SCD recipients were unavailable. Consequently, despite adjusting for available confounders, we determined that patient survival was not significantly different between the recent era (2010–2019) and the early era (2000–2009), but we did not have information to understand the reason for the lack of improvement in post-transplant outcomes in SCD recipients. With the above noted limitations on SCD severity data, we cannot conclude that there was no difference in the advances of SCD kidney transplant recipients' care. Physicians may have a lower threshold to offer kidney transplantation to SCD patients with more SCD-related organ involvements in the recent era, which could have affected patient outcomes. Future studies evaluating the effects of SCD severity and frequency of vaso-occlusive crises on outcomes of kidney transplant recipients with SCD are required. Finally, there are limited data on emerging treatments for SCD in the OPTN/UNOS database. Bone marrow transplantation has recently emerged as a novel treatment for SCD [49], and the beneficial effects of bone marrow transplantation in improving target organ damage have been reported [12,50,51]. Nevertheless, successful bone marrow transplant has only been reported in a limited number of dialysis and kidney transplant patients with SCD [50,52,53]. Additional studies are needed to assess its potential effects on clinical outcomes of SCD kidney transplant recipients [12]. In addition, while genetic approaches against SCD such as CRISPR gene correction therapy are promising, investigation in this area is still in the early stages [54].

5. Conclusions

In conclusion, patient and allograft survival in SCD kidney recipients were worse than that of recipients with other diagnoses. Overall SCD patient and allograft outcomes in the recent era did not improve from the early era. The findings of our study should not discourage kidney transplantation for ESKD patients with SCD due to the known survival benefits of transplantation compared with remaining on dialysis. Future studies are urgently needed to identify strategies to improve patient and allograft survival in SCD kidney recipients. In addition, it is reasonable to assign risk adjustment for SCD patients.

Author Contributions: Conceptualization, N.L., C.T., P.V., P.H., C.C.J., S.A.M., A.C., S.K., P.P., B.B., W.K., M.A.M., M.C. and W.C.; data curation, N.L. and C.T.; formal analysis, N.L.; investigation, N.L., C.T., P.H., P.P. and W.C.; methodology, N.L., C.T., C.C.J., S.A.M., P.P., B.B., M.A.M. and W.C.; project administration, S.K.; resources, N.L.; software, N.L.; supervision, P.V., S.A.M., W.K., M.A.M., M.C. and W.C.; validation, C.T. and W.C.; visualization, N.L.; writing—original draft, N.L., C.T. and W.C.; writing—review and editing, N.L., C.T., P.V., P.H., C.C.J., S.A.M., A.C., S.K., P.P., B.B., W.K., M.A.M., M.C. and W.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study, due to the use of publicly available de-identified data.

Informed Consent Statement: Patient consent was waived due to the minimal risk nature of observational study design.

Data Availability Statement: Data is available upon reasonable request to corresponding author.

Acknowledgments: The interpretation and reporting of this data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the OPTN or the United States government.

Conflicts of Interest: The authors deny any conflict of interest.

References

1. Maigne, G.; Ferlicot, S.; Galacteros, F.; Belenfant, X.; Ulinski, T.; Niaudet, P.; Ronco, P.; Godeau, B.; Durrbach, A.; Sahali, S.; et al. Glomerular lesions in patients with sickle cell disease. *Medicine* **2010**, *89*, 18–27. [CrossRef]
2. Nath, K.A.; Hebbel, R.P. Sickle cell disease: Renal manifestations and mechanisms. *Nat. Rev. Nephrol.* **2015**, *11*, 161–171. [CrossRef]
3. Warady, B.A.; Sullivan, E.K. Renal transplantation in children with sickle cell disease: A report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). *Pediatr. Transplant.* **1998**, *2*, 130–133.
4. Ataga, K.I.; Zhou, Q.; Derebail, V.K.; Saraf, S.L.; Hankins, J.S.; Loehr, L.R.; Garrett, M.E.; Ashley-Koch, A.E.; Cai, J.; Telen, M.J. Rapid decline in estimated glomerular filtration rate in sickle cell anemia: Results of a multicenter pooled analysis. *Haematologica* **2020**. [CrossRef]
5. Gosmanova, E.O.; Zaidi, S.; Wan, J.Y.; Adams-Graves, P.E. Prevalence and progression of chronic kidney disease in adult patients with sickle cell disease. *J. Investig. Med.* **2014**, *62*, 804–807. [CrossRef] [PubMed]
6. Powars, D.R.; Elliott-Mills, D.D.; Chan, L.; Niland, J.; Hiti, A.L.; Opas, L.M.; Johnson, C. Chronic renal failure in sickle cell disease: Risk factors, clinical course, and mortality. *Ann. Intern. Med.* **1991**, *115*, 614–620. [CrossRef] [PubMed]
7. Viner, M.; Zhou, J.; Allison, D.; Han, J.; Molokie, R.E.; Jain, S.; Gowhari, M.; Gordeuk, V.R.; Calip, G.; Saraf, S.L. The morbidity and mortality of end stage renal disease in sickle cell disease. *Am. J. Hematol.* **2019**, *94*, E138–E141. [CrossRef]
8. Ojo, A.O.; Govaerts, T.C.; Schmouder, R.L.; Leichtman, A.B.; Leavey, S.F.; Wolfe, R.A.; Held, P.J.; Port, F.K.; Agodoa, L.Y. Renal transplantation in end-stage sickle cell nephropathy. *Transplantation* **1999**, *67*, 291–295. [CrossRef] [PubMed]
9. Bae, S.; Johnson, M.; Massie, A.B.; Luo, X.; Haywood, C., Jr.; Lanzkron, S.M.; Grams, M.E.; Segev, D.L.; Purnell, T.S. Mortality and access to kidney transplantation in patients with sickle cell disease-associated kidney failure. *Clin. J. Am. Soc. Nephrol.* **2021**, *16*, 407–414. [CrossRef] [PubMed]
10. Bleyer, A.J.; Donaldson, L.A.; McIntosh, M.; Adams, P.L. Relationship between underlying renal disease and renal transplantation outcome. *Am. J. Kidney Dis.* **2001**, *37*, 1152–1161. [CrossRef] [PubMed]
11. Abbott, K.C.; Hypolite, I.O.; Agodoa, L.Y. Sickle cell nephropathy at end-stage renal disease in the United States: Patient characteristics and survival. *Clin. Nephrol.* **2002**, *58*, 9–15. [CrossRef]
12. Okafor, U.H.; Aneke, E. Outcome and challenges of kidney transplant in patients with sickle cell disease. *J. Transplant.* **2013**, *2013*, 614610. [CrossRef] [PubMed]
13. Huang, E.; Parke, C.; Mehrnia, A.; Kamgar, M.; Pham, P.T.; Danovitch, G.; Bunnapradist, S. Improved survival among sickle cell kidney transplant recipients in the recent era. *Nephrol. Dial. Transplant.* **2013**, *28*, 1039–1046. [CrossRef] [PubMed]
14. Wiseman, A.C. Induction therapy in renal transplantation: Why? What agent? What dose? We may never know. *Clin. J. Am. Soc. Nephrol.* **2015**, *10*, 923–925. [CrossRef] [PubMed]
15. Thongprayoon, C.; Hansrivijit, P.; Leeaphorn, N.; Acharya, P.; Torres-Ortiz, A.; Kaewput, W.; Kovvuru, K.; Kanduri, S.R.; Bathini, T.; Cheungpasitporn, W. Recent Advances and Clinical Outcomes of Kidney Transplantation. *J. Clin. Med.* **2020**, *9*, 1193. [CrossRef]
16. Cheungpasitporn, W.; Lentine, K.L.; Tan, J.C.; Kaufmann, M.; Caliskan, Y.; Bunnapradist, S.; Lam, N.N.; Schnitzler, M.; Axelrod, D.A. Immunosuppression Considerations for Older Kidney Transplant Recipients. *Curr. Transplant. Rep.* **2021**, *8*, 100–110. [CrossRef] [PubMed]
17. Lentine, K.L.; Cheungpasitporn, W.; Xiao, H.; McAdams-DeMarco, M.; Lam, N.N.; Segev, D.L.; Bae, S.; Ahn, J.B.; Hess, G.P.; Caliskan, Y.; et al. Immunosuppression Regimen Use and Outcomes in Older and Younger Adult Kidney Transplant Recipients: A National Registry Analysis. *Transplantation* **2020**. [CrossRef]
18. Arend, S.M.; Mallat, M.J.; Westendorp, R.J.; van der Woude, F.J.; van Es, L.A. Patient survival after renal transplantation; more than 25 years follow-up. *Nephrol. Dial. Transplant.* **1997**, *12*, 1672–1679. [CrossRef]
19. Meier-Kriesche, H.U.; Ojo, A.O.; Port, F.K.; Arndorfer, J.A.; Cibrik, D.M.; Kaplan, B. Survival improvement among patients with end-stage renal disease: Trends over time for transplant recipients and wait-listed patients. *J. Am. Soc. Nephrol.* **2001**, *12*, 1293–1296. [CrossRef]
20. Willis, J.C.; Awogbade, M.; Howard, J.; Breen, C.; Abbas, A.; Harber, M.; Shendi, A.M.; Andrews, P.A.; Galliford, J.; Thuraisingham, R.; et al. Outcomes following kidney transplantation in patients with sickle cell disease: The impact of automated exchange blood transfusion. *PLoS ONE* **2020**, *15*, e0236998. [CrossRef]
21. Sharpe, C.C.; Thein, S.L. How I treat renal complications in sickle cell disease. *Blood* **2014**, *123*, 3720–3726. [CrossRef]
22. Freedman, B.I.; Moxey-Mims, M.M.; Alexander, A.A.; Astor, B.C.; Birdwell, K.A.; Bowden, D.W.; Bowen, G.; Bromberg, J.; Craven, T.E.; Dadhania, D.M.; et al. APOL1 Long-term Kidney Transplantation Outcomes Network (APOLLO): Design and Rationale. *Kidney Int. Rep.* **2020**, *5*, 278–288. [CrossRef] [PubMed]
23. Pham, P.T.; Pham, P.C.; Wilkinson, A.H.; Lew, S.Q. Renal abnormalities in sickle cell disease. *Kidney Int.* **2000**, *57*, 1–8. [CrossRef]
24. Scheinman, J.I. Sickle cell disease and the kidney. *Nat. Clin. Pract. Nephrol.* **2009**, *5*, 78–88. [CrossRef] [PubMed]
25. Miner, D.J.; Jorkasky, D.K.; Perloff, L.J.; Grossman, R.A.; Tomaszewski, J.E. Recurrent sickle cell nephropathy in a transplanted kidney. *Am. J. Kidney Dis.* **1987**, *10*, 306–313. [CrossRef]
26. Wang, Y.; Doshi, M.; Khan, S.; Li, W.; Zhang, P.L. Utility of iron staining in identifying the cause of renal allograft dysfunction in patients with sickle cell disease. *Case Rep. Transplant.* **2015**, *2015*, 528792. [CrossRef]
27. O'Rourke, E.J.; Laing, C.M.; Khan, A.U.; Hussain, R.; Standish, R.A.; Buscombe, J.R.; Hilson, A.J.; Harber, M. The case. Allograft dysfunction in a patient with sickle cell disease. *Kidney Int.* **2008**, *74*, 1219–1220. [CrossRef]

28. Nangaku, M. Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *J. Am. Soc. Nephrol.* **2006**, *17*, 17–25. [CrossRef]
29. Fischer, G.B.; da Rosa, A.C. Thrombosis after kidney transplantation. *Blood Coagul. Fibrinolysis* **2009**, *20*, 456–457. [CrossRef]
30. Kim, L.; Garfinkel, M.R.; Chang, A.; Kadambi, P.V.; Meehan, S.M. Intragraft vascular occlusive sickle crisis with early renal allograft loss in occult sickle cell trait. *Hum. Pathol.* **2011**, *42*, 1027–1033. [CrossRef] [PubMed]
31. Laurin, L.P.; Nachman, P.H.; Desai, P.C.; Ataga, K.I.; Derebail, V.K. Hydroxyurea is associated with lower prevalence of albuminuria in adults with sickle cell disease. *Nephrol. Dial. Transplant.* **2014**, *29*, 1211–1218. [CrossRef]
32. Alvarez, O.; Miller, S.T.; Wang, W.C.; Luo, Z.; McCarville, M.B.; Schwartz, G.J.; Thompson, B.; Howard, T.; Iyer, R.V.; Rana, S.R.; et al. Effect of hydroxyurea treatment on renal function parameters: Results from the multi-center placebo-controlled BABY HUG clinical trial for infants with sickle cell anemia. *Pediatr. Blood Cancer* **2012**, *59*, 668–674. [CrossRef]
33. Steinberg, M.H.; Barton, F.; Castro, O.; Pegelow, C.H.; Ballas, S.K.; Kutlar, A.; Orringer, E.; Bellevue, R.; Olivieri, N.; Eckman, J.; et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: Risks and benefits up to 9 years of treatment. *JAMA* **2003**, *289*, 1645–1651. [CrossRef]
34. Ferrandiz, I.; Congy-Jolivet, N.; Del Bello, A.; Debiol, B.; Trébern-Launay, K.; Esposito, L.; Milongo, D.; Dörr, G.; Rostaing, L.; Kamar, N. Impact of early blood transfusion after kidney transplantation on the incidence of donor-specific Anti-HLA antibodies. *Am. J. Transplant.* **2016**, *16*, 2661–2669. [CrossRef]
35. Jalalonmuhali, M.; Carroll, R.P.; Tsiopelas, E.; Clayton, P.; Coates, P.T. Development of de novo HLA donor specific antibodies (HLA-DSA), HLA antibodies (HLA-Ab) and allograft rejection post blood transfusion in kidney transplant recipients. *Hum. Immunol.* **2020**, *81*, 323–329. [CrossRef]
36. Vichinsky, E.; Hoppe, C.C.; Ataga, K.I.; Ware, R.E.; Nduba, V.; El-Beshlawy, A.; Hassab, H.; Achebe, M.M.; Alkindi, S.; Brown, R.C.; et al. A phase 3 randomized trial of voxelotor in sickle cell disease. *N. Engl. J. Med.* **2019**, *381*, 509–519. [CrossRef]
37. Niihara, Y.; Miller, S.T.; Kanter, J.; Lanzkron, S.; Smith, W.R.; Hsu, L.L.; Gordeuk, V.R.; Viswanathan, K.; Sarnaik, S.; Osunkwo, I.; et al. A phase 3 trial of l-glutamine in sickle cell disease. *N. Engl. J. Med.* **2018**, *379*, 226–235. [CrossRef] [PubMed]
38. Han, J.; Saraf, S.L.; Gordeuk, V.R. Systematic review of crizanlizumab: A new parenteral option to reduce vaso-occlusive pain crises in patients with sickle cell disease. *Pharmacotherapy* **2020**, *40*, 535–543. [CrossRef] [PubMed]
39. Han, J.; Saraf, S.L.; Gordeuk, V.R. Systematic review of voxelotor: A first-in-class sickle hemoglobin polymerization inhibitor for management of sickle cell disease. *Pharmacotherapy* **2020**, *40*, 525–534. [CrossRef] [PubMed]
40. Chowdhury, T.A.; Wahba, M.; Mallik, R.; Peracha, J.; Patel, D.; De, P.; Fogarty, D.; Frankel, A.; Karalliedde, J.; Mark, P.B.; et al. Association of British Clinical Diabetologists and Renal Association guidelines on the detection and management of diabetes post solid organ transplantation. *Diabet. Med.* **2021**, e14523. [CrossRef]
41. Sharif, A.; Hecking, M.; de Vries, A.P.; Porrini, E.; Hornum, M.; Rasoul-Rockenschaub, S.; Berlakovich, G.; Krebs, M.; Kautzky-Willer, A.; Schernthaner, G.; et al. Proceedings from an international consensus meeting on posttransplantation diabetes mellitus: Recommendations and future directions. *Am. J. Transplant.* **2014**, *14*, 1992–2000. [CrossRef]
42. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care* **2021**, *44* (Suppl. 1), S15–S33. [CrossRef]
43. Darbari, D.S.; Kple-Faget, P.; Kwagyan, J.; Rana, S.; Gordeuk, V.R.; Castro, O. Circumstances of death in adult sickle cell disease patients. *Am. J. Hematol.* **2006**, *81*, 858–863. [CrossRef]
44. Manci, E.A.; Culberson, D.E.; Yang, Y.M.; Gardner, T.M.; Powell, R.; Haynes, J., Jr.; Shah, A.K.; Mankad, V.N. Causes of death in sickle cell disease: An autopsy study. *Br. J. Haematol.* **2003**, *123*, 359–365. [CrossRef]
45. Ku, E.; McCulloch, C.E.; Adey, D.B.; Li, L.; Johansen, K.L. Racial disparities in eligibility for preemptive waitlisting for kidney transplantation and modification of eGFR thresholds to equalize waitlist time. *J. Am. Soc. Nephrol.* **2021**, *32*, 677–685. [CrossRef]
46. Fox, M. Barriers to kidney transplantation in racial/ethnic minorities. *Clin. J. Am. Soc. Nephrol.* **2021**, *16*, 177–178. [CrossRef]
47. Wang, J.Y.; Lederer, S.E.; Ross, L.F. African-Americans with end stage renal disease in the early years of kidney transplantation. *J. Natl. Med. Assoc.* **2019**, *111*, 352–362. [CrossRef]
48. Weinhandl, E.; Snyder, J.; Israni, A.; Kasiske, B. Effect of comorbidity adjustment on CMS criteria for kidney transplant center performance. *Am. J. Transplant.* **2009**, *9*, 506–516. [CrossRef]
49. Saraf, S.L.; Ghimire, K.; Patel, P.; Sweiss, K.; Gowhari, M.; Molokie, R.E.; Gordeuk, V.R.; Rondelli, D. Improved health care utilization and costs in transplanted versus non-transplanted adults with sickle cell disease. *PLoS ONE* **2020**, *15*, e0229710. [CrossRef]
50. Knüppel, E.; Medinger, M.; Stehle, G.; Infanti, L.; Halter, J.; Burkhalter, F.; Mujagic, E.; Heim, D.; Passweg, J. Haploidentical hematopoietic bone marrow transplantation followed by living kidney transplantation from the same donor in a sickle cell disease patient with end-stage renal failure. *Ann. Hematol.* **2017**, *96*, 703–705. [CrossRef]
51. Bunin, N.; Guzikowski, V.; Rand, E.R.; Goldfarb, S.; Baluarte, J.; Meyers, K.; Olthoff, K.M. Solid organ transplants following hematopoietic stem cell transplant in children. *Pediatr. Transplant.* **2010**, *14*, 1030–1035. [CrossRef]
52. Horwitz, M.E.; Spasojevic, I.; Morris, A.; Telen, M.; Essell, J.; Gasparetto, C.; Sullivan, K.; Long, G.; Chute, J.; Chao, N.; et al. Fludarabine-based nonmyeloablative stem cell transplantation for sickle cell disease with and without renal failure: Clinical outcome and pharmacokinetics. *Biol. Blood Marrow Transplant.* **2007**, *13*, 1422–1426. [CrossRef] [PubMed]

53. van Besien, K.; Bartholomew, A.; Stock, W.; Peace, D.; Devine, S.; Sher, D.; Sosman, J.; Chen, Y.H.; Koshy, M.; Hoffman, R. Fludarabine-based conditioning for allogeneic transplantation in adults with sickle cell disease. *Bone Marrow Transplant.* **2000**, *26*, 445–449. [CrossRef] [PubMed]
54. Abbasi, J. Promising strategies for sickle cell disease and β-Thalassemia. *JAMA* **2021**, *325*, 121. [PubMed]

Journal of
Clinical Medicine

MDPI

Article

The Tacrolimus Metabolism Rate and Dyslipidemia after Kidney Transplantation

Gerold Thölking [1,2,*], Christian Schulte [1], Ulrich Jehn [2], Katharina Schütte-Nütgen [2], Hermann Pavenstädt [2], Barbara Suwelack [2] and Stefan Reuter [2]

[1] Department of Internal Medicine and Nephrology, University Hospital of Münster Marienhospital Steinfurt, 48565 Steinfurt, Germany; ch.schulte84@googlemail.com
[2] Department of Medicine D, Division of General Internal Medicine, Nephrology and Rheumatology, University Hospital of Münster, 48149 Münster, Germany; Ulrich.Jehn@ukmuenster.de (U.J.); Katharina.schuette-nuetgen@ukmuenster.de (K.S.-N.); Hermann.Pavenstaedt@ukmuenster.de (H.P.); Barbara.Suwelack@ukmuenster.de (B.S.); Stefan.Reuter@ukmuenster.de (S.R.)
* Correspondence: gerold.thoelking@ukmuenster.de; Tel.: +49-2552-791226; Fax: +49-2552-791181

Abstract: Fast tacrolimus (Tac) metabolism is associated with reduced survival rates after renal transplantation (RTx), mainly due to cardiovascular events. Because dyslipidemia is a leading cause of cardiovascular death, we hypothesized that most RTx patients do not achieve recommended target low-density lipoprotein cholesterol (LDL-C) levels (European cardiology society guidelines) and that fast Tac metabolizers have higher dyslipidemia rates. This study included RTx recipients who received initial immunosuppression with immediate-release tacrolimus (IR-Tac), mycophenolate, and prednisolone. Patients were grouped according to their Tac concentration-to-dose ratio (C/D ratio) 3 months after RTx. Dyslipidemia parameters were analyzed at RTx, 3 months, and 12 months after RTx. Statin use and renal function were documented in a 12-month follow-up, and death was documented in a 60-month follow-up. Ninety-six RTx recipients were divided into two groups: 31 fast Tac metabolizers (C/D ratio < 1.05 ng/mL·1/mg) and 65 slow metabolizers (C/D ratio ≥ 1.05 ng/mL·1/mg). There were no differences in triglyceride or cholesterol levels between groups at RTx, 3, and 12 months after RTx. A total of 93.5% of fast and 95.4% of slow metabolizers did not achieve target LDL-C levels ($p = 0.657$). Fast metabolizers developed lower renal function compared to slow metabolizers 12 months after RTx ($p = 0.009$). Fast metabolizers showed a 60 month survival rate of 96.8% compared to 94.7% in the slow metabolizer group ($p = 0.811$). As most RTx recipients do not reach recommended target LDL-C levels, individualized nutritional counseling and lipid-lowering therapy must be intensified. Fast Tac metabolism is associated with lower renal function after RTx, but does not play a significant role in dyslipidemia.

Keywords: kidney transplantation; tacrolimus; metabolism; C/D ratio; cholesterol; dyslipidemia; LDL-C

Citation: Thölking, G.; Schulte, C.; Jehn, U.; Schütte-Nütgen, K.; Pavenstädt, H.; Suwelack, B.; Reuter, S. The Tacrolimus Metabolism Rate and Dyslipidemia after Kidney Transplantation. *J. Clin. Med.* **2021**, *10*, 3066. https://doi.org/10.3390/jcm10143066

Academic Editors: Charat Thongprayoon, Wisit Cheungpasitporn and Wisit Kaewput

Received: 1 June 2021
Accepted: 7 July 2021
Published: 11 July 2021

1. Introduction

Renal transplantation (RTx) is the preferred renal replacement procedure compared to dialysis [1]. Nevertheless, many RTx recipients are considered high-risk patients for cardiovascular (CV) events [2]. Firstly, most RTx recipients do not reach estimated glomerular filtration rates (eGFRs) compared with healthy controls, which is important because the eGFR has an inverse relationship with cardiovascular disease [3,4]. Secondly, immunosuppression with corticosteroids or calcineurin inhibitors (CNIs) is often associated with the development of dyslipidemia. Thirdly, CV risk associated with cholesterol levels tended to be higher in transplant recipients than in the Framingham Heart Study population in [5]. Recently, the European Society of Cardiology (ESC) published target low-density lipoprotein cholesterol (LDL-C) levels for different CV risk groups [2]. Individuals at moderate CV risk are recommended to achieve LDL-C levels < 100 mg/dL, high-risk patients

< 70 mg/mL, and very-high-risk patients < 55 mg/dL. Therefore, most RTx recipients should achieve a target LDL-C level of at least < 70 mg/mL. Recently, the CKD-REIN study collaborators showed that patients with chronic kidney disease (CKD) in stages G3a–5 who are eligible for lipid-lowering therapy are frequently untreated, and those who receive therapy rarely achieve LDL-C targets [6].

Several studies showed an association between fast tacrolimus (Tac) metabolism and increased mortality [7,8]. As described for CKD patients [3], CV events were the main reason of death in these cohorts. Therefore, we initially hypothesized that most RTx recipients do not achieve recommended LDL-C levels as suggested by the ESC [9]. It was also shown that fast Tac metabolism is associated with an increased decline of renal function compared with slow metabolizers [7,10,11], but there is currently no data on Tac metabolism and dyslipidemia. Accordingly, we hypothesized that fast Tac metabolism is related to higher triglyceride and cholesterol levels, which may promote CV disease.

2. Patients and Methods

2.1. Patients and Study Design

This retrospective, observational study was performed considering patients who had undergone RTx at the University Hospital of Münster from 2007 to 2015. Figure 1 illustrates the enrollment of the subjects in the study. The study included 96 patients who met the inclusion criteria: age ≥ 18 years, intake of immediate-release Tac (IR-Tac) since RTx and available lipid status at RTx, 3, and 12 months after. The initial immunosuppressive regimen consisted of basiliximab, tacrolimus (target trough 6–12 ng/mL for 3 months, thereafter 4–8 ng/mL), mycophenolate mofetil, and prednisolone that was reduced to a maintenance dosage of 5 mg once daily (q.d.) at 3–6 months. Patient data were collected from the hospital's electronic health records. Blood analyses were performed using a Roche modular platform (Cobas, Roche Diagnostics, Mannheim, Germany), and renal function (based on enzymatic creatinine measures) was determined by calculating the eGFR using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

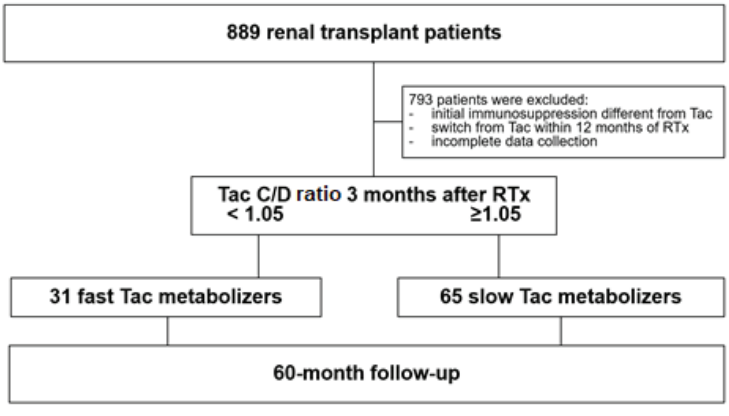


Figure 1. Study design and patient enrollment. Initially, a total of 889 renal transplant recipients were screened, but 793 patients were excluded because they did not meet the inclusion criteria. RTx recipients were defined as fast and slow Tac metabolizers 3 months after transplantation, and survival was observed in a 60-month follow-up. Abbreviations: Tac, tacrolimus; RTx, renal transplantation. C/D ratio values in ng/mL·1/mg.

The Tac concentration-to-dose ratio (C/D ratio, fast metabolizers C/D ratio < 1.05 ng/mL·1/mg, slow metabolizers C/D ratio ≥ 1.05 ng/mL·1/mg) was calculated to determine the Tac metabolism rate 3 months after RTx, as previously published by us and others [7,8]. The distribution of the C/D ratio in our cohort is shown in Figure 2.

The local ethics committee (No. 2014-381-f-N) approved the study. The methods in this study were performed in accordance with the current transplantation guidelines and the Declarations of Istanbul and Helsinki. All participants gave written informed consent for the collection of their clinical data at the time of transplantation. As recommended by the KDIGO (Kidney Disease: Improving Global Outcomes) guideline [12], the lipid profile was determined at months 3 and 12 after transplantation at our center. Depending on the result, we advised the patient (lifestyle management) and recommended statin therapy, which usually consisted of fluvastatin or pravastatin. Nevertheless, the suggested therapeutic approach was usually a recommendation that the patient previously discussed with their treating nephrologist. If the therapeutic goal was not achieved after 12 months, we usually increased the statin dose, supplemented ezetimibe, or recommended presentation to a lipid specialist.

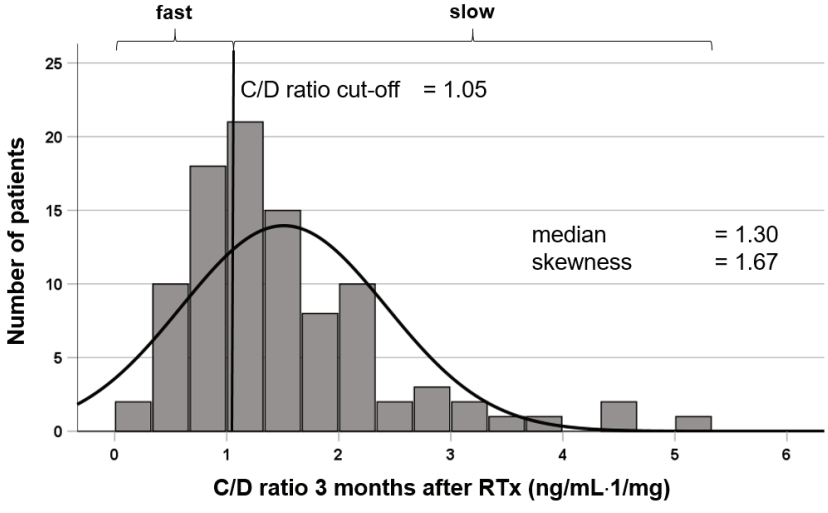


Figure 2. Histogram of the distribution of the tacrolimus C/D ratio (ng/mL·1/mg). The study cohort showed an asymmetric distribution relating to their C/D ratios and were categorized in two groups according to previous studies [7,8]. Fast metabolizers had a C/D ratio <1.05 and slow metabolizers ≥1.05 ng/mL·1/mg. RTx, renal transplantation.

Rates of calcineurin-inhibitor-induced nephrotoxicity (CNIT), BK viral nephropathy (BKVN), and acute rejection (AR) were assessed from indication biopsies. New-onset diabetes after transplantation (NODAT) data were obtained from the medical records, and the need for antidiabetic therapy or diabetes diet was assessed.

2.2. Statistical Analysis

SPSS® Statistics 27 for Windows (IBM Corporation, Somers, NY, USA) was used for statistical analyses. Normally distributed data are presented as mean ± standard deviation and non-normally distributed data are shown as median (minimum–maximum). The *t*-test was used for normally distributed data of unrelated groups. Non-normally distributed data were compared with the Mann–Whitney U test, and categorical variables with Fisher's exact test. Two-sided tests were applied in all statistical evaluations and a *p*-value of ≤0.05 was considered significant for all tests performed.

3. Results

The study cohort included 31 fast Tac metabolizers and 65 slow metabolizers. The fast metabolizer group included noticeably more patients with diabetes mellitus at RTx (Table 1).

Table 1. Patient characteristics and immunosuppression.

	Fast Metabolizers (n = 31)	Slow Metabolizers (n = 65)	p-Value
Age (years)	51.9 ± 12.6	51.0 ± 14.8	0.770 [a]
Sex (m/f)	22 (71%)/9 (29%)	45 (69%)/20 (31%)	1 [b]
Weight (kg)	79.7 ± 19.2	74.1 ± 14.7	0.158 [a]
Height (cm)	176 ± 11	172 ± 9	0.108 [a]
BMI (kg/m^2)	25.4 ± 4.4	24.7 ± 4.2	0.503 [a]
Living donor transplantation	10 (32%)	16 (25%)	0.467 [b]
ESP	4 (13%)	9 (14%)	1 [b]
Cold ischemic time (h)	8.1 ± 5.8	8.6 ± 4.7	0.698 [a]
Warm ischemic time (min)	31.6 ± 5.9	32.6 ± 7.5	0.483 [a]
DGF	2 (7%)	9 (14%)	0.494 [b]
Time on dialysis	47.5 ± 42.0	59.5 ± 44.6	0.206 [a]
Previous transplantation	5	5	0.284 [b]
One previous transplantation	3	5	0.168 [b]
Two previous transplantations	2	0	
Combined liver transplantation	1	2	1 [b]
Comorbidities			
Diabetes mellitus	5 (16%)	2 (3%)	0.034 [b]
Arterial hypertension	25 (81%)	59 (91%)	0.193 [b]
BMI ≥ 25 kg/m^2	18 (58%)	36 (55%)	0.830 [b]
Donor Characteristics			
Donor age	51.0 ± 15.3	53.4 ± 14.5	0.475 [a]
Donor sex (m/f)	10 (32%)/21 (68%)	30 (46%)/35 (54%)	0.269 [b]

BMI, body mass index; ESP, European Senior Program; DGF, delayed graft function. p-Values: [a] Welch's t-test; [b] Fisher's exact test.

At the 3 month mark after RTx, fast Tac metabolizers showed noticeably lower Tac trough levels (p = 0.004), had received higher Tac doses (p > 0.001) but lower prednisolone doses (p = 0.015), and had lower C/D ratios (p < 0.001) (Table 2). One year after RTx, Tac trough levels and prednisolone doses were comparable between groups, but Tac daily doses were higher in fast metabolizers, resulting in lower C/D ratios (0.96 vs. 1.59 ng/mL·1/mg, p < 0.001). Rates of CNIT, BKVN, AR, and NODAT always tended to show worse outcomes in fast metabolizers compared to slow metabolizers.

Table 2. Immunosuppression, statins, complications, cholesterol levels, and triglycerides.

	Fast Metabolizers (n = 31)	Slow Metabolizers (n = 65)	p-Values
Tac trough level M3 (ng/mL)	6.8 ± 2.4	8.4 ± 2.8	0.004 [a]
Tac daily dose M3 (mg)	9.0 (3–20)	5 (2–12)	<0.001 [b]
Tac C/D ratio M3 (ng/mL/mg)	0.79 (0.28–1.00)	1.57 (1.05–5.15)	<0.001 [b]
Tac daily dose M12 (mg)	7 (2–18)	3.75 (1–11)	<0.001 [b]
Tac trough level M12 (ng/mL)	6.8 ± 2.1	6.3 ± 2.1	0.401 [a]
Tac C/D ratio M12 (ng/mL/mg)	0.96 (0.28–2.85)	1.59 (0.24–7.60)	<0.001 [b]
Prednisolone M3 (mg)	7.5 (0–50)	10 (5–30)	0.015 [b]
Prednisolone M12 (mg)	5 (0–20)	5 (3–20)	0.594 [b]

Table 2. *Cont.*

	Fast Metabolizers (n = 31)	Slow Metabolizers (n = 65)	p-Values
Statin at discharge	8 (26%)	5 (8%)	0.024 [c]
Statin M3	10 (32%)	18 (28%)	0.640 [c]
Statin M12	18 (58%)	39 (60%)	1 [c]
Complications			
CNIT until M12	4 (12.9%)	4 (6.2%)	0.268 [c]
BKVN until M12	3 (9.7%)	1 (1.5%)	0.097 [c]
AR until M12	6 (19.4%)	6 (9.2%)	0.193 [c]
NODAT until M3	4 (12.9%)	7 (10.8%)	0.743 [c]
NODAT between M3 and M12	5 (16.1%)	10 (15.4%)	1 [c]
TC			
At RTx	195 (85–455)	200 (119–395)	0.422 [b]
3 months	215 (119–284)	224 (125–353)	0.285 [b]
12 months	209 (109–353)	202 (121–378)	0.443 [b]
LDL-C			
At RTx	102 (11–372)	111 (39–274)	0.565 [b]
3 months	117 (57–194)	122 (56–229)	0.347 [b]
12 months	116 (43–269)	114 (47–266)	0.919 [b]
HDL-C			
At RTx	45 (25–87)	48 (22–119)	0.464 [b]
3 months	49 (31–91)	52 (28–91)	0.426 [b]
12 months	46 (31–108)	51 (24–104)	0.148 [b]
TG			
At RTx	158 (57–469)	159 (67–885)	0.763 [b]
3 months	221 (90–545)	186 (46–1326)	0.283 [b]
12 months	206 (68–774)	160 (77–663)	0.138 [b]

Tac, tacrolimus; M, month; C/D ratio, concentration/dose ratio; CNIT, calcineurin inhibitor-induced nephrotoxicity; BKVN, BK virus nephropathy; AR, acute rejection; NODAT, new-onset diabetes after transplantation; RTx, renal transplantation; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. p-Values: [a] Welch's t-test; [b] Mann–Whitney U test; [c] Fisher's exact test.

Statin use was more frequent in fast metabolizers (p = 0.024) but—similarly to TC, LDL-C, HDL-C, and triglyceride levels—did not differ between groups at 3 and 12 months after RTx (Table 2). In a subgroup analysis, the lipid profiles between female and male patients and patients <50 and ≥50 years of age in fast and slow metabolizer groups were analyzed. There were no differences between these subgroups (data not shown).

According to current ESC guidelines, most RTx recipients in our cohort were defined as "high-risk" or "very-high-risk" patients [2], with no differences between metabolizer groups (p = 0.259, Table 3). Only 6.5% of fast metabolizers and 4.6% of slow metabolizers achieved their individual guideline-compliant LDL-C target value. While 1 out of 4 fast Tac metabolizers in the "moderate-risk" group reached their LDL-C target at all three time points, only 18.5% of slow metabolizers did so. In "high-risk" patients, the current LDL-C target values were rarely reached by patients (6.5% vs. 1.5%) and the "very-high-risk" target values were not reached by any of the patients (Table 3; overall p = 0.342).

Table 3. Cardiovascular risk and achieved LDL-C levels.

	Fast Metabolizers (n = 31)	Slow Metabolizers (n = 65)	p-Values
CV risk level according to the ESC guidelines			
Moderate-risk	4 (12.9%)	7 (10.8%)	0.259
High-risk	17 (54.8%)	34 (52.3%)	
Very-high-risk	10 (32.3%)	14 (21.5%)	
LDL-C target level achieved *			
Individual level achieved	2 (6.5%)	3 (4.6%)	0.657
Moderate-risk achieved	8 (25.8%)	12 (18.5%)	0.342
High-risk level achieved	2 (6.5%)	1 (1.5%)	
Very-high-risk level achieved	0	0	

CV, cardiovascular risk; LDL-C, low-density lipoprotein cholesterol. p-Values: Fisher's exact test. * According to current European Society of Cardiology guidelines 2019.

During the follow-up (M12-60), one patient in the fast metabolizer group (3%) and three slow metabolizers (5%) died, mainly from CV events (3 of 4). Three months after RTx, fast metabolizers developed a greater decrease of eGFR by trend (51.1 ± 19.4 vs. 43.7 ± 16.5 mL/min/1.73 m^2; Figure 3; p = 0.057) and a noticeably lower eGFR at 12 months after RTx (53.6 ± 20.9 vs. 44.3 ± 12.9 mL/min/1.73 m^2; p = 0.009) compared to slow metabolizers.

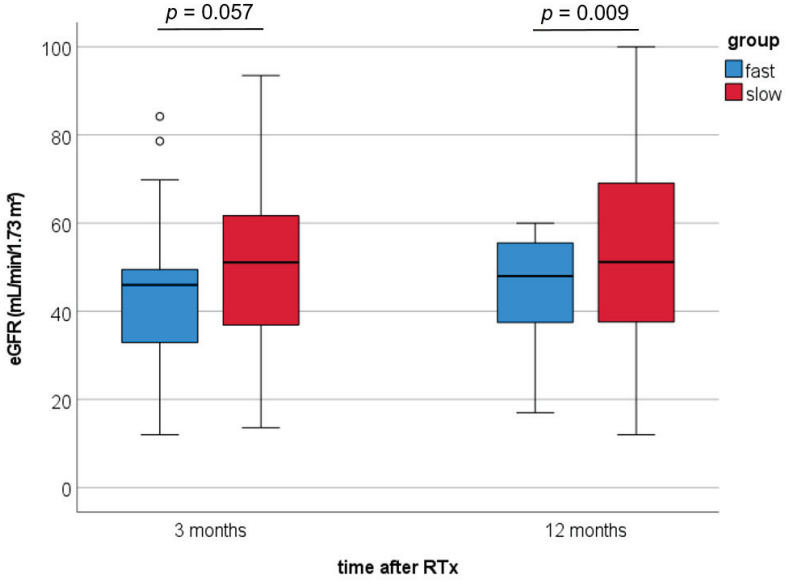


Figure 3. Renal function of fast and slow tacrolimus metabolizers three and twelve months after transplantation. eGFR, estimated glomerular filtration rate; RTx, renal transplantation.

4. Discussion

Herein, we investigated whether Tac metabolism rate is associated with lipid status because we and others had previously observed that mortality was higher in fast metabolizers than in slow metabolizers [7,8]. In addition, it was observed that a lower C/D ratio (<1.8 ng/mL·1/mg) resulted in an increased rate of de novo dyslipidemia one year after liver transplantation [13]. Because CV events are the major cause of death in fast

metabolizers and dyslipidemia is clearly associated with mortality in a severity-dependent manner, we conducted the present study [2,14].

The Tac metabolism rate is associated with renal function after transplantation [15]. One year after RTx, fast metabolizers showed lower eGFR values than slow metabolizers. This is in line with recent studies and potentially related to increased rejection rates, BK virus nephropathy, and CNI nephrotoxicity [7,10,16–18]. Rates of CNIT, BKVN, and AR always displayed worse outcomes in fast metabolizers as was previously shown in larger cohorts, but the differences did not reach significance in our study cohort, most likely because of small patient numbers [10,17,19]. Renal function after RTx is strongly associated with patient and graft survival [20]. For example, patients with lower eGFR show higher blood pressure values and poorer blood pressure control despite the increased number of antihypertensive medications [21]. However, in this study cohort, the diagnosis of arterial hypertension did not differ between groups. This could be related to the fact that a very large number of patients (>80%) in both groups required blood pressure treatment, which is not unusual because hypertension is common in RTx patients [22].

Interestingly, the rate of diabetes before transplantation was higher in the fast metabolizer group, while the rate of NODAT at three and twelve months after RTx only tended to be higher in the fast metabolizer group (Table 2). This could be important because diabetes influences dyslipidemia [2]. However, diabetic metabolism and dyslipidemia are not always revealed because LDL-C levels may be within the normal range. More typical results are elevated TGs or low HDL-C. Similar findings for dyslipidemia are described in relation to renal function, which was lower in fast metabolizers in our cohort [2]. Interestingly, three and twelve months after RTx, higher TG levels were found more often in fast than in slow metabolizers, whereas the other lipid parameters were relatively similar between groups. Consistent with data from other cohorts of CKD patients who did not achieve target LDL-C levels [6], most RTx recipients in our study also did not achieve their individual goals set by current or previous ESC guidelines [2,23].

CNIs and steroids are known to impact lipid metabolism [24]. However, cyclosporine A appears to be less effective than Tac, as it has been shown that switching from cyclosporine A to Tac can improve dyslipidemia [25–27]. The reduction in serum LDL-C after switching to Tac may be (partly) caused by removing the inhibitory effect of cyclosporin A on LDL-C receptor production. Interestingly, LDL-C reduction was found only in patients who were not treated with statins [25]. Furthermore, lowering Tac trough levels from 9.5 to 6.4 ng/mL (a Tac level range comparable to that of our patients) did not significantly lower TC, LDL-C, or TG levels in renal transplant recipients, in contrast to steroid withdrawal, which resulted in a slight improvement in lipids [28]. Others found no correlation between Tac trough level, exposure, or Tac dosage and the lipid parameters [29,30]. Unfortunately, we cannot comment on the influence of Tac AUC values in this regard, but we and others have previously shown that Tac AUC is comparable in fast and slow metabolizers [16,31]. The choice of the 3 month time point for the calculation of the C/D ratio was a compromise. We already know from previous analyses that the calculation of the C/D ratio at very early time points (postoperative day 1–10) is not able to predict the metabolism type at all [32]. However, we found acceptable correlations between the 3 month time point and the calculation at 1 month or 6 months [7]. Jouve et al. analyzed the C/D ratio at 3, 6, and 12 months using the same cut-off (1.05 ng/mL·1/mg) and observed no statistically or clinically significant intrapatient evolution of the C/D ratio over time [8]. Rostaing et al. observed that from the third month after transplantation, the C/D ratio was relatively constant over time [33]. The choice of the C/D ratio cut off level can be very relevant [34]. In our first study on the C/D ratio, we assessed the outcomes of three different C/D ratio groups (<1.05, ≥1.05 and <1.54, and ≥1.55 ng/mL·1/mg). Since kidney transplant recipients with a C/D ratio between 1.05 and 1.54 ng/mL·1/mg showed comparable results to patients with a CD ratio ≥1.55 ng/mL·1/mg, we chose to combine both groups in later analyses. However, others chose different C/D ratio cut-off values, which may lead to different definitions and outcomes [18,35].

Consistent with the literature on Tac and lipid metabolism, in the present study we excluded relevant effects of Tac metabolic type on lipid metabolism within 12 months after RTx.

The strengths of the current study included the complete data set of each patient and the use of real-world data that better reflected the reality of treatment. Limitations of this study included (i) the retrospective nature of the study, (ii) the small number of patients, (iii) the lack of adherence data, (iv) the low achievement of therapeutic lipid targets based on current guidelines, and (v) the lack of clear differentiation between effects of renal function, Tac, or co-medication on lipids. However, a prospective multicenter study focusing on the C/D ratio would be desirable to prospectively validate the hypotheses obtained from retrospective studies.

In conclusion, although dyslipidemia after RTx is common (at least at our center), treatment according to current guidelines is suboptimal and individualized nutritional counseling and lipid-lowering therapy must be intensified. The Tac metabolism type does not seem to be a crucial parameter regarding dyslipidemia after RTx. This study is intended to raise awareness of lipid management under real-life conditions and to call for (re-)evaluation of the procedure for individual centers.

Author Contributions: Conceptualization, G.T. and S.R.; data curation, C.S.; formal analysis, C.S., U.J., K.S.-N., and G.T.; methodology, G.T. and S.R.; resources, H.P. and B.S.; supervision, G.T.; writing—original draft, G.T. and S.R.; writing—review and editing, K.S.-N., H.P., and B.S. All authors have read and agreed to the published version of the manuscript.

Funding: The APC was funded by the Open Access Fund of the University of Münster.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee (Ethik Kommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität, No. 2014-390-f-N).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of this study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Wolfe, R.A.; Ashby, V.B.; Milford, E.L.; Ojo, A.O.; Ettenger, R.E.; Agodoa, L.Y.; Held, P.J.; Port, F.K. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N. Engl. J. Med.* **1999**, *341*, 1725–1730. [CrossRef] [PubMed]
2. Task Force Members; ESC Committee for Practice Guidelines (CPG); ESC National Cardiac Societies. 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Atherosclerosis* **2019**, *290*, 140–205. [CrossRef] [PubMed]
3. Go, A.S.; Chertow, G.M.; Fan, D.; McCulloch, C.E.; Hsu, C.Y. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med.* **2004**, *351*, 1296–1305. [CrossRef] [PubMed]
4. Chronic Kidney Disease Prognosis Consortium; Matsushita, K.; van der Velde, M.; Astor, B.C.; Woodward, M.; Levey, A.S.; de Jong, P.E.; Coresh, J.; Gansevoort, R.T. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: A collaborative meta-analysis. *Lancet* **2010**, *375*, 2073–2081. [CrossRef]
5. Kasiske, B.L.; Chakkera, H.A.; Roel, J. Explained and unexplained ischemic heart disease risk after renal transplantation. *J. Am. Soc. Nephrol.* **2000**, *11*, 1735–1743. [CrossRef]
6. Massy, Z.A.; Ferrieres, J.; Bruckert, E.; Lange, C.; Liabeuf, S.; Velkovski-Rouyer, M.; Stengel, B.; Collaborators, C.-R. Achievement of Low-Density Lipoprotein Cholesterol Targets in CKD. *Kidney Int. Rep.* **2019**, *4*, 1546–1554. [CrossRef]
7. Schutte-Nutgen, K.; Tholking, G.; Steinke, J.; Pavenstadt, H.; Schmidt, R.; Suwelack, B.; Reuter, S. Fast Tac Metabolizers at Risk—It is Time for a C/D Ratio Calculation. *J. Clin. Med.* **2019**, *8*, 587. [CrossRef]
8. Jouve, T.; Fonrose, X.; Noble, J.; Janbon, B.; Fiard, G.; Malvezzi, P.; Stanke-Labesque, F.; Rostaing, L. The TOMATO study (TacrOlimus MetabolizAtion in kidney TransplantatiOn): Impact of the concentration-dose ratio on death-censored graft survival. *Transplantation* **2019**. [CrossRef]

9. Mach, F.; Baigent, C.; Catapano, A.L.; Koskinas, K.C.; Casula, M.; Badimon, L.; Chapman, M.J.; De Backer, G.G.; Delgado, V.; Ference, B.A.; et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur. Heart J.* **2020**, *41*, 111–188. [CrossRef]
10. Tholking, G.; Fortmann, C.; Koch, R.; Gerth, H.U.; Pabst, D.; Pavenstadt, H.; Kabar, I.; Husing, A.; Wolters, H.; Reuter, S.; et al. The tacrolimus metabolism rate influences renal function after kidney transplantation. *PLoS ONE* **2014**, *9*, e111128. [CrossRef]
11. Thongprayoon, C.; Hansrivijit, P.; Kovvuru, K.; Kanduri, S.R.; Bathini, T.; Pivovarova, A.; Smith, J.R.; Cheungpasitporn, W. Impacts of High Intra- and Inter-Individual Variability in Tacrolimus Pharmacokinetics and Fast Tacrolimus Metabolism on Outcomes of Solid Organ Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 2193. [CrossRef]
12. Kasiske, B.; Zeier, M.; Craig, J.; Ekberg, H.; Garvey, C.; Green, M.; Abariga, S. Kidney Disease: Improving Global Outcomes Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am. J. Transplant.* **2009**, *9* (Suppl. 3), S1–S155. [CrossRef]
13. Bardou, F.N.; Guillaud, O.; Erard-Poinsot, D.; Chambon-Augoyard, C.; Thimonier, E.; Vallin, M.; Boillot, O.; Dumortier, J. Tacrolimus exposure after liver transplantation for alcohol-related liver disease: Impact on complications. *Transpl. Immunol.* **2019**, *56*, 101227. [CrossRef] [PubMed]
14. Matas, A.J.; Gillingham, K.J.; Humar, A.; Kandaswamy, R.; Sutherland, D.E.; Payne, W.D.; Dunn, T.B.; Najarian, J.S. 2202 kidney transplant recipients with 10 years of graft function: What happens next? *Am. J. Transplant.* **2008**, *8*, 2410–2419. [CrossRef]
15. Schutte-Nutgen, K.; Tholking, G.; Suwelack, B.; Reuter, S. Tacrolimus—Pharmacokinetic Considerations for Clinicians. *Curr. Drug Metab.* **2018**, *19*, 342–350. [CrossRef] [PubMed]
16. Tholking, G.; Schutte-Nutgen, K.; Schmitz, J.; Rovas, A.; Dahmen, M.; Bautz, J.; Jehn, U.; Pavenstadt, H.; Heitplatz, B.; Van Marck, V.; et al. A Low Tacrolimus Concentration/Dose Ratio Increases the Risk for the Development of Acute Calcineurin Inhibitor-Induced Nephrotoxicity. *J. Clin. Med.* **2019**, *8*, 1586. [CrossRef] [PubMed]
17. Tholking, G.; Schmidt, C.; Koch, R.; Schuette-Nuetgen, K.; Pabst, D.; Wolters, H.; Kabar, I.; Husing, A.; Pavenstadt, H.; Reuter, S.; et al. Influence of tacrolimus metabolism rate on BKV infection after kidney transplantation. *Sci. Rep.* **2016**, *6*, 32273. [CrossRef]
18. Nowicka, M.; Gorska, M.; Nowicka, Z.; Edyko, K.; Edyko, P.; Wislicki, S.; Zawiasa-Bryszewska, A.; Strzelczyk, J.; Matych, J.; Kurnatowska, I. Tacrolimus: Influence of the Posttransplant Concentration/Dose Ratio on Kidney Graft Function in a Two-Year Follow-Up. *Kidney Blood Press Res.* **2019**, *44*, 1075–1088. [CrossRef]
19. Egeland, E.J.; Robertsen, I.; Hermann, M.; Midtvedt, K.; Storset, E.; Gustavsen, M.T.; Reisaeter, A.V.; Klaasen, R.; Bergan, S.; Holdaas, H.; et al. High Tacrolimus Clearance Is a Risk Factor for Acute Rejection in the Early Phase After Renal Transplantation. *Transplantation* **2017**, *101*, e273–e279. [CrossRef]
20. Schnitzler, M.A.; Johnston, K.; Axelrod, D.; Gheorghian, A.; Lentine, K.L. Associations of renal function at 1-year after kidney transplantation with subsequent return to dialysis, mortality, and healthcare costs. *Transplantation* **2011**, *91*, 1347–1356. [CrossRef]
21. Jehn, U.; Schutte-Nutgen, K.; Strauss, M.; Kunert, J.; Pavenstadt, H.; Tholking, G.; Suwelack, B.; Reuter, S. Antihypertensive Treatment in Kidney Transplant Recipients-A Current Single Center Experience. *J. Clin. Med.* **2020**, *9*, 3969. [CrossRef]
22. Weir, M.R.; Burgess, E.D.; Cooper, J.E.; Fenves, A.Z.; Goldsmith, D.; McKay, D.; Mehrotra, A.; Mitsnefes, M.M.; Sica, D.A.; Taler, S.J. Assessment and management of hypertension in transplant patients. *J. Am. Soc. Nephrol.* **2015**, *26*, 1248–1260. [CrossRef] [PubMed]
23. Reiner, Z.; Catapano, A.L.; De Backer, G.; Graham, I.; Taskinen, M.R.; Wiklund, O.; Agewall, S.; Alegria, E.; Chapman, M.J.; Durrington, P.; et al. ESC/EAS Guidelines for the management of dyslipidaemias: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur. Heart J.* **2011**, *32*, 1769–1818. [CrossRef]
24. Marchetti, P.; Navalesi, R. The metabolic effects of cyclosporin and tacrolimus. *J. Endocrinol. Investig.* **2000**, *23*, 482–490. [CrossRef]
25. Artz, M.A.; Boots, J.M.; Ligtenberg, G.; Roodnat, J.I.; Christiaans, M.H.; Vos, P.F.; Blom, H.J.; Sweep, F.C.; Demacker, P.N.; Hilbrands, L.B. Improved cardiovascular risk profile and renal function in renal transplant patients after randomized conversion from cyclosporine to tacrolimus. *J. Am. Soc. Nephrol.* **2003**, *14*, 1880–1888. [CrossRef] [PubMed]
26. McCune, T.R.; Thacker, L.R., II; Peters, T.G.; Mulloy, L.; Rohr, M.S.; Adams, P.A.; Yium, J.; Light, J.A.; Pruett, T.; Gaber, A.O.; et al. Effects of tacrolimus on hyperlipidemia after successful renal transplantation: A Southeastern Organ Procurement Foundation multicenter clinical study. *Transplantation* **1998**, *65*, 87–92. [CrossRef]
27. Claes, K.; Meier-Kriesche, H.U.; Schold, J.D.; Vanrenterghem, Y.; Halloran, P.F.; Ekberg, H. Effect of different immunosuppressive regimens on the evolution of distinct metabolic parameters: Evidence from the Symphony study. *Nephrol. Dial. Transplant.* **2012**, *27*, 850–857. [CrossRef]
28. Boots, J.M.; van Duijnhoven, E.M.; Christiaans, M.H.; Wolffenbuttel, B.H.; van Hooff, J.P. Glucose metabolism in renal transplant recipients on tacrolimus: The effect of steroid withdrawal and tacrolimus trough level reduction. *J. Am. Soc. Nephrol.* **2002**, *13*, 221–227. [CrossRef] [PubMed]
29. Ciftci, H.S.; Ayna, T.K.; Caliskan, Y.K.; Turkmen, A.; Gurtekin, M. Lipid parameters, doses and blood levels of calcineurin inhibitors in renal transplant patients. *Indian J. Clin. Biochem.* **2013**, *28*, 164–168. [CrossRef]
30. Kuypers, D.R.; Claes, K.; Evenepoel, P.; Maes, B.; Vanrenterghem, Y. Clinical efficacy and toxicity profile of tacrolimus and mycophenolic acid in relation to combined long-term pharmacokinetics in de novo renal allograft recipients. *Clin. Pharmacol. Ther.* **2004**, *75*, 434–447. [CrossRef]

31. Kuypers, D.R.; Naesens, M.; de Jonge, H.; Lerut, E.; Verbeke, K.; Vanrenterghem, Y. Tacrolimus dose requirements and CYP3A5 genotype and the development of calcineurin inhibitor-associated nephrotoxicity in renal allograft recipients. *Ther. Drug Monit.* **2010**, *32*, 394–404. [CrossRef]
32. Bartmann, I.; Schutte-Nutgen, K.; Suwelack, B.; Reuter, S. Early postoperative calculation of the tacrolimus concentration-to-dose ratio does not predict outcomes after kidney transplantation. *Transpl. Int.* **2020**, *33*, 689–691. [CrossRef] [PubMed]
33. Rostaing, L.; Bunnapradist, S.; Grinyo, J.M.; Ciechanowski, K.; Denny, J.E.; Silva, H.T., Jr.; Budde, K.; Envarsus Study, G. Novel Once-Daily Extended-Release Tacrolimus Versus Twice-Daily Tacrolimus in De Novo Kidney Transplant Recipients: Two-Year Results of Phase 3, Double-Blind, Randomized Trial. *Am. J. Kidney Dis.* **2016**, *67*, 648–659. [CrossRef] [PubMed]
34. Tholking, G.; Reuter, S. Alternative Viewpoint on Tacrolimus Concentration-to-Dose Ratios in Kidney Transplant Recipients and Relationship to Clinical Outcomes. *Pharmacotherapy* **2019**, *39*, 1036–1037. [CrossRef]
35. Bartlett, F.E.; Carthon, C.E.; Hagopian, J.C.; Horwedel, T.A.; January, S.E.; Malone, A. Tacrolimus Concentration-to-Dose Ratios in Kidney Transplant Recipients and Relationship to Clinical Outcomes. *Pharmacotherapy* **2019**, *39*, 827–836. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Association of Hematuria with Renal Progression and Survival in Patients Who Underwent Living Donor Liver Transplant

Kai-Chieh Chang [1,†], Yao-Peng Hsieh [1,2,†], Huan-Nung Chao [1,3], Chien-Ming Lin [1], Kuo-Hua Lin [4], Chun-Chieh Tsai [1], Chia-En Heish [4], Pei-Ru Lin [5], Chew-Teng Kor [5], Yao-Li Chen [4,6,*,‡] and Ping-Fang Chiu [1,7,8,*,‡]

1 Division of Nephrology, Department of Internal Medicine, Changhua Christian Hospital, Changhua 50006, Taiwan; 200648@cch.org.tw (K.-C.C.); 102407@CCH.ORG.TW (Y.-P.H.); 1310008@cch.org.tw (H.-N.C.); 719696@cch.org.tw (C.-M.L.); 144942@cch.org.tw (C.-C.T.)
2 School of Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan
3 Division of Nephrology, Hanming Christian Hospital, Changhua 50058, Taiwan
4 Department of General Surgery, Changhua Christian Hospital, Changhua 50006, Taiwan; 120380@cch.org.tw (K.-H.L.); 69918@cch.org.tw (C.-E.H.)
5 Big Data Center, Changhua Christian Hospital, Changhua 50006, Taiwan; 183778@cch.org.tw (P.-R.L.); 179297@cch.org.tw (C.-T.K.)
6 Transplant Medicine & Surgery Research Centre, Changhua Christian Hospital, Changhua 50006, Taiwan
7 School of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan
8 Department of Hospitality Management, Ming Dao University, Changhua 52345, Taiwan
* Correspondence: 31560@cch.org.tw (Y.-L.C.); 68505@cch.org.tw (P.-F.C.)
† These first authors contributed equally to the work.
‡ These corresponding authors contributed equally to the work.

Citation: Chang, K.-C.; Hsieh, Y.-P.; Chao, H.-N.; Lin, C.-M.; Lin, K.-H.; Tsai, C.-C.; Heish, C.-E.; Lin, P.-R.; Kor, C.-T.; Chen, Y.-L.; et al. Association of Hematuria with Renal Progression and Survival in Patients Who Underwent Living Donor Liver Transplant. *J. Clin. Med.* **2021**, *10*, 4345. https://doi.org/10.3390/jcm10194345

Academic Editor: Charat Thongprayoon

Received: 10 September 2021
Accepted: 19 September 2021
Published: 24 September 2021

Abstract: Background: This study aimed to determine the association between episodic or persistent hematuria after liver transplantation and long-term renal outcomes. Methods: Patients who underwent living donor liver transplantation between July 2005 and June 2019 were recruited and divided into two groups based on the finding of microscopic or gross hematuria after transplantation. All patients were followed up from the index date until the end date in May 2020. The risks of chronic kidney disease, death, and 30% and 50% declines in estimated glomerular filtration rate (eGFR) were compared between groups. Results: A total of 295 patients underwent urinalysis for various reasons after undergoing transplantation. Hematuria was detected in 100 patients (group A) but was not present in 195 patients (group B). Compared with group B, group A had a higher risk of renal progression, including eGFR decline >50% [aHR = 3.447 (95%CI: 2.24~5.30), $p < 0.001$] and worse survival. In addition, patients who took non-steroidal anti-inflammatory drugs (NSAIDs) continuously for over seven days within six months before transplant surgery had high risks of rapid renal progression, including a >30% decline in eGFR [aHR = 1.572 (95%CI: 1.12~2.21), $p = 0.009$)]. Conclusion: Development of hematuria after surgery in patients who underwent living donor liver transplant and were exposed to NSAIDs before surgery were associated with worse long-term renal dysfunction and survival.

Keywords: liver transplantation; hematuria; chronic kidney disease; survival

1. Introduction

Hematuria is a sign of various conditions of kidney disease and can be of glomerular or non-glomerular origin. The common causes of glomerular hematuria include systemic lupus erythematosus, vasculitis, IgA nephropathy, and thin basement membrane nephropathy [1]. Non-glomerular hematuria can originate from the urinary system, which comprises the renal pelvis, ureter, and bladder. In men, the prostate gland communicates with the urinary system. Cancer, infection, and urolithiasis are common causes of non-glomerular hematuria. Several methods help distinguish between glomerular and non-glomerular

hematuria; these include the presence of dysmorphic red blood cells or nephrotic proteinuria. However, regardless of its origin, hematuria has been reported to influence patient outcomes and renal function in the general population. Hematuria is associated with the incidence of acute kidney injury (AKI) [2] and chronic kidney disease (CKD) [3]. Moreover, a long-term follow-up study revealed that persistent asymptomatic hematuria correlated with increased risk of renal progression [4]. In patients who underwent kidney transplantation, the frequent incidence of urothelial malignancy may be attributed to an immunocompromised status and the underlying etiology of end-stage renal disease (ESRD). On the other hand, in adult patients who have undergone liver transplantation, no study has focused on the effects of hematuria on renal outcomes. Accordingly, we conducted the present study to investigate the effects of hematuria on urinalysis and renal outcomes.

2. Materials and Methods

Patients who underwent living donor liver transplant at Changhua Christian Hospital between July 2005 and June 2019 were enrolled. These patients had either liver failure or hepatocellular carcinoma that fulfilled the Milan criteria for liver transplantation. The index date was defined as the date of achieving stable renal function after surgery, around three months after transplantation. Patients who died during the hospitalization for transplantation and those who developed AKI immediately or within a short period after surgery and recovered with an estimated glomerular filtration rate (eGFR) of <90% were also excluded. This study was approved by the institutional review board of Changhua Christian Hospital, Taiwan (IRB 210131).

The enrolled patients were divided into two groups: those who had persistent or episodic hematuria on their urinalyses after the index date (group A) and those who did not have hematuria (group B). The diagnostic criteria for hematuria were defined as five or more red blood cells/HPF (high-power field, X 400) in the urinary sediment sample. The following variables were compared between the groups: age; comorbidity; previous exposure to medications; and laboratory data, including complete blood count, albumin level, baseline liver and renal function test results, and electrolytes. All patients were followed up from the index date until the end of May 2020. In addition, the risks of CKD (eGFR < 60 mL/min/1.73 m^2) occurrence, death, and eGFR decline by 30% or 50% were compared between groups. The endpoints, including 30% and 50% decline of eGFR, were defined as surrogate outcomes, because few patients progressed to ESRD. Both surrogate endpoints that were applied simultaneously were practical for the enrolled patients who presented with varying renal function trajectories, especially the acute on CKD pattern [5]. In addition, the risk factors for the renal outcomes were evaluated. Sensitivity analysis was conducted for subgroups of patients who had no CKD (eGFR $\geq$ 60 mL/min/1.73 m^2) and those in whom the identified causes of hematuria were urinary tract infection (UTI) and urolithiasis.

Data are presented as mean $\pm$ standard deviation for continuous variables and as numbers (percentages) for categorical variables. Student's *t*-test and χ^2 test were used to compare the continuous and categorical variables, respectively, between the two groups. The survival curves of one of the outcomes (i.e., decline in eGFR over time) in groups A and B were estimated using the Simon and Makuch method, which is an alternative to the Kaplan–Meier estimation, because the cases in group A included a time-varying exposure. Mantel and Bayer's tests was used to compare the survival curves between the groups. Considering the immortal time bias for group A and the competing risk of death, we used time-dependent Cox models to determine the association between infections and renal function decline. The results were reported as hazard ratios (HRs) with 95% confidence intervals (CIs). Confounders, which were the variables with $p < 0.05$, were selected on the basis of crude HRs that were adjusted for in the multivariate Cox analysis to estimate the adjusted HRs (aHRs). All statistical analyses were conducted using the statistical package SPSS (v20; IBM Corporation, Chicago, IL, USA). Values with two-sided $p < 0.05$ were considered statistically significant.

3. Results

In total, 295 patients met the eligibility criteria and were categorized into two groups according to the development of urinalysis-confirmed hematuria secondary to various causes after the index date: group A (n = 100) and group B (n = 195). Table 1 presents the following demographic characteristics of the patients in both groups: sex; age; comorbidities; baseline liver and renal function test results; and prescribed medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) and angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (ACE-I/ARBs). The mean eGFR on the index date was 75.5 ± 32.8 mL/min/1.73 m^2 in group A and 75.2 ± 25.8 mL/min/1.73 m^2 in group B.

Table 1. Demographic data.

	Group A (Hematuria+)	Group B (Hematuria-)	*p*-Value
Patients (*n*)	100	195	
Age	55.1 ± 7.6	55.6 ± 7.9	0.555
Gender, Male	70 (70%)	154 (79%)	0.088
Comorbidity			
CCI	4.1 ± 4.2	4 ± 3.6	0.774
Diabetes mellitus	17 (17%)	31 (15.9%)	0.808
Hypertension	8 (8%)	24 (12.3%)	0.260
Hyperlipidemia	3 (3%)	10 (5.1%)	0.399
Hepatitis B	37 (37%)	71 (36.4%)	0.921
Hepatitis C	30 (30%)	44 (22.6%)	0.163
Cirrhosis	51 (51%)	98 (50.3%)	0.904
CHF	9 (9%)	14 (7.2%)	0.581
CAD	1 (1%)	3 (1.5%)	0.705
Medication before surgery			
NSAID	23 (23%)	39 (20%)	0.549
ACE-I/ARB	7 (7%)	16 (8.2%)	0.715
Laboratory data at surgery			
Hemoglobin (g/dL)	9.4 ± 1.6	9.8 ± 1.7	0.053
Albumin (g/dL)	2.86 ± 0.72	2.94 ± 0.69	0.326
AST (U/L)	179.0 ± 99.5	173.3 ± 106.1	0.653
ALT (U/L)	112.3 ± 59	115.3 ± 73.1	0.723
PT (second)	18.8 ± 4.9	17.9 ± 4.1	0.135
INR	1.7 ± 0.4	1.6 ± 0.4	0.231
APTT (second)	39.9 ± 11.2	37.5 ± 8.5	0.063
Platelet (10^3)	84.6 ± 34.3	85.8 ± 42.7	0.798
Creatinine (mg/dL)	1.24 ± 0.79	1.22 ± 0.82	0.898
Laboratory data at index date			
BUN (mg/dL)	21.0 ± 16.9	17.1 ± 12	0.045
Creatinine (mg/dL)	1.10 ± 0.44	1.07 ± 0.36	0.569
eGFR (mL/min/1.73^2)	75.5 ± 32.8	75.2 ± 25.8	0.930
Events after index date during follow-up			
Liver rejection	9(9%)	11(5.6%)	0.277
Average tacrolimus level (ng/mL)	5.3 ± 2.2	5.2 ± 1.5	0.785
[1] NSAID exposure	27 (27%)	48 (24.6%)	0.656
Outcome after index date			
Mortality	27 (27%)	28 (14.4%)	0.008
eGFR decline > 30%	74 (74%)	108 (55.4%)	0.002
eGFR decline > 50%	50 (50%)	49 (25.1%)	<0.001
CKD (eGFR < 60)	65 (65%)	110 (56.4%)	0.155
Renal composite outcomes	88 (88%)	148 (75.9%)	0.014
Follow-up time			
Time to CKD (years)	1.4 ± 1.7	1.8 ± 1.8	0.046
Time to renal composite outcomes (years)	0.7 ± 1.2	1.3 ± 1.7	<0.001

[1] NSAIDs were prescribed continuously for over seven days before surgery. SI: bloodstream infection; CCI: Charlson comorbidity index; CKD: chronic kidney disease; CHF: congestive heart failure; CAD: coronary artery disease. NSAIDs: nonsteroidal anti-inflammatory drugs; ACE-I/ARBs: angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PT: prothrombin time; aPTT: activated partial thromboplastin time; CRP: C-reactive protein.

No significant differences between the groups were observed in terms of sex, Charlson comorbidity index score, diabetes, cirrhosis, coronary artery disease, congestive heart failure, and hepatitis B (Table 1). In addition, the number of patients who had continuous exposure to NSAIDs for over seven days within six months before surgery was comparable between the groups. The two groups exhibited similar eGFR, hemoglobin, platelet, albumin, electrolyte levels, and coagulation test results.

On five-year follow-up, the risk of a 50% decline in eGFR was 50 (50%) in group A and 49 (25.1%) in group B ($p < 0.001$) and that of a 30% decline in eGFR was 74 (74%) in group A and 108 (55.4%) in group B ($p = 0.002$). In addition, the interval of occurrence of renal outcomes was shorter in group A than in group B (0.7 ± 1.2 years vs. 1.3 ± 1.7 years). The development of hematuria was not associated with the incidence of liver allograft rejection during follow-up (9 (9%) vs. 11 (5.6%), $p = 0.277$; Table 1). At the end of observation, mortality was higher in group A than in group B (27 (27%) vs. 28 (14.4%), $p = 0.008$). (Table 1, Figure 1).

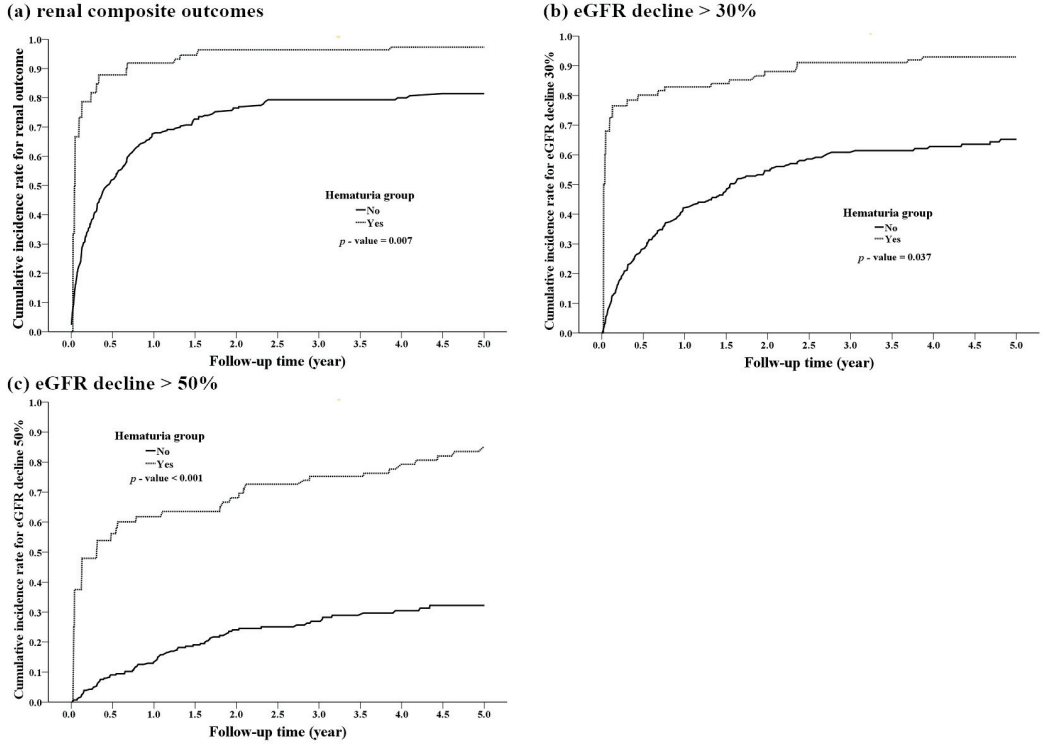


Figure 1. Patients with hematuria had rapid renal disease progression. (**a**) renal composite outcomes, (**b**) eGFR decline >30%, (**c**) eGFR decline >50%.

When using a Cox proportional hazard regression to determine the association between transplant procedures with or without infections and renal outcomes, after adjustment for confounders (Tables 2–4, and Figure 1), group A, compared with group B, had higher a risk of renal dysfunction (aHR: 1.939, 95% CI: 1.23–3.06, $p = 0.005$), >30% or >50% decline in eGFR (aHR: 3.447, 95% CI: 2.24–5.30, $p < 0.001$), CKD, and composite outcomes (aHR: 1.926; 95% CI: 1.11–3.51, $p = 0.027$). Moreover, women and patients with relatively low albumin levels at baseline had a relatively high risk of renal composite outcomes (Table 2). Patients who took NSAIDs continuously for over seven days within six months

before surgery had a relatively high risk of rapid renal dysfunction progression, including a >30% decline in eGFR and renal composite outcomes. Similarly, patients who took ACE-I/ARBs continuously for over 28 days within six months before surgery had a relatively high risk of composite outcomes. However, the number of patients with NSAID exposure after liver transplant was comparable between both groups (27 (27%) vs. 48 (24.6%)). NSAID intake after surgery had less influence on the final renal function at the end of the observation period than NSAID intake before surgery (>30% or >50% decline in eGFR, $p = 0.33$ and 0.76, respectively, not shown).

Table 2. Effects of hematuria on renal composite outcomes during a five-year follow-up.

	cHR (95% CI)	*p*-Value	aHR (95% CI)	*p*-Value
Hematuria	2.239 (1.30, 3.87)	0.004	1.926 (1.08, 3.44)	0.027
Age	1.021 (1.00, 1.04)	0.013	1.017 (1.00, 1.04)	0.062
Sex, Male	0.612 (0.46, 0.82)	0.001	0.681 (0.5, 0.93)	0.014
Diabetes mellitus	1.440 (1.03, 2.02)	0.035	1.193 (0.84, 1.69)	0.321
BUN	1.012 (1.00, 1.02)	0.002	1.01 (1.00, 1.02)	0.017
Albumin	0.783 (0.65, 0.95)	0.011	0.768 (0.62, 0.94)	0.012
NSAID (7 days)	1.505 (1.12, 2.03)	0.007	1.556 (1.15, 2.12)	0.005
ACE-I/ARB	1.77 (1.14, 2.75)	0.011	1.751 (1.11, 2.76)	0.016

Renal composite outcomes (30% or 50% decline in eGFR, CKD, or death). NSAIDs were prescribed continuously for over seven days before surgery; ACE-I/ARBs were prescribed continuously for over 28 days before surgery. CKD: chronic kidney disease; NSAIDs: nonsteroidal anti-inflammatory drugs; ACE-I/ARBs: angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; BUN: blood urea nitrogen.

Table 3. Effects of hematuria on a 30% decline in eGFR during a five-year follow-up.

	cHR (95% CI)	*p*-Value	aHR (95% CI)	*p*-Value
Hematuria	2.063 (1.31, 3.24)	0.002	1.939 (1.23, 3.06)	0.005
Sex, Male	0.652 (0.48, 0.89)	0.008	0.714 (0.52, 0.98)	0.038
CCI	1.042 (1.01, 1.08)	0.019	1.030 (0.99, 1.07)	0.115
Albumin	0.776 (0.63, 0.96)	0.020	0.788 (0.63, 0.99)	0.037
NSAID	1.706 (1.24, 2.35)	0.001	1.572 (1.12, 2.21)	0.009

Table 4. Effects of hematuria on a 50% decline in eGFR during a five-year follow-up.

	cHR (95% CI)	*p*-Value	aHR (95% CI)	*p*-Value
Hematuria	3.839 (2.55, 5.77)	<0.001	3.447 (2.24, 5.30)	<0.001
Platelet	1.005 (1.00, 1.01)	0.007	1.005 (1.00, 1.01)	0.010
CRP	1.052 (1.01, 1.10)	0.027	1.043 (0.997, 1.09)	0.066
NSAID	1.584 (1.07, 2.35)	0.022	1.389 (0.92, 2.10)	0.119

Further analysis of the causes of hematuria revealed that 26 patients had renal stones and that 49 patients had episodes of UTI. Moreover, 17 patients developed both UTI and urolithiasis. Two patients had prostate hypertrophy. Long-term antiplatelet medications were prescribed for 17 patients with coronary artery or cerebrovascular disease, and anticoagulants were used to treat atrial fibrillation in three patients. A total of 19 patients with UTI or urolithiasis received antiplatelet medication; however, no patient died from proven urinary tract malignancy. After completion of the study, we could not determine the etiology of hematuria in 31 patients (31%).

Table 5 shows the results of the sensitivity analysis of different subgroups according to infection, after excluding patients with eGFR of <60 mL/min/1.73 m^2 (aHR: 2.913, 95% CI: 1.38–6.13; $p = 0.005$). In addition, we observed significant results after controlling for UTI or urolithiasis. Hematuria was independently associated with negative outcomes (aHR: 2.596, 95% CI: 1.04–6.49, $p = 0.041$; aHR: 2.86, 95% CI: 1.50–5.45, $p < 0.001$).

Table 5. Sensitivity analysis.

	Renal Composite Outcomes		eGFR Decline 30%		eGFR Decline 50%	
	aHR (95% CI)	***p*-Value**	**aHR (95% CI)**	***p*-Value**	**aHR (95% CI)**	***p*-Value**
Excluding eGFR < 60 (N = 205)						
Hematuria	2.913 (1.38, 6.13)	0.005	3.199 (1.58, 6.46)	0.001	2.773 (1.59, 4.83)	<0.001
Excluding UTI (N = 246)						
Hematuria	2.596 (1.04, 6.49)	0.041	2.330 (1.20, 4.54)	0.013	4.137 (2.26, 7.59)	<0.001
Excluding stone (N = 269)						
Hematuria	2.861 (1.50, 5.45)	0.001	2.930 (1.66, 5.16)	<0.001	4.633 (2.77, 7.74)	<0.001

Further investigation focused on the influence of proteinuria in urinalysis on renal outcomes. Of 295 patients, 164 had proteinuria on urinalysis. Patients with incidental proteinuria with or without hematuria had more rapid progression of renal composite outcomes compared with that in patients who did not have proteinuria (aHR = 1.688, 95% CI: 1.04–2.75; $p = 0.036$).

4. Discussion

In addition to long-term administration of calcineurin inhibitors, diabetes mellitus, hypertension, and kidney dysfunction before liver transplant were the reported factors associated with CKD incidence following liver transplant [6]. In addition, the risk of CKD was reported to be significantly associated with advanced age, female sex, and hepatitis C carrier status before transplantation [7]. Lee et al. found that the overall risk of CKD (eGFR < 60 mL/min per 1.73 m^2) correlated with low pretransplant eGFR values, pretransplant hepatorenal syndrome, pretransplant proteinuria levels, and higher Child–Pugh and MELD scores [8].

In a 22-year follow-up study, the presence of isolated microscopic hematuria was found to be associated with a significantly increased risk of ESRD4 in the general population. In addition, hematuria was associated with a significantly high risk of death in the first two years of follow-up [9]. Hematuria had been considered an indicator of activity of glomerular nephropathy in vasculitis, lupus nephritis, or IgA nephropathy [10–12]. In addition, persistent hematuria was reported to be associated with an increased risk of ESRD in IgA nephropathy [13]. The degree of proteinuria definitely correlates with the risk of diabetic kidney; however, microscopic hematuria is also a dependent risk factor for ESRD in diabetic nephropathy [14]. These suggest that the presence of nondiabetic renal disease in patients with diabetic CKD carries a relatively high risk of renal progression [15]. Furthermore, glomerular hematuria can lead to AKI, with the pathogenesis primarily involving bouts of gross hematuria, including intratubular obstruction of blood casts with consequent acute tubular necrosis, and direct toxic tubular effects of hemoglobin. In addition, heme may trigger oxidative stress and erythrophagocytosis through renal tubular cells1.

Non-glomerular hematuria includes UTI, urolithiasis, and prostatic disorders. Among liver transplant recipients, UTI in the first year was identified as an independent risk factor for CKD stage progression [16]. Several studies have consistently observed a relationship between a history of nephrolithiasis and a two-fold increase in the risk of both CKD and ESRD [17]. After stone formation in patients with CKD, the specific complications include obstructive uropathy, recurrent UTI, and struvite stones. Urolithiasis is managed through modalities, such as extracorporeal shock wave lithotripsy and ureterolithotripsy, which can further cause kidney injury. In part, this finding indicates that kidney stone formation is common in patients with hypertension, gout, and diabetes mellitus. In renal transplant patients, the incidence of renal allograft stones has been associated with reduced graft survival [18]. Frequent urinalysis in such patients enables early and easy recognition of obstructive uropathy. On the other hand, in patients who have undergone liver transplant, the diagnosis of obstructive uropathy is probably delayed.

Patients on antithrombotic agents may have increased incidence of gross hematuria [19]. Anticoagulants and their corresponding medical conditions are commonly referred to as anticoagulant-related nephropathy, which was found to be correlated with the progression of kidney dysfunction [20]. Moreover, the incidence of microscopic hematuria was found to be elevated in elderly patients who received regular doses of aspirin [21], a low dose aspirin may play a role in renal progression in patients with CKD [22]. However, contrasting results were observed in patients who were older [23] or had diabetes [24]. A further cohort study is ongoing to clarify this issue [25].

Rare etiologies of hematuria have been reported in patients who have received organ transplants. In a liver transplant recipient, hemorrhagic cystitis was reported to have developed secondary to BK virus infection [26]. In our study, although hematuria has several causes, its appearance was consistently shown to predict renal outcomes in patients who received living donor liver transplants. Routine urinalysis and early surveillance of the underlying disease in such patients were the essential tasks. In this study, the long-term renal outcomes were affected by hypoalbuminemia and continuous exposure to NSAIDs within seven days before surgery or to ACE-I/ARBs within 28 days before surgery, in addition to the hematuria-related disorders. Development of adverse renal outcomes with NSAID use after liver transplant is well known. However, exposure to NSAIDs or ACE-I/ARBs before the associated poor renal outcomes was an interesting finding. Perhaps patients with liver failure are always in a state of low effective intravascular volume. ACE-I/ARBs and NSAIDs may cause further deterioration of the renal injury. Male sex appeared to be a protective factor in patients who received a living donor liver transplant; this finding may be attributed to the higher mean eGFR on the index date in men than in women (77.3 ± 26.6 vs. 68.9 ± 32.5; $p = 0.029$). One review showed that asymptomatic hematuria in adults had unknown etiology in 43%–68% [27]; for these 31 patients (31%) without determinate etiology, regular and close follow-up every three to six months may be considered.

The current study had several limitations. First, this was a single-center study that enrolled a limited number of patients. Second, we were unable to further distinguish between gross and microscopic hematuria because of patient subjectivity. Quantitative analysis of proteinuria (proteinuria-creatinine ratio or 24 h urine sample) was not monitored routinely. Third, we surveyed surrogate outcomes instead of definite outcomes (i.e., death, ESRD, and renal transplant), because only few patients achieved these solid endpoints. Fourth, the data of deceased patients who had received liver transplant were not considered in this study because of the various conditions that may have been acquired from the donors.

5. Conclusions

The appearance of hematuria consistently predicted the renal outcomes and survival of patients who underwent living donor liver transplant. Routine urinalysis and early surveillance of the underlying disease is essential for such patients.

Author Contributions: K.-C.C., Y.-P.H. and P.-F.C. contributed to the design of the study and wrote the manuscript. C.-T.K. and P.-R.L. contributed to the analysis of the statistics. Y.-L.C. and K.-H.L., H.-N.C. participated in the implementation of the research. C.-M.L., C.-C.T. and C.-E.H. provided ideas and directed consultations. All authors provided critical feedback on the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by grants 109-CCH-IRP-020 and 110-CCH-IRP-027 from the Changhua Christian Hospital Research Foundation.

Institutional Review Board Statement: This study was approved by the institutional review board of Changhua Christian Hospital, Taiwan (IRB 210131).

Informed Consent Statement: Patient consent was waived due to the type of study being a retrospective analysis, which did not require us to carry out any extraordinary interventions different from usual practice and no patient interviews were required. Personal data were codified in order to guarantee confidentiality.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Moreno, J.A.; Sevillano, Á.; Gutiérrez, E.; Guerrero-Hue, M.; Vázquez-Carballo, C.; Yuste, C.; Herencia, C.; García-Caballero, C.; Praga, M.; Egido, J. Glomerular Hematuria: Cause or Consequence of Renal Inflammation? *Int. J. Mol. Sci.* **2019**, *20*, 2205. [CrossRef] [PubMed]
2. Moreno, J.A.; Martín-Cleary, C.; Gutierrez, J.A.M.; Toldos, O.; Blanco-Colio, L.M.; Praga, M.; Ortiz, A.; Egido, J. AKI Associated with Macroscopic Glomerular Hematuria: Clinical and Pathophysiologic Consequences. *Clin. J. Am. Soc. Nephrol.* **2012**, *7*, 175–184. [CrossRef] [PubMed]
3. Kim, H.; Lee, M.; Cha, M.-U.; Nam, K.H.; An, S.Y.; Park, S.; Jhee, J.H.; Yun, H.-R.; Kee, Y.K.; Park, J.T.; et al. Microscopic hematuria is a risk factor of incident chronic kidney disease in the Korean general population: A community-based prospective cohort study. *Qjm Int. J. Med.* **2018**, *111*, 389–397. [CrossRef] [PubMed]
4. Vivante, A.; Afek, A.; Frenkel-Nir, Y.; Tzur, D.; Farfel, A.; Golan, E.; Chaiter, Y.; Shohat, T.; Skorecki, K.; Calderon-Margalit, R. Persistent Asymptomatic Isolated Microscopic Hematuria in Israeli Adolescents and Young Adults and Risk for End-Stage Renal Disease. *JAMA* **2011**, *306*, 729–736. [CrossRef]
5. Rosansky, S.J.; Glassock, R.J. Is a decline in estimated GFR an appropriate surrogate end point for renoprotection drug trials? *Kidney Int.* **2014**, *85*, 723–727. [CrossRef]
6. Bahirwani, R.; Reddy, K.R. Outcomes after liver transplantation: Chronic kidney disease. *Liver Transplant.* **2009**, *15*, S70–S74. [CrossRef]
7. Ojo, A.O.; Held, P.J.; Port, F.K.; Wolfe, R.A.; Leichtman, A.B.; Young, E.W.; Arndorfer, J.; Christensen, L.; Merion, R.M. Chronic Renal Failure after Transplantation of a Nonrenal Organ. *N. Engl. J. Med.* **2003**, *349*, 931–940. [CrossRef]
8. Lee, J.P.; Heo, N.J.; Joo, K.W.; Yi, N.J.; Suh, K.S.; Moon, K.C.; Kim, S.G.; Kim, Y.S. Risk factors for consequent kidney impairment and differential impact of liver transplantation on renal function. *Nephrol. Dial. Transplant.* **2010**, *25*, 2772–2785. [CrossRef]
9. Orlandi, P.F.; Fujii, N.; Roy, J.; Chen, H.Y.; Hamm, L.L.; Sondheimer, J.H.; He, J.; Fischer, M.J.; Rincon-Choles, H.; Krishnan, G.; et al. Hematuria as a risk factor for progression of chronic kidney disease and death: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. *BMC Nephrol.* **2018**, *19*, 150. [CrossRef]
10. Ding, J.Y.; Ibañez, D.; Gladman, D.D.; Urowitz, M.B. Isolated hematuria and sterile pyuria may indicate systemic lupus erythematosus activity. *J. Rheumatol.* **2015**, *42*, 437–440. [CrossRef]
11. Rhee, R.L.; Davis, J.C.; Ding, L.; Fervenza, F.C.; Hoffman, G.S.; Kallenberg, C.G.; Langford, C.A.; McCune, W.J.; Monach, P.A.; Seo, P.; et al. The Utility of Urinalysis in Determining the Risk of Renal Relapse in ANCA-Associated Vasculitis. *Clin. J. Am. Soc. Nephrol.* **2018**, *13*, 251–257. [CrossRef]
12. Berthoux, F.; Mohey, H.; Laurent, B.; Mariat, C.; Afiani, A.; Thibaudin, L. Predicting the Risk for Dialysis or Death in IgA Nephropathy. *J. Am. Soc. Nephrol.* **2011**, *22*, 752–761. [CrossRef] [PubMed]
13. Coppo, R.; Fervenza, F.C. Persistent Microscopic Hematuria as a Risk Factor for Progression of IgA Nephropathy: New Floodlight on a Nearly Forgotten Biomarker. *J. Am. Soc. Nephrol.* **2017**, *28*, 2831–2834. [CrossRef]
14. Okada, S.; Samejima, K.-I.; Matsui, M.; Morimoto, K.; Furuyama, R.; Tanabe, K.; Eriguchi, M.; Akai, Y.; Saito, Y.; Tsuruya, K. Microscopic hematuria is a risk factor for end-stage kidney disease in patients with biopsy-proven diabetic nephropathy. *BMJ Open Diabetes Res. Care* **2020**, *8*, e001863. [CrossRef]
15. Lin, H.Y.-H.; Niu, S.-W.; Kuo, I.-C.; Lim, L.-M.; Hwang, D.-Y.; Lee, J.-J.; Hwang, S.-J.; Chen, H.-C.; Hung, C.-C. Hematuria and Renal Outcomes in Patients with Diabetic Chronic KidneyDisease. *Am. J. Med Sci.* **2018**, *356*, 268–276. [CrossRef]
16. Sánchez, M.P.R.; Rubio, D.C.A.; Luna, I.M.; Padilla, P.K.G.; Villamizar, K.M.C.; González, C.A.G.; Trejos, J.A.P. Impact of Complicated Urinary Tract Infection on Renal Graft Function. *Transpl. Proc.* **2020**, *52*, 1173–1177. [CrossRef]
17. Keddis, M.T.; Rule, A.D. Nephrolithiasis and loss of kidney function. *Curr. Opin. Nephrol. Hypertens.* **2013**, *22*, 390–396. [CrossRef]
18. Rezaee-Zavareh, M.S.; Ajudani, R.; Binabaj, M.R.; Heydari, F.; Einollahi, B. Kidney Allograft Stone after Kidney Transplantation and its Association with Graft Survival. *Int. J. Organ Transplant. Med.* **2015**, *6*, 114–118.
19. Bhatt, N.R.; Davis, N.F.; Nolan, W.J.; Flynn, R.J.; McDermott, T.; Thomas, A.Z.; Manecksha, R.P. Incidence of Visible Hematuria among Antithrombotic Agents: A Systematic Review of Over 175,000 Patients. *Urology* **2018**, *114*, 27–32. [CrossRef] [PubMed]
20. Brodsky, S.; Eikelboom, J.; Hebert, L.A. Anticoagulant-Related Nephropathy. *J. Am. Soc. Nephrol.* **2018**, *29*, 2787–2793. [CrossRef] [PubMed]
21. Moudi, E.; Hosseini, S.R.; Bijani, A. Higher rate of microscopic hematuria in elderly patients who take regular doses of aspirin: Result from AHAP Study. *Casp. J. Intern. Med.* **2016**, *7*, 278–282.

22. Kim, A.J.; Lim, H.J.; Ro, H.; Ko, K.P.; Han, S.Y.; Chang, J.H.; Lee, H.H.; Chung, W.; Jung, J.Y. Low-Dose Aspirin for Prevention of Cardiovascular Disease in Patients with Chronic Kidney Disease. *PLoS ONE* **2014**, *9*, e104179.
23. Akinwusi, P.; Oluyombo, R.; Ogunro, P.S.; Adeniji, A.; Okunola, O.O.; Ayodele, O.E. Low dose aspirin therapy and renal function in elderly patients. *Int. J. Gen. Med.* **2013**, *6*, 19–24. [CrossRef] [PubMed]
24. Gaede, P.; Hansen, H.P.; Parving, H.H.; Pedersen, O. Impact of low-dose acetylsalicylic acid on kidney function in type 2 diabetic patients with elevated urinary albumin excretion rate. *Nephrol. Dial. Transplant.* **2003**, *18*, 539–542. [CrossRef]
25. Violi, F.; Targher, G.; Vestri, A.; Carnevale, R.; Averna, M.; Farcomeni, A.; Lenzi, A.; Angelico, F.; Cipollone, F.; Pastori, D. Effect of aspirin on renal disease progression in patients with type 2 diabetes: A multi-center, double-blind, placebo-controlled, randomized trial. The renaL disEase progression by aspirin in diabetic pAtients (LEDA) trial. Rationale and study design. *Am. Heart J.* **2017**, *189*, 120–127. [CrossRef] [PubMed]
26. Kamal, F.; Ali, B.; Barnes, M.; Kamal, S.; Nair, S.; Gonzalez, H. Hemorrhagic Cystitis in a Liver Transplant Recipient Secondary to BK Virus. *ACG Case Rep. J.* **2017**, *4*, e67. [CrossRef] [PubMed]
27. Sharp, V.J.; Barnes, K.T.; Erickson, B. Assessment of asymptomatic microscopic hematuria in adults. *Am. Fam. Physician* **2013**, *88*, 747–754. [PubMed]

Journal of
Clinical Medicine

MDPI

Review

Tocilizumab and Desensitization in Kidney Transplant Candidates: Personal Experience and Literature Review

Jules Weinhard [1], Johan Noble [1], Thomas Jouve [1,2], Paolo Malvezzi [1] and Lionel Rostaing [1,2,*]

[1] Service de Néphrologie, Hémodialyse, Aphérèses, et Transplantation Rénale, CHU Grenoble-Alpes, 38700 Grenoble, France; jweinhard@chu-grenoble.fr (J.W.); jnoble@chu-grenoble.fr (J.N.); tjouve@chu-grenoble.fr (T.J.); pmalvezzi@chu-grenoble.fr (P.M.)
[2] Faculté de Médecine, Université Grenoble-Alpes, 38700 Grenoble, France
* Correspondence: lrostaing@chu-grenoble.fr; Tel.: +33-476-768-945

Abstract: Desensitization (DES) allows kidney transplantation for highly HLA-sensitized subjects. Due to the central role of IL-6 in the immunological response, tocilizumab may improve DES efficacy. Thus, we conducted a PubMed systematic review using the MeSH terms tocilizumab, interleukin-6, kidney transplantation, and desensitization. Tocilizumab (TCZ) was first studied for DES as the second-line treatment after failure of a standard DES protocol (SP) (apheresis, rituximab +/− IVIg). Although TCZ (as a monotherapy) attenuated anti-HLA antibody rates, it did not permit transplantation. However, lymphocyte immuno-phenotyping has shown that TCZ hinders B-cell maturation and thus could improve the long-term efficacy of DES by limiting anti-HLA rebound and so avoid antibody-mediated rejection. This hypothesis is supported by a recent study where clazakizumab, a monoclonal antibody directed against IL-6, was continued after kidney transplantation in association with an SP. Nine out of ten patients were then eligible for transplantation, and there were no donor-specific antibodies at 6 months post-transplantation. In association with an SP, tocilizumab does not seem to significantly improve kidney-allograft access (short-term efficacy) vs. a SP only. However, it could improve the long-term prognosis of HLA-incompatible transplantation by hindering B-cell maturation and, thereby, avoiding donor-specific antibody rebounds post-transplantation.

Keywords: tocilizumab; clazakizumab; desensitization; kidney transplantation; anti-HLA alloantibody

Citation: Weinhard, J.; Noble, J.; Jouve, T.; Malvezzi, P.; Rostaing, L. Tocilizumab and Desensitization in Kidney Transplant Candidates: Personal Experience and Literature Review. *J. Clin. Med.* **2021**, *10*, 4359. https://doi.org/10.3390/jcm10194359

Academic Editors: Wisit Cheungpasitporn, Charat Thongprayoon and Wisit Kaewput

Received: 30 August 2021
Accepted: 20 September 2021
Published: 24 September 2021

1. Introduction

Kidney transplantation is recognized as the best replacement therapy for end-stage renal disease in terms of survival and quality of life, but also from a medical and economic standpoint [1–3]. However, a shortage of kidney grafts increases time on a waiting list, and human leukocyte antigen (HLA)-sensitized kidney transplant candidates (KTCs) are the most affected. As these subjects have developed anti-HLA antibodies during previous allogeneic exposures due to solid-organ transplantation, pregnancy, or blood transfusion, the number of potentially compatible grafts is limited. To improve access to transplantation, a desensitization (DES) protocol can be undertaken to eliminate anti-HLA antibodies and to block their production.

Initially developed in the context of HLA- and ABO-incompatible living donations, DES is now considered to increase the chances of obtaining a graft from a deceased donor. The current standard protocol (SP) for DES has been empirically developed and validated by studies [4–6] that describe combining apheresis (plasma exchange, double-filtration plasmapheresis, semispecific immunoadsorption), rituximab (anti-CD20 monoclonal antibody), conventional triple immunosuppression (anti-calcineurin, mycophenolic acid, and steroids), and possibly intravenous polyvalent immunoglobulin (IVIg).

The results of these DES protocols are primarily supported in the living donor transplant setting, and much less so in the deceased donor setting. Montgomery et al. have

shown that HLA-incompatible recipients who underwent a DES protocol and received a graft from a living donor had better survival than highly sensitized patients who remained on dialysis or eventually obtained a compatible graft from a deceased donor [7]. The results from this single-center study were supported by a larger multicenter study confirming a survival benefit at 8 years [8]. Both studies used a US-based population. In contrast, in a UK-based study, Manook et al. showed no survival benefit in highly sensitized patients who received an HLA-incompatible living donor transplant compared with patients remaining on dialysis while waiting for a compatible graft from a deceased donor [9]. This difference between the US and UK studies may be explained by the decreased survival rates of dialysis subjects in the US compared to those in the UK. Nevertheless, the results of Manook et al. can be seen from two different angles: the absence of improved survival by the practice of HLA-incompatible transplantation and the absence of excess mortality in DES protocols, which allow improved access to transplantation for highly HLA-sensitized patients and therefore improved quality of life.

In some patients, these DES protocols do not succeed in decreasing the level of anti-HLA antibodies sufficiently to allow the transplantation of grafts carrying the corresponding HLA specificities. One of the hypotheses explaining this is the absence (with the current protocols) of action on plasma cells, particularly on memory plasma cells. With rituximab, this may seem obvious since it is an anti-CD20 monoclonal antibody, and this CD20 marker is no longer expressed by plasma cells. Perry et al. have shown in vitro that rituximab as well as IVIg and anti-thymocyte globulin (ATG; used as a transplant induction therapy) were not able to induce plasma cell apoptosis or block antibody production [10]. Vo et al. showed that in their cohort of 600 highly sensitized patients who received an IVIg + rituximab desensitization protocol, DES failure was more frequent in patients with a high level of memory plasma cells with anti-HLA specificities [11]. Therefore, the idea arose to use treatments that could better target plasma cells.

First, bortezomib was studied, as an analogy to the treatment of myeloma (precisely due to the monoclonal proliferation of plasma cells). In vivo, in highly sensitized primates, bortezomib decreased the level of antibody-producing cells and CD38 + CD19 + CD20 plasma cells in the bone marrow. However, it did not decrease the level of donor-specific antibodies (DSAs) [12]. In vivo, bortezomib has been shown to decrease DSA levels in highly sensitized patients, but without achieving cross-match negativity through microlymphocytotoxicity, and therefore without improving access to transplantation [13,14]. Due to a lack of evidence of major efficacy and a poor safety profile, bortezomib has not found its place in the field of DES.

In the challenge of finding a molecule that could better target all facets of the immune response responsible for the production of anti-HLA antibodies, the study of the IL-6 blockade is relevant because this cytokine plays a central role in the regulation of the humoral and cellular immune responses. Therefore, we carried out a literature review of studies that specify the place of the anti-IL-6R monoclonal antibody tocilizumab (TCZ) in DES protocols. We first present the studies that justify a pathophysiological rationale for the use of TCZ, and then detail the recent clinical studies that assess TCZ's efficacy and safety as a DES treatment.

2. Materials and Methods

We performed a systematic review of the literature using PubMed with the following MeSH terms: tocilizumab, interleukin-6, kidney transplantation, desensitization.

3. Results and Discussion

3.1. Pathophysiological Rationale: IL-6 and the Immune Response

Interleukin-6 (IL-6) is a pleiotropic cytokine whose sites of action are as numerous as the possible therapeutic effects of tocilizumab: impact on hematopoiesis (anemia caused by inflammatory syndrome), osteoclast differentiation (osteoporosis), keratocyte proliferation (psoriasis and skin disorders of the scleroderma), and regulation of the hypothalamo–

hypophyseal axis [15]. IL-6's role in the immune response is also decisive. It is interesting to note that it was the ability of IL-6 to induce antibody production by B cells that led to its discovery in 1985 [16]. It was then called B-cell stimulating factor 2 (BSF-2) or B cell differentiation factor (BCDF). IL-6's central role in both the innate and adaptive immune response is now well documented.

3.1.1. IL-6 and the Innate Immune Response

In the early phases of the innate immune system response, IL-6 is released by monocytes, macrophages, lymphocytes, endothelial cells, and dendritic cells after recognition of the danger signals sent out by the toll-like receptors(TLRs). These signals can be induced by an aggressive infection (pathogen-associated molecular patterns) or not (danger-associated molecular patterns). IL-6 also stimulates the hepatocyte production of inflammation proteins such as C-reactive protein, serum amyloid A protein, haptoglobin, and fibrinogen.

3.1.2. IL-6 and the Humoral Adaptive Immune Response: B-Cell Response

IL-6 plays a role in the differentiation of a mature B cell into a cell capable of secreting antibodies. It promotes the differentiation of CD4+ T cells into the T follicular helper (Tfh) cells, which release the IL-21 necessary for the differentiation of mature B cells into plasma cells [17,18]. It has been shown that plasmablasts have the capacity to produce IL-6 and thus to generate numerous T follicular helper cells. Moreover, circulating levels of these two cell populations are not only higher in patients with rheumatoid arthritis than in the general population, but they are also lower after initiation of tocilizumab [19]. Furthermore, a study in mutated mice that do not express the IL-6 gene showed a clear decline in the immune B-cell response with decreases of blood IgG1, IgG2a, and IgG3 rates [20]. Thus, it is clear that IL-6 is essential for antibody production through inducing plasma cell differentiation, and it plays a role in the generation and regulation of memory B cells. Indeed, the IL-6 blockade by TCZ in rheumatoid arthritis is associated with a significant decrease in pre-switch (IgM+) and post-switch (IgG+) memory B cells [21,22]. IL-6 has also been shown in vivo in mice to participate in the survival of the long-lived plasma cells nested in the bone marrow [23].

3.1.3. IL-6 and the Cellular Adaptive Immune Response: T-Cell Response

Naive CD4+ T cells differentiate into one or another of these effectors (Th1, Th2, Th17, or Treg), each of which will have a different role, depending on the cytokine environment: IL-12 shifts towards a Th1 profile; IL-4 shifts towards a Th2 profile; TGF-β shifts towards a Treg profile (regulatory T cells) profile; and the association of TGF-β and IL-6 shifts towards a Th17 profile [24]. It has also been shown that the orientation towards Th17 or Treg is 'mutually exclusive.' Whereas Treg generation requires the exclusive presence of TGF-β, the presence of IL-6 in association with TGF-β will orient towards a Th17 profile while preventing differentiation into Treg [25]. Yet, while Tregs induce immune-tolerance and decrease autoimmune and alloimmune responses, Th17 is associated with the development of autoimmune and chronic inflammatory diseases. It also seems to be involved in the pathophysiology of acute T-cell-mediated rejection after kidney transplantation and in chronic rejection after cardiac transplantation [24,26,27].

The differentiation of CD8+ T cells into cytotoxic T cells is also promoted by IL-6 [28,29]. In addition, the importance of IL-6 in the differentiation and survival of long-term memory T cells has also been demonstrated [30]. Thus, the use of TCZ as a treatment for desensitization could be relevant because of the centrality of IL-6 in the adaptive immune response on both the humoral and cellular levels.

3.2. Tocilizumab and Kidney Transplantation

3.2.1. Tocilizumab

Tocilizumab (TCZ) is a monoclonal antibody that is specifically directed against the IL-6 receptor, either the membrane-bound or the soluble form. It was initially approved

for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis. Due to the multiple sites of action of IL-6 and a favorable safety profile in early rheumatology cohorts, tocilizumab has been studied for many other indications over the past 15 years, including refractory Takayasu disease [31], systemic lupus erythematosus [32], giant cell arteritis [33], highly relapsing neuromyelitis optica spectrum disorder [34], acute graft-versus-host disease after hematopoietic stem-cell transplantation [35], and even more recently in severe acute respiratory syndrome coronavirus 2 pneumonia [36].

3.2.2. Tocilizumab and Antibody-Mediated Rejection

In the field of kidney transplantation, TCZ was first studied in antibody-mediated rejection (AMR). Chronic active antibody-mediated rejection (cAMR) is a major therapeutic issue in kidney transplantation, as it is one of the main causes of graft loss. However, no treatment has been proven to be superior. Choi et al. used tocilizumab in 36 patients with cAMR who failed the SOC and obtained encouraging results: there was 91% patient survival (overall survival) and 80% graft survival at 6 years, associated with a decrease in DSAs and stable kidney function at 2 years post-transplantation [37]. The researchers observed four instances of cAMR-related graft loss. These occurred in four patients for whom TCZ had been stopped prematurely (one for medical reasons and three for financial reasons), suggesting a rebound effect related to the accumulation of IL-6 under treatment by blocking its receptor with TCZ [38]. To avoid this, clazakizumab, a monoclonal antibody that directly inhibits IL-6, was tested in a randomized controlled trial in 20 patients with late active or chronic active antibody-mediated rejection [39]. After 12 weeks of treatment, the clazakizumab group had a significant decrease in DSA mean fluorescence intensity and a smaller decline in graft function compared to the placebo group. However, the safety analysis (primary endpoint) showed severe infectious complications in five patients (25%) and complicated colonic diverticulitis in two patients (10%). The encouraging efficacy data must be contrasted with the results from a series of nine patients with SOC-resistant cAMR treated with tocilizumab: compared with a historical cohort on SOC alone, tocilizumab did not significantly improve graft function or reduce histological damage [40].

To determine whether the potentially beneficial effect of tocilizumab described by Choi et al. requires prior exposure to other immunosuppressive therapies, Lavacca et al. used tocilizumab as a first-line therapy in 15 kidney transplant patients with severe cAMR (60% with transplant glomerulopathy classified as cg3 according to Banff classification) with satisfactory results. Over a follow-up period of 20.7 months, there was a stabilization of renal function, increased proteinuria, a significant decrease in DSAs, improvement of microvascular inflammation lesions on systematic biopsy at 6 months, and stabilization of other histological lesions [41]. However, data on the use of tocilizumab to treat acute active humoral rejection are limited [42].

Chandran et al. performed a randomized controlled trial that included 30 patients with stable graft function, with subclinical inflammation defined on routine biopsy as moderate interstitial inflammation (Banff classification i or ti 1–2 and t0) [43]. Subjects were randomized between a treatment group (tocilizumab 8 mg/kg monthly, 6 injections) and a placebo group. The authors showed a significant decrease in interstitial inflammation associated with an increased level of Tregs in 62.5% of the subjects treated with tocilizumab versus 21.4% in the control group (p = 0.03). Given this study's results, tocilizumab could be of interest for use in the early stages of graft inflammation and even before the rejection stage.

3.2.3. Tocilizumab and Desensitization

Few studies have focused on the use of TCZ in DES protocols in highly sensitized KTCs. An in vivo study in HLA-incompatible (HLA-A2) skin graft sensitized mice suggested that TCZ not only decreases the level of anti-HLA-A2 antibodies, but also the number of plasma cells producing these antibodies in the bone marrow and spleen [44,45]. In humans, Vo et al. tested TCZ as a second-line DES therapy in 10 highly sensitized KTCs who had failed

in the standard desensitization protocol (IVIg + rituximab +/− plasma exchange) [11]. The safety profile (primary endpoint) of the treatment was favorable, and 5 of 10 patients were able to receive a transplant after a mean time of 8.1 months, compared to a mean time of 25 months since the first desensitization attempt. There was no AMR on a routine biopsy conducted at 6 months and no development of DSAs. There was AMR at 12 months in one patient, who nonetheless responded well to treatment. Thus, this small study suggests the value of adding TCZ to the standard DES protocol in the patients who are the most difficult to desensitize (see Table 1).

Table 1. Key safety and efficacy studies using tocilizumab as a desensitization therapy.

Study	Design/Population	Intervention	Outcomes	Main Results
Kim et al. [44] Transplantation 2014	Pre-clinical study, in vivo HLA-incompatible (HLA-A2) skin graft sensitized mice	TCZ intraperitoneal (10–30 mg/kg ×3/week, during 4 weeks) vs. placebo	MFI anti-HLA-A2 Ab Rates of - T fh - T fh 17 - T reg - long-term PC	↘ MFI anti-HLA-A2 Ab ($p = 0.0076$) ↘ rates: - T fh - T fh 17 - long-term PC ↗ T reg
Vo et al. [11] Transplantation 2015	Phase I/II monocentric, uncontrolled study 10 HS patients After failure of DES with RTX + IVIg +/− PE	TCZ IV 8 mg/kg at J15 then 1/month during 6 months + IVIg at J0 and J15 (2 g/kg)	Efficacy: - % of patients receiving a transplant - Rejection at M6 biopsy - DSA at M6 Safety	5/10 patients received a transplant (mean delay of 8.1 months post-1st TCZ) At M6: no DSA, no AMR At M12: 1 AMR (good response to treatment); no graft loss 2 serious AEs: - Acute pulmonary edema (dialysis insufficiency) with epilepsy (not related to TCZ) - Colonic diverticulitis with perforation (possibly related to TCZ)
Daligault et al. [46] Transplantation Direct 2021	Phase II monocentric, uncontrolled study HS patients; First DES attempt	TCZ IV 8 mg/kg (1/month; during ≥6 months) No other prior or concurrent DES procedures	Efficacy: - MFI of anti-HLA immunodominant Ab - Number of anti-HLA Ab with MFI > 10,000 - % of patients received a transplant Safety	↘: - MFI of anti-HLA immunodominant Ab ($p < 0.05$) - Number of anti-HLA Ab with MFI > 10 000 ($p < 0.05$) Not clinically relevant: only 1 patient received a transplant 1 serious AE: spondylodiscitis

Table 1. *Cont.*

Study	Design/Population	Intervention	Outcomes	Main Results
Jouve et al. [47] AJT 2021	Monocentric, controlled, non-randomized study HS patients (first DES attempt) Control groups: - HS patients remaining on dialysis without DES attempt - Healthy subjects	TCZ IV 8 mg/kg (1/month; during ≥6 months) No other prior or concurrent DES procedures	Rates evolution of: - T fh 1 ; T fh 2 ; T fh 17 ; T reg - Plasmablasts, plasma-cells, B memory cells Evolution of anti-HLA Ab MFI	T population: No significant change: T fh 1; T fh 2; T fh 17; T reg B population Blocking of maturation ↘: - Post-germinative B-cells - Plasma-blasts - Plasma-cells Anti-HLA Ab MFI: Same observation as Daligault et al. (same cohort)

Abbreviations: Ab: antibody; AE: adverse event; AMR: antibody-mediated rejection; DES: desensitization; IV: intravenous; IVIG: intravenous polyvalent immunoglobulin; HS: highly sensitized; MFI: mean fluorescence intensity; PE: plasma exchange; PC: plasma cells; RTX: rituximab; T fh: T follicular helper cells; T reg: T regulatory cells; ↘ : decrease ; ↗ : increase.

In order to assess the efficacy and safety of a DES protocol based exclusively on tocilizumab Daligault and our team conducted a single-center, non-randomized study in 14 highly sensitized candidates awaiting kidney transplantation who had not previously received any other DES treatment [46]. While having a favorable safety profile (only one serious adverse event of an infectious nature), there was also a significant decrease in the MFI of the immunodominant anti-HLA antibody and in the number of antibodies with MFI > 10,000. However, this decrease was not clinically significant as the MFIs remained at levels incompatible with transplantation, and only one patient could receive a transplant. In contrast, when 11 out of the 14 patients were started on a standard DES protocol (apheresis, rituximab, triple immunosuppression (tacrolimus, mycophenolic acid, steroids)) following tocilizumab therapy, 8 subsequently became eligible for a transplant. Transplantation was performed, on average, 9.6 months after the start of TCZ, whereas waiting times from the date of last enrollment to the start of TCZ averaged 90.2 months.

In comparison, Noble and our team recently published the results of the standard DES protocol used at the University Hospital of Grenoble (apheresis; rituximab; immunosuppression with tacrolimus, mycophenolate mofetil, and steroids) between 2016 and 2020. Forty-five subjects were treated (18 with a living donor, 27 from a deceased donor), including the 13 patients in the Daligault et al. study who had previously benefited from tocilizumab [48]. With the removal of those patients from the sample, 32 patients were treated, 29 of whom were able to undergo transplantation (91%). These studies (Daligault et al. and Noble et al.) therefore suggest that, in terms of improving transplantation access (which could be seen as the 'short-term efficacy' of DES), TCZ alone is not sufficient, and the addition of TCZ upstream of a standard DES protocol does not seem to do any better than the standard protocol alone.

However, TCZ may be of interest due to its longer-term effects. Using a cohort of patients in Grenoble treated with TCZ alone, Jouve and our team used flow cytometry to characterize the lymphocyte profiles of these subjects in comparison with highly sensitized patients on dialysis and with healthy subjects [47]. The research showed that TCZ could inhibit B-cell maturation, as evidenced by the significant decrease in post-germinative B cells, plasma cells, and plasmablasts. By hindering B-cell maturation, it is possible that TCZ in association with the standard DES protocol limits the rebound of anti-HLA antibodies after transplantation and thus improves the long-term outcome of DES protocols. Rather than improving access to transplantation ('short-term efficacy'), tocilizumab would therefore mainly improve long-term graft survival by limiting the risk of post-transplant anti-HLA rebound and, therefore, AMR.

Therefore, the question could arise as to the value of continuing tocilizumab after HLA-incompatible transplantation. To investigate this, a study was conducted using clazakizumab, a monoclonal antibody directed directly against IL-6 and studied in rheumatoid arthritis (not yet marketed) [49]. Vo et al. used clazakizumab as a DES therapy in 10 highly sensitized KTCs receiving IVIg and plasma exchange in parallel. While having a favorable safety profile, a significant decrease in anti-HLA antibodies was observed between the beginning and end of the treatment in 9 out of 10 patients, all of whom could then undergo transplantation. Clazakizumab was continued after transplantation and none of the patients had a DSA at 6 months post-transplantation. In addition, these patients had a significant increase of FoxP3-expressing Tregs at 6 months post-transplantation, suggesting that clazakizumab could orient the T-cell response towards a tolerogenic profile [50]. Moreover, Jouve et al. noted no significant increase in this lymphocyte population with tocilizumab [47].

4. Conclusions

Continued research into new treatments and DES procedures is a major challenge towards improving access to transplantation for highly sensitized subjects while guaranteeing not only safety, but also efficacy. The efficacy of DES has to be judged at the individual level (access to transplantation, long-term graft function) and at the collective level (respect for the principle of utility) Desensitization is sufficiently effective such that offering a graft to a highly sensitized subject does not shorten the graft's lifespan compared to that expected in a non-sensitized subject. Tocilizumab is a relevant candidate because of the centrality of IL-6 in the regulation of a cellular and humoral immune response. When used as a single agent without any other DES treatment, tocilizumab reduces anti-HLA antibodies, but this is not sufficient to allow transplantation. However, the TCZ-induced blockage of B-cell maturation suggests that its addition to the standard DES protocol could improve the long-term outcome of HLA-incompatible transplants by decreasing post-transplant antibody rebound. The continuation of TCZ after transplantation could then be justified to decrease the risk of humoral rejection. The efficacy and safety of such a procedure remain to be demonstrated in a study that compares this strategy to the standard DES protocol.

Author Contributions: J.W. conducted the literature screening and the literature analysis, in addition to writing the initial draft. J.N. and T.J. conceptualized the study. Review and editing were conducted by P.M. and L.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Port, F.K.; Wolfe, R.A.; Mauger, E.A.; Berling, D.P.; Jiang, K. Comparison of Survival Probabilities for Dialysis Patients vs Cadaveric Renal Transplant Recipients. *JAMA* **1993**, *270*, 1339–1343. [CrossRef]
2. Laupacis, A.; Keown, P.; Pus, N.; Krueger, H.; Ferguson, B.; Wong, C.; Muirhead, N. A study of the quality of life and cost-utility of renal transplantation. *Kidney Int.* **1996**, *50*, 235–242. [CrossRef]
3. Wong, G.; Howard, K.; Chapman, J.R.; Chadban, S.; Cross, N.; Tong, A.; Webster, A.; Craig, J. Comparative Survival and Economic Benefits of Deceased Donor Kidney Transplantation and Dialysis in People with Varying Ages and Co-Morbidities. *PLoS ONE* **2012**, *7*, e29591. [CrossRef]
4. Jordan, S.C.; Tyan, D.; Stablein, D.; McIntosh, M.; Rose, S.; Vo, A.; Toyoda, M.; Davis, C.; Shapiro, R.; Adey, D.; et al. Evaluation of Intravenous Immunoglobulin as an Agent to Lower Allosensitization and Improve Transplantation in Highly Sensitized Adult Patients with End-Stage Renal Disease: Report of the NIH IG02 Trial. *J. Am. Soc. Nephrol.* **2004**, *15*, 3256–3262. [CrossRef]
5. Vo, A.A.; Choi, J.; Cisneros, K.; Reinsmoen, N.; Haas, M.; Ge, S.; Toyoda, M.; Kahwaji, J.; Peng, A.; Villicana, R.; et al. Benefits of Rituximab Combined with Intravenous Immunoglobulin for Desensitization in Kidney Transplant Recipients. *Transplantation* **2014**, *98*, 312–319. [CrossRef]
6. Loupy, A.; Suberbielle-Boissel, C.; Zuber, J.; Anglicheau, D.; Timsit, M.-O.; Martinez, F.; Thervet, E.; Bruneval, P.; Charron, D.; Hill, G.S.; et al. Combined Posttransplant Prophylactic IVIg/Anti-CD 20/Plasmapheresis in Kidney Recipients with Preformed Donor-Specific Antibodies: A Pilot Study. *Transplantation* **2010**, *89*, 1403–1410. [CrossRef] [PubMed]
7. Montgomery, R.A.; Lonze, B.E.; King, K.E.; Kraus, E.S.; Kucirka, L.M.; Locke, J.E. Desensitization in HLA-incompatible kidney recipients and survival. *N. Engl. J. Med.* **2011**, *365*, 318. [CrossRef] [PubMed]

8. Orandi, B.J.; Luo, X.; Massie, A.B.; Garonzik-Wang, J.M.; Lonze, B.; Ahmed, R.; Van Arendonk, K.J.; Stegall, M.D.; Jordan, S.C.; Oberholzer, J.; et al. Survival Benefit with Kidney Transplants from HLA-Incompatible Live Donors. *N. Engl. J. Med.* **2016**, *374*, 940–950. [CrossRef] [PubMed]
9. Manook, M.; Koeser, L.; Ahmed, Z.; Robb, M.; Johnson, R.; Shaw, O.; Kessaris, N.; Dorling, A.; Mamode, N. Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: A matched cohort analysis. *Lancet* **2017**, *389*, 727–734. [CrossRef]
10. Perry, D.K.; Burns, J.M.; Pollinger, H.S.; Amiot, B.P.; Gloor, J.M.; Gores, G.J.; Stegall, M.D. Proteasome Inhibition Causes Apoptosis of Normal Human Plasma Cells Preventing Alloantibody Production. *Am. J. Transplant.* **2009**, *9*, 201. [CrossRef]
11. Vo, A.A.; Choi, J.; Kim, I.; Louie, S.; Cisneros, K.; Kahwaji, J.; Toyoda, M.; Ge, S.; Haas, M.; Puliyanda, D.; et al. A Phase I/II Trial of the Interleukin-6 Receptor–Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients. *Transplantation* **2015**, *99*, 2356–2363. [CrossRef]
12. Kwun, J.; Burghuber, C.; Manook, M.; Iwakoshi, N.; Gibby, A.; Hong, J.J.; Knechtle, S. Humoral Compensation after Bortezomib Treatment of Allosensitized Recipients. *J. Am. Soc. Nephrol.* **2017**, *28*, 1991–1996. [CrossRef] [PubMed]
13. Gonzales, M.A.M.; Gandhi, M.J.; Schinstock, C.A.; Moore, N.A.; Smith, B.H.; Braaten, N.Y.; Stegall, M.D. 32 Doses of Bortezomib for Desensitization Is Not Well Tolerated and Is Associated with Only Modest Reductions in Anti-HLA Antibody. *Transplantation* **2017**, *101*, 1222–1227. [CrossRef] [PubMed]
14. Guthoff, M.; Schmid-Horch, B.; Weisel, K.C.; Häring, H.-U.; Königsrainer, A.; Heyne, N. Proteasome inhibition by bortezomib: Effect on HLA-antibody levels and specificity in sensitized patients awaiting renal allograft transplantation. *Transpl. Immunol.* **2012**, *26*, 171–175. [CrossRef]
15. Tanaka, T.; Kishimoto, T. Targeting Interleukin-6: All the Way to Treat Autoimmune and Inflammatory Diseases. *Int. J. Biol. Sci.* **2012**, *8*, 1227–1236. [CrossRef]
16. Hirano, T.; Yasukawa, K.; Harada, H.; Taga, T.; Watanabe, Y.; Matsuda, T.; Kashiwamura, S.-I.; Nakajima, K.; Koyama, K.; Iwamatsu, A.; et al. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* **1986**, *324*, 73–76. [CrossRef]
17. Kishimoto, T. IL-6: From its discovery to clinical applications. *Int. Immunol.* **2010**, *22*, 347–352. [CrossRef] [PubMed]
18. Dienz, O.; Eaton, S.M.; Bond, J.P.; Neveu, W.; Moquin, D.; Noubade, R.; Briso, E.M.; Charland, C.; Leonard, W.J.; Ciliberto, G.; et al. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. *J. Exp. Med.* **2009**, *206*, 69–78. [CrossRef] [PubMed]
19. Chavele, K.-M.; Merry, E.; Ehrenstein, M.R. Cutting Edge: Circulating Plasmablasts Induce the Differentiation of Human T Follicular Helper Cells via IL-6 Production. *J. Immunol.* **2015**, *194*, 2482–2485. [CrossRef]
20. Jones, S.A. Directing Transition from Innate to Acquired Immunity: Defining a Role for IL-6. *J. Immunol.* **2005**, *175*, 3463–3468. [CrossRef] [PubMed]
21. Roll, P.; Muhammad, K.; Schumann, M.; Kleinert, S.; Einsele, H.; Dörner, T.; Tony, H.-P. In vivo effects of the anti-interleukin-6 receptor inhibitor tocilizumab on the B cell compartment. *Arthritis Rheum.* **2011**, *63*, 1255–1264. [CrossRef]
22. Muhammad, K.; Roll, P.; Seibold, T.; Kleinert, S.; Einsele, H.; Dörner, T.; Tony, H.-P. Impact of IL-6 receptor inhibition on human memory B cells in vivo: Impaired somatic hypermutation in preswitch memory B cells and modulation of mutational targeting in memory B cells. *Ann. Rheum. Dis.* **2011**, *70*, 1507–1510. [CrossRef] [PubMed]
23. Cassese, G.; Arce, S.; Hauser, A.E.; Lehnert, K.; Moewes, B.; Mostarac, M.; Muehlinghaus, G.; Szyska, M.; Radbruch, A.; Manz, R.A. Plasma Cell Survival Is Mediated by Synergistic Effects of Cytokines and Adhesion-Dependent Signals. *J. Immunol.* **2003**, *171*, 1684–1690. [CrossRef] [PubMed]
24. Afzali, B.; Lombardi, G.; Lechler, R.I.; Lord, G. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. *Clin. Exp. Immunol.* **2007**, *148*, 32–46. [CrossRef] [PubMed]
25. Bettelli, E.; Carrier, Y.; Gao, W.; Korn, T.; Strom, T.B.; Oukka, M.; Weiner, H.L.; Kuchroo, V.K. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **2006**, *441*, 235–238. [CrossRef]
26. Faust, S.M.; Lu, G.; Marini, B.L.; Zou, W.; Gordon, D.; Iwakura, Y.; Laouar, Y.; Bishop, D.K. Role of T Cell TGFβ Signaling and IL-17 in Allograft Acceptance and Fibrosis Associated with Chronic Rejection. *J. Immunol.* **2009**, *183*, 7297–7306. [CrossRef]
27. Van Kooten, C.; Boonstra, J.G.; Paape, M.E.; Fossiez, F.; Banchereau, J.; Lebecque, S. Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *J. Am. Soc. Nephrol.* **1998**, *9*, 1526–1534. [CrossRef]
28. Kimura, A.; Kishimoto, T. IL-6: Regulator of Treg/Th17 balance. *Eur. J. Immunol.* **2010**, *40*, 1830–1835. [CrossRef]
29. Sarantopoulos, A.; Tselios, K.; Gkougkourelas, I.; Pantoura, M.; Georgiadou, A.-M.; Boura, P. Tocilizumab Treatment Leads to a Rapid and Sustained Increase in Treg Cell Levels in Rheumatoid Arthritis Patients: Comment on the Article by Thiolat et al. *Arthritis Rheumatol.* **2014**, *66*, 2638. [CrossRef]
30. Rochman, I.; Paul, W.E.; Ben-Sasson, S.Z. IL-6 Increases Primed Cell Expansion and Survival. *J. Immunol.* **2005**, *174*, 4761–4767. [CrossRef]
31. Nakaoka, Y.; Isobe, M.; Tanaka, Y.; Ishii, T.; Ooka, S.; Niiro, H.; Tamura, N.; Banno, S.; Yoshifuji, H.; Sakata, Y.; et al. Long-term efficacy and safety of tocilizumab in refractory Takayasu arteritis: Final results of the randomized controlled phase 3 TAKT study. *Rheumatology* **2020**, *59*, 2427–2434. [CrossRef] [PubMed]

32. Illei, G.G.; Shirota, Y.; Yarboro, C.H.; Daruwalla, J.; Tackey, E.; Takada, K.; Fleisher, T.; Balow, J.E.; Lipsky, P.E. Tocilizumab in systemic lupus erythematosus: Data on safety, preliminary efficacy, and impact on circulating plasma cells from an open-label phase I dosage-escalation study. *Arthritis Rheum.* **2010**, *62*, 542–552. [CrossRef] [PubMed]
33. Calderón-Goercke, M.; Castañeda, S.; Aldasoro, V.; Villa, I.; Prieto-Peña, D.; Atienza-Mateo, B.; Patiño, E.; Moriano, C.; Romero-Yuste, S.; Narvaez, J.; et al. Tocilizumab in giant cell arteritis: Differences between the GiACTA trial and a multicentre series of patients from the clinical practice. *Clin. Exp. Rheumatol.* **2020**, *38* (Suppl. 124), 32441643.
34. Zhang, C.; Zhang, M.; Qiu, W.; Ma, H.; Zhang, X.; Zhu, Z.; Yang, C.-S.; Jia, D.; Zhang, T.-X.; Yuan, M.; et al. Safety and efficacy of tocilizumab versus azathioprine in highly relapsing neuromyelitis optica spectrum disorder (TANGO): An open-label, multicentre, randomised, phase 2 trial. *Lancet Neurol.* **2020**, *19*, 391–401. [CrossRef]
35. Ganetsky, A.; Frey, N.V.; Hexner, E.O.; Loren, A.W.; Gill, S.I.; Luger, S.M.; Mangan, J.K.; Martin, M.E.; Babushok, D.V.; Drobyski, W.R.; et al. Tocilizumab for the treatment of severe steroid-refractory acute graft-versus-host disease of the lower gastrointestinal tract. *Bone Marrow Transplant.* **2019**, *54*, 212–217. [CrossRef]
36. Salama, C.; Han, J.; Yau, L.; Reiss, W.G.; Kramer, B.; Neidhart, J.D.; Criner, G.J.; Kaplan-Lewis, E.; Baden, R.; Pandit, L.; et al. Tocilizumab in Patients Hospitalized with COVID-19 Pneumonia. *N. Engl. J. Med.* **2021**, *384*, 20–30. [CrossRef]
37. Choi, J.; Aubert, O.; Vo, A.; Loupy, A.; Haas, M.; Puliyanda, D.; Kim, I.; Louie, S.; Kang, A.; Peng, A.; et al. Assessment of Tocilizumab (Anti-Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients. *Arab. Archaeol. Epigr.* **2017**, *17*, 2381–2389. [CrossRef]
38. Nishimoto, N.; Terao, K.; Mima, T.; Nakahara, H.; Takagi, N.; Kakehi, T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti–IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood* **2008**, *112*, 3959–3964. [CrossRef]
39. Doberer, K.; Duerr, M.; Halloran, P.F.; Eskandary, F.; Budde, K.; Regele, H.; Reeve, J.; Borski, A.; Kozakowski, N.; Reindl-Schwaighofer, R.; et al. A Randomized Clinical Trial of Anti–IL-6 Antibody Clazakizumab in Late Antibody-Mediated Kidney Transplant Rejection. *J. Am. Soc. Nephrol.* **2021**, *32*, 708–722. [CrossRef] [PubMed]
40. Massat, M.; Congy-Jolivet, N.; Hebral, A.; Esposito, L.; Marion, O.; Delas, A.; Colombat, M.; Faguer, S.; Kamar, N.; Del Bello, A. Do anti-IL-6R blockers have a beneficial effect in the treatment of antibody-mediated rejection resistant to standard therapy after kidney transplantation? *Am. J. Transplant.* **2021**, *21*, 1641–1649. [CrossRef]
41. Lavacca, A.; Presta, R.; Gai, C.; Mella, A.; Gallo, E.; Camussi, G.; Abbasciano, I.; Barreca, A.; Caorsi, C.; Fop, F.; et al. Early effects of first-line treatment with anti-interleukin-6 receptor antibody tocilizumab for chronic active antibody-mediated rejection in kidney transplantation. *Clin. Transplant.* **2020**, *34*, e13908. [CrossRef]
42. Pottebaum, A.A.; Venkatachalam, K.; Liu, C.; Brennan, D.C.; Murad, H.; Malone, A.F.; Alhamad, T. Efficacy and Safety of Tocilizumab in the Treatment of Acute Active Antibody-mediated Rejection in Kidney Transplant Recipients. *Transplant. Direct* **2020**, *6*, e543. [CrossRef] [PubMed]
43. Chandran, S.; Leung, J.; Hu, C.; Laszik, Z.G.; Tang, Q.; Vincenti, F.G. Interleukin-6 blockade with tocilizumab increases Tregs and reduces T effector cytokines in renal graft inflammation: A randomized controlled trial. *Am. J. Transplant.* **2021**, *21*, 2543–2554. [CrossRef] [PubMed]
44. Kim, I.; Wu, G.; Chai, N.-N.; Klein, A.S.; Jordan, S. Anti–Interleukin 6 Receptor Antibodies Attenuate Antibody Recall Responses in a Mouse Model of Allosensitization. *Transplantation* **2014**, *98*, 1262–1270. [CrossRef] [PubMed]
45. Wu, G.; Chai, N.; Kim, I.; Klein, A.; Jordan, S. Monoclonal anti-interleukin-6 receptor antibody attenuates donor-specific antibody responses in a mouse model of allosensitization. *Transpl. Immunol.* **2013**, *28*, 138–143. [CrossRef] [PubMed]
46. Daligault, M.; Bardy, B.; Noble, J.; Bourdin, A.; Masson, D.; Bennani, H.N.; Bugnazet, M.; Malvezzi, P.; Rostaing, L.; Jouve, T. Marginal Impact of Tocilizumab Monotherapy on Anti-HLA Alloantibodies in Highly Sensitized Kidney Transplant Candidates. *Transplant. Direct* **2021**, *7*, e690. [CrossRef]
47. Jouve, T.; Laheurte, C.; Noble, J.; Weinhard, J.; Daligault, M.; Renaudin, A.; Bennani, H.N.; Masson, D.; Gravelin, E.; Bugnazet, M.; et al. Immune responses following tocilizumab therapy to desensitize HLA-sensitized kidney transplant candidates. *Am. J. Transplant.* **2021**. [CrossRef]
48. Noble, J.; Metzger, A.; Bennani, H.N.; Daligault, M.; Masson, D.; Terrec, F.; Imerzoukene, F.; Bardy, B.; Fiard, G.; Marlu, R.; et al. Apheresis Efficacy and Tolerance in the Setting of HLA-Incompatible Kidney Transplantation. *J. Clin. Med.* **2021**, *10*, 1316. [CrossRef]
49. Vo, A.; Ammerman, N.; Huang, E.; Toyoda, M.; Ge, S.; Peng, A.; Najjar, R.; Williamson, S.; Myers, C.; Sethi, S.; et al. Clazakizumab® (Anti-Il-6) For Desensitization of Highly-Hla Sensitized Patients Awaiting Kidney Transplant (Nct03380962). *Transplantation* **2020**, *104*, S104–S105. [CrossRef]
50. Jordan, S.; Ge, S.; Ammerman, N.; Toyoda, M.; Huang, E.; Peng, A.; Najjar, R.; Sethi, S.; Williamson, S.; Myers, K.; et al. Clazakizumab (Anti-Il-6) Induces Foxp3+ Tregs In Highly Hla Sensitized Patients Receiving HLAI Kidney Transplantation (NCT03380962). *Transplantation* **2020**, *104*, S105. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Exploring the Level of Post Traumatic Growth in Kidney Transplant Recipients via Network Analysis

Yuri Battaglia [1,*], Luigi Zerbinati [2], Martino Belvederi Murri [2], Michele Provenzano [3], Pasquale Esposito [4], Michele Andreucci [3], Alda Storari [1] and Luigi Grassi [2]

[1] Nephrology and Dialysis Unit, St. Anna University Hospital, 44124 Ferrara, Italy; a.storari@ospfe.it
[2] Department of Neuroscience and Rehabilitation, Institute of Psychiatry, University of Ferrara, 44124 Ferrara, Italy; zrblgu@unife.it (L.Z.); martino.belvederimurri@unife.it (M.B.M.); luigi.grassi@unife.it (L.G.)
[3] Nephrology and Dialysis Unit, Department of Health Sciences, Magna Graecia University, 88100 Catanzaro, Italy; michiprov@hotmail.it (M.P.); andreucci@unicz.it (M.A.)
[4] Department of Internal Medicine, Division of Nephrology, Dialysis and Transplantation, University of Genoa and IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; pasqualeesposito@hotmail.com
* Correspondence: yuri.battaglia@ospfe.it

Abstract: Although kidney transplant can lead to psychiatric disorders, psychosocial syndromes and demoralization, a positive post-traumatic growth (PTG) can occur in kidney transplant recipients (KTRs). However, the PTG-Inventory (PTGI), a reliable tool to measure PTG is scarcely used to explore the effect of this stressful event in KTRs. Thus, the purpose of our study was to assess the level of PTG and its correlation with demoralization, physical and emotional symptoms or problems via network analysis in KTRs. Additionally, we aimed at exploring the association of PTG with psychiatric diagnoses, Diagnostic Criteria for Psychosomatic Research (DCPR) conditions, and medical variables. A total of 134 KTRs were tested using MINI International Neuropsychiatric Interview 6.0 (MINI 6.0), DCPR interview, PTGI, Edmonton Symptom Assessment System (ESAS), Canadian Problem Checklist (CPC) and Demoralization scale (DS-IT). PTGI was used to investigate the positive psychological experience of patients after KT. It consists of 21 items divided in five factors. Routine biochemistry, immunosuppressive agents, socio-demographic and clinical data were collected. A symptom network analysis was conducted among PTGI, ESAS and DS-IT. Mean score of PTGI total of sample was 52.81 $\pm$ 19.81 with higher scores in women (58.53 $\pm$ 21.57) than in men (50.04 $\pm$ 18.39) ($p < 0.05$). PTGI-Relating to Others (16.50 $\pm$ 7.99) sub-score was markedly higher than other PTGI factor sub-scores. KTRs with DCPR-alexithymia or International Classification of Diseases, tenth revision (ICD-10) anxiety disorders diagnosis had lower PTGI total score and higher PTGI-Personal Strength sub-score, respectively ($p < 0.05$). The network analysis identified two communities: PTGI and ESAS with DS-IT. DS-IT Disheartenment, DS-IT Hopelessness and PTGI Relating to Others were the most central items in the network. After 1000 bootstrap procedures, the Exploratory graph analysis revealed the presence of a median of two communities in the network in 97.5% of the bootstrap iterations. A more extensive use of PTGI should be encouraged to identify and enhance the positive psychological changes after KT.

Keywords: post traumatic growth; psychiatric morbidity; kidney transplantation; network analysis; ESAS; MINI; CPC; DCPR; distress; demoralization; alexithymia; anxiety

Citation: Battaglia, Y.; Zerbinati, L.; Belvederi Murri, M.; Provenzano, M.; Esposito, P.; Andreucci, M.; Storari, A.; Grassi, L. Exploring the Level of Post Traumatic Growth in Kidney Transplant Recipients via Network Analysis. *J. Clin. Med.* **2021**, *10*, 4747. https://doi.org/10.3390/jcm10204747

Academic Editors: Charat Thongprayoon, Wisit Cheungpasitporn, Wisit Kaewput and Eytan Mor

Received: 3 September 2021
Accepted: 14 October 2021
Published: 16 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Kidney transplantation (KT) is the best treatment option for people affected by end stage chronic kidney disease (ESRD) [1]. However, KT can be considered a "traumatic event" for kidney transplant recipients (KTRs), leading to the development of intra-psychological conflicts and existential crisis [2]. Indeed, psychiatric disorders, especially

anxiety and depression, are presented in KTRs with a prevalence from 10% to 40%, depending on the measures used, such as the International Classification of Diseases (ICD) or the Diagnostic and Statistical manual for Mental disorders (DSM) and cut-off points adopted [3–5].

Furthermore, other clinically significant psychosocial syndromes, not diagnosable with traditional psychiatric tools, were found in about 65% of KTRs. More specifically, abnormal illness behavior, irritability, alexithymia and somatization were the most frequent syndromes assessed by Diagnostic Criteria for Psychosomatic Research (DCPR), a useful interview for identifying sub-threshold or undetected syndromes [6].

Recently, demoralization, a psychological syndrome distinct from depression [7], has been diagnosed in a high percentage of medically ill patients [8], particularly with a high prevalence of up to 86% in KTRs [9]. That is a condition described as a state of existential distress, characterized by loss of meaning and purpose in life, hopelessness and helplessness and feelings of "being trapped" because of persistently being unable to cope with a particular stressful situation [10].

On the other hand, the stressful event of KT can promote positive psychological changes, which are at the basis of concept of post-traumatic growth (PTG). According to Tedeschi and Calhun [11], PTG is defined as a positive cognitive effect due to an extreme crisis, and not merely a coping mechanism in ordinary stress condition. Basically, PTG, induced by the activation of latent intrapersonal resources of the subject, might increase the level of mental functioning after a serious experience. In order to assess and screen the PTG, a specific psychometric tool, PTG Inventory (PTGI) has been developed [12]. It is able to capture five psychological dimensions: relating to others, new possibilities, personal strength, spiritual change and appreciation of life. Notably, although PTGI has been extensively used in traumatized healthy people [13], it has been also validated in several settings of medicine, such as psycho-oncology [14], neurology [15], hematopoietic stem cell [16] or liver transplantation [17] and dialysis [18]. Demoralization and PTG can be conceptualized as opposite reactions to a stressful event and, to date, little is known about the relationship between these two dimensions [9]. Thus, a network approach to psychopathology represents an intriguing option to investigate the associations between these two dimensions [19,20]. Rather than seeing mental disorders as the cause of different symptoms, this theory conceptualizes them as systems arising from the complex and mutual interaction of different symptoms [21,22]. This approach can be furtherly expanded with the use of Exploratory Graph Analysis (EGA) [23], allowing the identification of clusters of symptoms ("communities") that are more related to each other.

To date, only few studies [24,25] have been focused on the use of PTGI in KTRs; thus, the primary aim of our study was to evaluate the levels of PTG and to examine the relationship of PTG dimensions with demoralization, physical and emotional symptoms or problems via network analysis in KTRs. The secondary aim was to assess any association of the PTGI with psychiatric diagnoses, DCPR conditions and medical variables.

2. Materials and Methods

Ongoing KTRs, followed by the Nephrology Unit of the Ferrara University-Hospital, were enrolled in the study, during one of their routine follow up nephrological visits. Inclusion criteria were: (1) being a recipient of kidney from cadaveric or living donor and (2) age $\geq$ 18 years. Exclusion criteria were: (1) Karnofsky Performance Status Scale (KPS), indicating an insufficient level of autonomy (score < 50), and (2) presence of cognitive disorders (Mini Mental State Examination < 24). All patients gave their written informed consent, and the research protocol obtained the ethical approval from the Hospital Ethics Committee for Human Research (Code: 151297, on 17 March 2016). The procedures agreed with the Declaration of Helsinki. The same psychiatrist of the Consultation-Liaison Psychiatric Service, University Psychiatry Unit of the same Hospital tested each KTR. Two individual interviews, such as Mini-International Neuropsychiatric Interview (MINI6.0) and Diagnostic Criteria for Psychosomatic Research Interview (DCPR), as well as four

self-report tools, including Post-Traumatic Growth Inventory (PTGI), Edmonton Symptom Assessment System (ESAS-Revised) and Canadian Problem Checklist (CPC) within the COMPASS (Comprehensive Problem and Symptom Screening) tool [26] and Italian version of Demoralization Scale (DS-IT), were administered.

2.1. PTGI

It is a screening measurement tool used to investigate the positive changes experienced in the aftermath of a traumatic event [27]. It consists of 21 items divided in five factors: New Possibilities, Relating to Others, Personal Strength, Spiritual Change and Appreciation of Life. Each item scored on 6-point Likert scale from 0 (I did not experience this change as a result of my crisis) to 5 (I experienced this change to a very great degree as a result of my crisis). A total score of posttraumatic growth was calculated [28].

2.2. Other Instruments

- The Mini International Neuropsychiatric Interview (MINI 6.0) [29,30] is a diagnostic interview, expensively validated for DSM diagnoses and for ICD-10 diagnoses. In order to formulate a psychiatric diagnosis, the ICD-10 classification was used in this study.
- The Diagnostic Criteria for Psychosomatic Research Structured Interview (DCPR-SI) [31,32] is a semi-structured interview used to evaluate a set of 12 syndromes grouped in 3 different clusters: abnormal illness behavior (i.e., disease phobia, thanatophobia, health anxiety, illness denial); somatization and its different expressions (i.e., persistent somatization, functional somatic symptoms secondary to a psychiatric disorder, conversion symptoms, anniversary reaction); irritability (i.e., irritable mood and type A behavior), and in two clinical constructs (i.e., demoralization and alexithymia). It is able to identify psychological dimensions to a much greater extent than the DSM or ICD criteria [6,33].
- The Edmonton Symptom Assessment System (ESAS-Revised) [34,35] is a reliable assessment tool to explore the severity of six physical (i.e., pain, tiredness, nausea, drowsiness, lack of appetite, shortness of breath), three psychological (i.e., depression, anxiety, feeling of not being well) and one optional symptom (i.e., emotional distress corresponding to the Distress Thermometer) [36,37]. The Global Distress score (ESAS-TOTAL) was obtained by summing up all the scores on the single ESAS symptoms. Analogously, a physical distress sub-score (ESAS-PHYS) and a psychological distress sub-score (ESAS-EMOTIONAL) were computed as a sum of scores for the six physical symptoms and for the four psychological symptoms, respectively. The Italian version shows an acceptable level of validity and good psychometric properties in KTRs [38,39].
- The Canadian Problem Checklist (CPC) [40] is a useful instrument used to screen a list of 21 problems the patient has to deal with. It is divided into six categories (practical, social/family, emotional, spiritual, informational and physical problems) and the severity of each problem is rated in a yes/no (0–1) format.
- The Italian Version of the Demoralization Scale (DS-IT) [41] is a widely valid tool for measuring the demoralization in medical setting and also more recently in KTRs, over the past 2 weeks [9]. It consists in 24 items which compose four subscales: loss of meaning and purpose, dysphoria, disheartenment, and sense of failure. Each item ranges on 6-point Likert scale (0 = never; 5 = all the time) and the sum of the single subscales scores provides a total score.

Demographic data, clinical characteristics and routine biochemistry, determined using standard auto analyzer techniques, were collected.

2.3. Statistical Analysis

Categorical variables were shown as frequencies (%). Continuous variables were reported as either mean ± standard deviation (SD) or median and interquartile range

(IQR) based on their distribution. T-test and chi-square test were employed to determine the associations of PTGI groups with DCPR/ICD-10 diagnosis, ESAS/CPC scores and clinical variables.

Exploratory Graph Analysis

Exploratory graph analysis (EGA) was used to estimate the number of dimensions in multivariate data using undirected network models [23]. EGA first applied a network estimation method followed by a community detection algorithm for weighted networks [42].

- Network Estimation Method.

This study applied the graphical least absolute shrinkage and selection operator [43], which estimated a Gaussian Graphical Model [44] where nodes (circles) represented variables and edges (lines) represented the conditional dependence (or partial correlations) between nodes given all other nodes in the network. The ratio of the minimum and maximum λλ was set to 0.1 in order to control the sparsity of the network in the LASSO. EBICglasso was applied using the qgraph package in R and γγ was set to 0.5 [45]

- Community Detection Algorithm.

The Walktrap algorithm was applied to detect the community and was implemented using the igraph package in R [46].

- Data Analysis.

EGA was applied using the EGAnet package (version 0.9.8) in R (version 4.0.3) and its associated results were visualized using the GGally (version 2.1.1), ggplot2 (version 3.3.3), and qgraph (version 1.6.5) packages in R [47].

- Bootstrap Exploratory Graph Analysis.

Bootstrap exploratory graph analysis (bootEGA) was used to estimate and to evaluate the dimensional structure estimated using EGA. The parametric bootstrap procedure was implemented in this study. The procedure begun by estimating a network using EGA and then generating new replicate data from a multivariate normal distribution (with the same number of cases as the original data). EGA was then applied to the replicate data, continuing iteratively until the desired number of samples was achieved (e.g., 1000). The result was a sampling distribution of EGA networks. From this sampling distribution, descriptive statistics, namely median number of dimensions, 95% confidence intervals around the median and the number of times a certain number of dimensions replicates, were obtained. In addition, a median (or typical) network structure was estimated by computing the median value of each edge across the replicate networks, resulting in a single network. Such a network represented the "typical" network structure of the sampling distribution. The community detection algorithm was then applied, resulting in dimensions that would be expected for a typical network from the EGA sampling distribution. BootEGA was applied using the EGAnet package (version 0.9.8) in R (version 4.0.3) and its associated results were visualized using the GGally (version 2.1.1) and ggplot2 (version 3.3.3) packages in R [48].

3. Results

3.1. Characteristics of the Sample

In total, 134 consecutive KTRs were included in this study; nine patients declined to participate (six for work or family reasons and three because of health reasons) and none was excluded. Ninety patients were men, and the average age was 56.1 (SD = 12) years. The median time since transplantation was 85 months (ranged from 34 months to 178 months). Almost all patients (94%) received kidney from cadaveric donor and a short time in dialysis before KT (median = 22; IQR = 3–166 months) was found. More than half of KTRs (83) were on triple antirejection agents, including steroids (84.3%), calcineurin inhibitors (90.3%), mycophenolate (67.7%), mTOR inhibitors (8.3%), azathioprine (10.4%).

Further detailed socio-demographic and clinical characteristics of the cohort are presented in Table 1.

Table 1. Socio-demographic characteristics, clinical variables and ranking order of DCPR and ICD diagnoses of the sample.

Variables		Variables	
Age, years	56.1 ± 12	*Occupation*	
Females, n (%)	44 (32.8)	Employed, *n* (%)	65 (48.5)
Race-Caucasian, *n* (%)	126 (94)	Retired, *n* (%)	59 (44.1)
Education, years	11.5 ± 4.52	Unemployed, *n* (%)	8 (6.0)
Smokers, *n* (%)	14 (10.4)	Unemployable, *n* (%)	2 (1.4)
Second Transplant, *n* (%)	10 (7.4)		
Acute Rejection, *n* (%)	12 (8.9)	*Marital Status*	
		Single, *n* (%)	29 (21.5)
Cause of CKD		Married, *n* (%)	89 (66)
Glomerulonephritis, *n* (%)	55 (41)	Divorced, *n* (%)	10 (7.5)
ADPKD, *n* (%)	25 (18.7)		
Diabetes Mellitus, *n* (%)	6 (4.5)	*DCPR Diagnosis*	
Hypertension, *n* (%)	4 (3)	Cluster AIB, *n* (%)	43 (31.3)
Other, *n* (%)	44 (32.8)	Cluster Irritability, *n* (%)	42 (31.3)
		Cluster Somatization, *n* (%)	26 (19.3)
Living Situation		Alexithymia, *n* (%)	31 (23.1)
Family, *n* (%)	93 (69.4)	Demoralization, *n* (%)	23 (17.2)
Parents, *n* (%)	22 (16.4)		
Alone, *n* (%)	11 (8.2)	*ICD-10 Diagnosis*	
Others, *n* (%)	8 (5.9)	No diagnosis, *n* (%)	88 (65.7)
		Anxiety disorders, *n* (%)	14 (10.4)
Blood Test Values		Mood [affective] disorders, *n* (%)	11 (8.2)
Creatinine serum, mg/dL	1.4 ± 0.54	Reaction to severe stress and adjustment disorders, *n* (%)	21 (15.7)
GFR-MDRD, mL/min	53.2 ± 17.5		

ADPKD: Autosomal Dominant Polycystic Kidney Disease; AIB: Abnormal Illness Behavior; BMI: Body Mass Index; CKD: Chronic Kidney Disease; DCPR: Diagnostic Criteria for Psychosomatic Research; ICD: International Classification of Diseases; GFR-MDRD: Glomerular Filtration Rate according to the equation from the Modification of Diet in Renal Disease Study.

3.2. Level of Post Traumatic Growth Inventory and Its Relationship with ICD Diagnoses and DCPR Diagnoses

The mean score of PTGI total was 52.81 (SD = 19.81). PTGI-Relating to Others (M = 16.50; SD = 7.99) sub-score was markedly higher than other PTGI factor sub-scores, including PTGI-New Possibilities (M = 11.42, SD = 5.43), PTGI-Personal Strength (M11.08, SD = 5.19), PTGI-Appreciation of Live (M = 9.92, SD = 3.55) and PTGI-Spiritual Changes (M = 4.33, SD = 2.91).

No significant differences of PTG total scores and sub-scores were found with socio-demographic (marital status, housing, occupation, kidney graft months, age), clinical characteristics (pre-emptive transplant, second transplant, immunosuppressive therapy, previous psychiatric ICD diagnosis and cause of nephropathy) and blood chemistry (hemoglobin, eGFR and creatinine), expect for sex and schooling. Women (M = 58.53, SD 21.57) had higher scores of PTGI than men (M = 50.04, SD 18.39) ($t = 2.34$, df = 130, $p < 0.05$) and a positive correlation was found between PTGI-New Possibilities and schooling (Pearson = 0.27, $p < 0.05$).

The specific characteristic of the DCPR and ICD diagnoses are extensively reported elsewhere [6], and briefly summarized in the Table 1.

Regarding ICD-10 psychiatric diagnosis (as assessed through the MINI6.0 interview), KTRs with anxiety ICD diagnosis had higher PTGI-Personal Strength sub-score (M 13.07 with SD 2.30) than ones without anxiety ICD diagnosis (M 10.84 with SD 5.39) ($t = -2.81$, df = 33.87, $p < 0.05$).

Regarding DCPR-diagnosis compared to the whole sample, both PTGI total score and all PTGI factors sub-scores was significantly lower in patients with alexithymia (M = 44.93,

SD = 18.21, t = 2.53, df = 130, $p < 0.05$) and higher in nosophobic patients (M = 69.75, SD = 14.08, $t = -1.99$, $p < 0.05$).

3.3. Relationship of the PTG Level with DS-IT and COMPASS

The network of PTGI with DS-IT and ESAS is reported graphically in Figure 1, depicting the connection between physical and phycological symptoms [49].

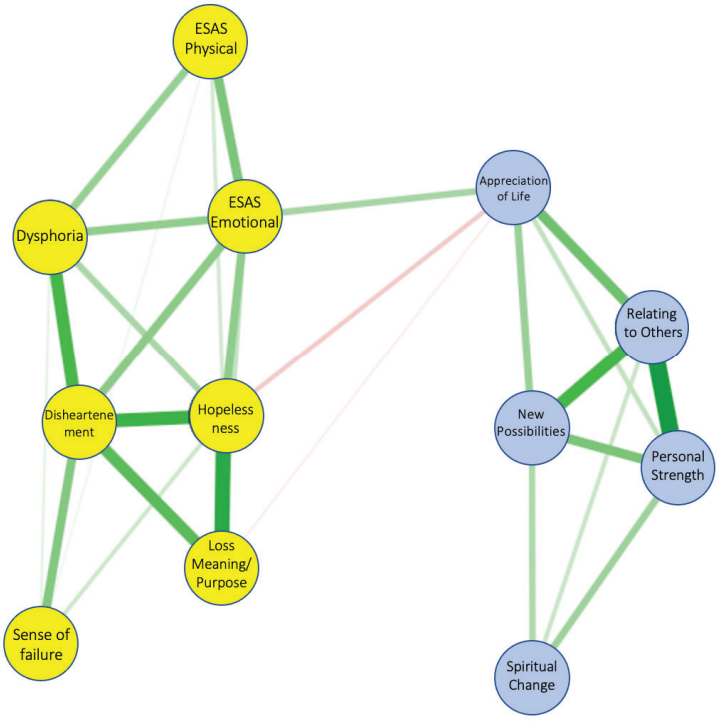


Figure 1. The network represents the relationships of PTGI factors with ESAS symptoms and DS-IT dimensions. Lines between symptoms (edges) are colored in green when they represent positive correlations and in red when they represent negative correlations. The magnitude of color represents the degree of the relationship between symptoms. ESAS: Edmonton Symptom Assessment System; ESAS-PHYS: physical distress sub-score; ESAS-Emotional: psychological distress sub-score.

In a first analysis, practical, social/family and informational problems from the CPC were included (emotional and physical problems were considered redundant in the model), but resulted in no connections and thus were not included in a second analysis. The most central items in the network were DS-IT Dishartenment, DS-IT Hopelessness and PTGI Relating to Others. The strength of each node as a measure of centrality is shown in Figure 2.

The values of edge weights in the network are reported in Table 2. After 1000 bootstrap procedures, the EGA revealed the presence of two communities in the network in 97.5% of the bootstrap iterations (95% CI 1.69–2.3).

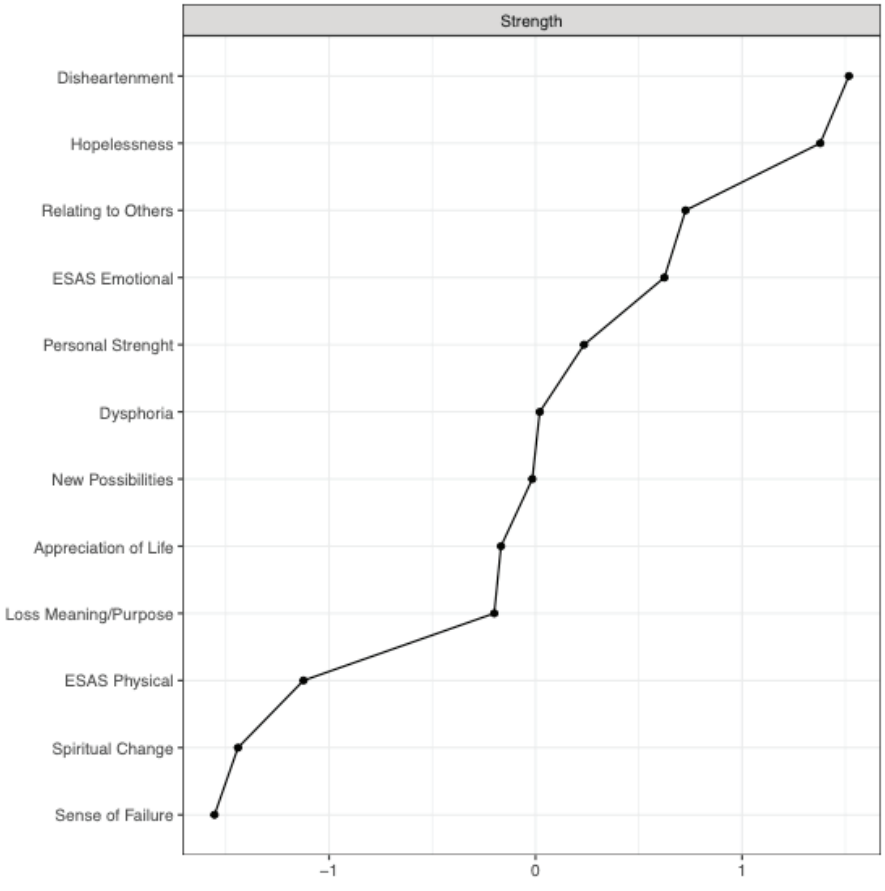


Figure 2. The centrality measure: the raw values of node strength are represented on horizontal axis, while the physical and phycological symptoms are represented on vertical axis.

Table 2. Edge weights, reflecting the connection strength of PTGI factors with ESAS Scores and DS-IT dimensions, in the network.

	ESAS-PHYS	ESAS-Emotional	Relating to Others *	New Possibilities *	Personal Strength *	Spiritual Change *	Appreciation of Life *	Loss of Meaning and Purpose **	Dysphoria **	Disheartenment **	Hopelessness **	Sense of Failure **
ESAS-PHYS		0.21							0.16		0.07	0.03
ESAS-Emotional	0.21						0.14	0.09	0.17	0.18	0.17	
Relating to Others *				0.29	0.40	0.08	0.22					
New Possibilities *			0.29		0.21	0.13	0.16					

Table 2. *Cont.*

	ESAS-PHYS	ESAS-Emotional	Relating to Others *	New Possibilities *	Personal Strength *	Spiritual Change *	Appreciation of Life *	Loss of Meaning and Purpose **	Dysphoria **	Disheartenment **	Hopelessness **	Sense of Failure **
Personal Strength *			0.40	0.21		0.15	0.09					
Spiritual Change *			0.08	0.13	0.15		0.01					
Appreciation of Life *		0.14	0.22	0.16	0.09	0.01		−0.03			−0.08	
Loss of Meaning and Purpose **		0.09					−0.03			0.26	0.34	
Dysphoria **	0.16	0.17								0.28	0.13	0.04
Disheartenment **		0.18						0.26	0.28		0.30	0.19
Hopelessness **	0.07	0.17					−0.08	0.34	0.13	0.30		0.08
Sense of Failure **	0.03								0.04	0.19	0.08	

* PTGI factors; ** DS-IT dimensions. The connections are highlighted in dark grey (strong) and light grey (moderate) for ease of visual comparison. ESAS: Edmonton Symptom Assessment System; ESAS-PHYS: physical distress sub-score; ESAS-EMOTIONAL: psychological distress sub-score; PTGI: Post Traumatic Growth Inventory.

4. Discussion

In this study, we report the level of Post Traumatic Growth by assessing PTG Inventory among KTRs and characterized, for the first time, the relationship of PTG with DS-IT, physical/emotional symptoms or problems via network analysis among patients who were submitted to kidney transplantation. Additionally, the effect of psychiatric and psychosocial variables on the development of PTG was identified.

A first result indicates a mean PTGI score of 52.81 (SD = 19.81). These findings are in line with the reported mean value of PTGI total score of a 3-year longitudinal study performed in 53 KTRs [50], supporting the evidence that posttraumatic growth often occur in patients after kidney transplant.

A plausible explanation could be due to the fact that KTRs are directly exposed to traumatic events, such as the transplant surgery, the relapse of previous kidney disease, the acute rejection, the infections and the side effects of immunosuppressive drugs, which can mobilize positive energy after kidney transplant. Besides, PTG might be also induced by the activation of other intrapersonal resources as consequence of a sense of gratitude towards the deceased donor and the medical team. Indeed, kidney transplantation is considered the beginning of a new life by KTRs, after a period of physical suffering for dialysis dependence and fear of remaining on dialysis for all life.

In depth, when investigating the different factors of PTGI, our data show PTGI-Relating to others sub-scale had the highest score among different dimensions of PTGI, putting in evidence another relevant aspect, the relationships of KTRs with their family. A strong familiar support could become crucial to overcome the extreme stress of transplant event, especially in presence of kidney living donor. These results are consistent with a recent study in which higher PTGI score in recipients from a living donor (M = 74, SD = 16) compared to from a cadaveric donor (M = 65, SD = 21) were reported, highlighting the influence of family members to enhance PTG in the post-transplant period [25]. On the other hand, we do not find correlation between the extent of post traumatic growth and months since transplantation. This finding seems to indicate that PTG is a phenomenon relatively stable overtime, similarly to other studies in patients with liver transplant [28], breast cancer [51] and colon-rectal cancer [52].

In relation to the network developed, the results reveal the presence of two distinct communities. More specifically, ESAS physical/emotional symptoms with DS-IT dimensions and PTGI factors were the first and second cluster, respectively. The symptom associations are not equally strong in the network and the edges between symptoms within each community were higher than edges between communities. DS-IT Appreciation of life, DS-IT Hopelessness and ESAS-Emotional were the three symptoms which moderately connected the two communities. These findings seem to indicate that KTRs with high burden of physiological symptoms, including distress, in the presence of low levels of hopelessness, are able to develop a higher level of appreciation of life after the kidney transplant. However, the relationship of distress with PTG is still debated in literature. Although many studies supported an inverse correlation between distress and PTG, the presence of distress symptoms could enhance or reduce the changes in the personality-related domains on the base of the time elapsed since traumatic event and the type of event (acute vs. chronic disease) [53].

Furthermore, our data seem to suggest that interventions aimed to promote appreciation of life could increase PTGI and decrease demoralization: interventions with a focus on valued-living are gaining importance, as the two dimensions might have possible common underlying processes; also, valued living has been shown to be correlated with PTGI-Appreciation of Life [54]. Other approaches might include those derived from positive psychology, like well-being therapy, which has been shown to increase PTGI in traumatized patients [55].

Other intriguing results emerged when the relationship of PTGI with ICD-10 was assessed. Paradoxically, the diagnosis of ICD-10 anxiety was positively associated with high PTGI-Personal Strength score. This result might be interpreted by the fact that higher self-awareness to handle difficulties is induced by anxiety. This cognitive state could be considered as a positive response of patients to the new organ's integration [56]. However, this finding is in contrast with a previous report in which a persistent state of anxiety, due to the partial psychological integration of kidney, contributed to reduce the level of PTG. In addition, lower PTGI score was a significant predictor of graft rejection episodes at 3 years in cadaveric kidney transplant recipients (Odds Ratio = 0.963, 95% CI = 0.929–0.999, $p < 0.05$). In other words, the higher the psychological growing after KT, the lower rates of graft rejection [49].

On the other hand, it is worth noting that the presence of DCPR alexithymia, shown in about one-quarter of KTRs and in almost two-third of KTRs with another DCPR diagnosis [6], was a strong predictor of low PTGI score. Alexithymia is characterized by the inability to recognize and express own emotions [57]. This lack of symbolizing the feelings, a defense mechanism against a traumatic event as kidney transplant [58], could play a role in reducing the development of positive changes after KT. These data are consistent with the only other study, recently published, in which the levels of alexithymia, assessed by using TAS20 questionnaire [59], were negatively and moderately associated with PTGI total score and sub-scores, such as changes in self-perception and appreciation of life, in cadaveric donor recipients [50].

The strength of this study is that it evaluated, in KTRs, the level of the post-traumatic growth level as well as its correlations with DS-IT and emotional symptoms, using PTGI and network analysis, respectively. This research helps to promote the use of PTGI after the experience of kidney transplantation, as proved in heart, lung and liver transplantation [60–62]. Furthermore, the network analysis, a consolidated approach in psychopathology, represents an innovative technique in the kidney transplant research.

The limitations of our study are: (1) the small sample size of our population that does not allow us to generalize our results and (2) the lack of control group of patients with other kidney conditions. Further multicenter studies on larges cohort of KTRs could be conducted using a short form of PTGI [8,63,64], after its validation in the kidney transplant setting. Furthermore, (3) in our cohort the main cause of CKD was quite different from other investigated populations, however this variable seems not to be correlated with

PTG score [24,25]. Another limit (4) is the use of the old version of DCPR-SI, due to the absence of the new version (DCRP-R) when the study started. In addition, although the administration of vitamin D is associated with an improvement of mental health [65,66], (5) we did not evaluate the effect of vitamin D on PTG. Additionally, (6) we did not consider the intensity of physical activity, a rising variable which can modify psychosocial and physical risks factors [67–70].

5. Conclusions

This study showed a moderate level of PTG in kidney transplant recipients, suggesting that health professionals responsible for treatment of KTRs should be sensitive to promote the awareness of these complex psychological changes after kidney transplant in their patients.

Furthermore, the network analysis identified two communities, ESAS physical/emotional symptoms with DS-IT dimensions and PTGI factors, which were more strongly connected within-cluster than between-clusters. This result suggests that a psychological intervention could be an appropriate means of addressing psychological distress, reducing hopelessness and improving the appreciation of life, among kidney transplant recipients. However, the connection between these three relevant dimensions, should be considered for further research to better define this relationship and its consequence in term of outcomes in KTRs.

Author Contributions: Conceptualization Y.B.; drafting of the manuscript Y.B. and L.Z.; formal analysis Y.B. and L.Z.; methodology M.P. and M.B.M.; supervision, P.E., M.A. and A.S.; validation, L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Hospital Ethics Committee for Human Research (Code: 151297, on 17 March 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abecassis, M.; Bartlett, S.T.; Collins, A.J.; Davis, C.L.; Delmonico, F.L.; Friedewald, J.; Hays, R.; Howard, A.; Jones, E.; Leichtman, A.B.; et al. Kidney transplantation as primary therapy for end-stage renal disease: A National Kidney Foundation/Kidney Disease Outcomes Quality Initiative (NKF/KDOQITM) conference. *Clin. J. Am. Soc. Nephrol.* **2008**, *3*, 471–480. [CrossRef] [PubMed]
2. Müller, H.H.; Englbrecht, M.; Wiesener, M.S.; Titze, S.; Heller, K.; Groemer, T.W.; Schett, G.; Eckardt, K.U.; Kornhuber, J.; Maler, J.M. Depression, Anxiety, Resilience and Coping Pre and Post Kidney Transplantation—Initial Findings from the Psychiatric Impairments in Kidney Transplantation (PI-KT)-Study. *PLoS ONE* **2015**, *10*, e0140706. [CrossRef]
3. Chilcot, J.; Spencer, B.W.J.; Maple, H.; Mamode, N. Depression and Kidney Transplantation. *Transplantation* **2014**, *97*, 717–721. [CrossRef] [PubMed]
4. Fukunishi, I.; Sugawara, Y.; Takayama, T.; Makuuchi, M.; Kawarasaki, H.; Surman, O.S. Psychiatric disorders before and after living-related transplantation. *Psychosomatics* **2001**, *42*, 337–343. [CrossRef] [PubMed]
5. Noohi, S.; Khaghani-Zadeh, M.; Javadipour, M.; Assari, S.; Najafi, M.; Ebrahiminia, M.; Pourfarziani, V. Anxiety and Depression Are Correlated With Higher Morbidity after Kidney Transplantation. *Transplant. Proc.* **2007**, *39*, 1074–1078. [CrossRef] [PubMed]
6. Battaglia, Y.; Martino, E.; Piazza, G.; Cojocaru, E.; Massarenti, S.; Peron, L.; Storari, A.; Grassi, L. Abnormal Illness Behavior, Alexithymia, Demoralization, and Other Clinically Relevant Psychosocial Syndromes in Kidney Transplant Recipients: A Comparative Study of the Diagnostic Criteria for Psychosomatic Research System versus ICD-10 Psychiatric Nosology. *Psychother. Psychosom.* **2018**, *87*, 375–376.
7. Murri, M.B.; Caruso, R.; Ounalli, H.; Zerbinati, L.; Berretti, E.; Costa, S.; Recla, E.; Folesani, F.; Kissane, D.; Nanni, M.G.; et al. The relationship between demoralization and depressive symptoms among patients from the general hospital: Network and exploratory graph analysis. *J. Affect. Disord.* **2020**, *276*, 137–146. [CrossRef]

8. Belvederi Murri, M.; Zerbinati, L.; Ounalli, H.; Kissane, D.; Casoni, B.; Leoni, M.; Rossi, G.; Dall'Olio, R.; Caruso, R.; Nanni, M.G.; et al. Assessing demoralization in medically ill patients: Factor structure of the Italian version of the demoralization scale and development of short versions with the item response theory framework. *J. Psychosom. Res.* **2020**, *128*, 109889. [CrossRef]
9. Battaglia, Y.; Zerbinati, L.; Piazza, G.; Martino, E.; Massarenti, S.; Provenzano, M.; Esposito, P.; Andreucci, M.; Storari, A.; Grassi, L. The Use of Demoralization Scale in Italian Kidney Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 2119. [CrossRef]
10. Kissane, D.W.; Clarke, D.M.; Street, A.F. Demoralization syndrome: A relevant psychiatric diagnosis for palliative care. *J. Palliat. Care* **2001**, *17*, 12–21. [CrossRef]
11. Tedeschi, R.G.; Calhoun, L.G. Clinical approach to growth after traumatic experiences. In *Positive Psychology in Practice*; Linley, P.A., Joseph, S., Eds.; Polish Scientific Publishers PWN: Warszawa, Poland, 2007; Volume 25, pp. 230–248.
12. Schubert, C.F.; Schmidt, U.; Rosner, R. Posttraumatic Growth in Populations with Posttraumatic Stress Disorder-A Systematic Review on Growth-Related Psychological Constructs and Biological Variables. *Clin. Psychol. Psychother.* **2016**, *23*, 469–486. [CrossRef]
13. Pollard, C.; Kennedy, P. A longitudinal analysis of emotional impact, coping strategies and post-traumatic psychological growth following spinal cord injury: A 10-year review. *Br. J. Health Psychol.* **2007**, *12 Pt 3*, 347–362. [CrossRef]
14. Gori, A.; Topino, E.; Sette, A.; Cramer, H. Pathways to post-traumatic growth in cancer patients: Moderated mediation and single mediation analyses with resilience, personality, and coping strategies. *J. Affect. Disord.* **2021**, *279*, 692–700. [CrossRef] [PubMed]
15. Kelly, G.; Morris, R.; Shetty, H. Predictors of post-traumatic growth in stroke survivors. *Disabil. Rehabil.* **2018**, *40*, 2916–2924. [CrossRef] [PubMed]
16. Dyer, G.; Gilroy, N.; Brice, L.; Kabir, M.; Gottlieb, D.; Huang, G.; Hogg, M.; Brown, L.; Greenwood, M.; Larsen, S.R.; et al. A survey of infectious diseases and vaccination uptake in long-term hematopoietic stem cell transplant survivors in Australia. *Transpl. Infect. Dis.* **2019**, *21*, e13043. [CrossRef]
17. Martín-Rodríguez, A.; Pérez-San-Gregorio, M.Á.; Avargues-Navarro, M.L.; Borda-Mas, M.; Pérez-Bernal, J.; Gómez-Bravo, M.Á. How Thinking About the Donor Influences Post-traumatic Growth in Liver Transplant Recipients. *Transplant. Proc.* **2018**, *50*, 610–612. [CrossRef] [PubMed]
18. de Alegría, B.R.; Basabe, N.; De Lorenzo, E. Evolution of post-traumatic growth during the first 12 months of dialysis: A longitudinal study. *J. Ren. Care* **2017**, *43*, 108–113. [CrossRef]
19. Cramer, A.; Waldorp, L.; Van der Maas, H.; Borsboom, D. Comorbidity: A network perspective. *Behav. Brain Sci.* **2010**, *33*, 137–150. [CrossRef]
20. Fried, E.I. Problematic assumptions have slowed down depression research: Why symptoms, not syndromes are the way forward. *Front. Psychol.* **2015**, *6*, 309. [CrossRef]
21. Contreras, A.; Nieto, I.; Valiente, C.; Espinosa, R.; Vazquez, C. The Study of Psychopathology from the Network Analysis Perspective: A Systematic Review. *Psychother. Psychosom.* **2019**, *88*, 71–83. [CrossRef]
22. Borsboom, D. A network theory of mental disorders. *World Psychiatry* **2017**, *16*, 5–13. [CrossRef]
23. Golino, H.F.; Epskamp, S. Exploratory Graph Analysis: A new approach for estimating the number of dimensions in psychological research. *PLoS ONE* **2017**, *12*, e0174035. [CrossRef] [PubMed]
24. Yucetin, L.; Bozoklar, C.A.; Yanik, O.; Tekin, S.; Tuncer, M.; Demirbas, A. An Investigation of Post-Traumatic Growth Experiences Among Living Kidney Donors. *Transplant. Proc.* **2015**, *47*, 1287–1290. [CrossRef] [PubMed]
25. Tomaszek, A.; Wróblewska, A.; Zdankiewicz-Ścigała, E.; Rzońca, P.; Gałązkowski, R.; Gozdowska, J.; Lewandowska, D.; Kosson, D.; Kosieradzki, M.; Danielewicz, R. Post-Traumatic Growth among Patients after Living and Cadaveric Donor Kidney Transplantation: The Role of Resilience and Alexithymia. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2164. [CrossRef] [PubMed]
26. Howell, D.; Keshavarz, H.; Esplen, M.J.; Hack, T.; Hamel, M.; Howes, J.; Jones, J.; Li, M.; Manii, D.; McLeod, D.; et al. A Pan Canadian Practice Guideline: Screening, Assessment and Care of Psychosocial Distress, Depression, and Anxiety in Adults with Cancer. In *The Canadian Association of Psychosocial Oncology (CAPO)*, 2nd ed.; Canadian Association of Psychosocial Oncology: Toronto, ON, Canada, 2015. Available online: https://www.capo.ca/resources/Documents/Guidelines/3APAN-~1.PDF (accessed on 1 May 2016).
27. Prati, G.; Pietrantoni, L. Italian Adaptation and Confirmatory Factor Analysis of the Full and the Short Form of the Posttraumatic Growth Inventory. *J. Loss Trauma* **2014**, *19*, 12–22. [CrossRef]
28. Pérez-San-Gregorio, M.Á.; Martín-Rodríguez, A.; Borda-Mas, M.; Avargues-Navarro, M.L.; Pérez-Bernal, J.; Conrad, R.; Gómez-Bravo, M.Á. Post-traumatic growth and its relationship to quality of life up to 9 years after liver transplantation: A cross-sectional study in Spain. *BMJ Open* **2017**, *7*, e017455. [CrossRef]
29. Faravelli, C.; Abrardi, L.; Bartolozzi, D.; Cecchi, C.; Iacono, B.L.; Ravaldi, C.; Scarpato, M.A.; Truglia, E.; Prodi, P.M.R.; Rosi, S.; et al. The Sesto Fiorentino study: Point and one-year prevalences of psychiatric disorders in an Italian community sample using clinical interviewers. *Psychother. Psychosom.* **2004**, *73*, 226–234. [CrossRef]
30. Rossi, A.; Alberio, R.; Porta, A.; Sandri, M.; Tansella, M.; Amaddeo, F. The reliability of the Mini-International Neuropsychiatric Interview–Italian version. *J. Clin. Psychopharmacol.* **2004**, *24*, 561–563. [CrossRef]
31. Porcelli, P.; Rafanelli, C. Criteria for Psychosomatic Research (DCPR) in the Medical Setting. *Curr. Psychiatry Rep.* **2010**, *12*, 246–254. [CrossRef]
32. Grassi, L.; Sabato, S.; Rossi, E.; Biancosino, B.; Marmai, L. The use of the Diagnostic Criteria for Psychosomatic Research (DCPR) in oncology. *Psychother. Psychosom.* **2005**, *74*, 100–107. [CrossRef]

33. Battaglia, Y.; Zerbinati, L.; Martino, E.; Piazza, G.; Massarenti, S.; Storari, A.; Grassi, L. Psychosocial Dimensions in Hemodialysis Patients on Kidney Transplant Waiting List: Preliminary Data. *Transplantology* **2020**, *1*, 123–134. [CrossRef]
34. Bruera, E.; Kuehn, N.; Miller, M.J.; Selmser, P.; Macmillan, K. The Edmonton Symptom Assessment System (ESAS): A Simple Method for the Assessment of Palliative Care Patients. *J. Palliat. Care* **1991**, *7*, 6–9. [CrossRef] [PubMed]
35. Davison, S.N.; Jhangri, G.S.; Johnson, J.A. Longitudinal validation of a modified Edmonton symptom assessment system (ESAS) in haemodialysis patients. *Nephrol. Dial. Transplant.* **2006**, *21*, 3189–3195. [CrossRef] [PubMed]
36. Ma, X.; Zhang, J.; Zhong, W.; Shu, C.; Wang, F.; Wen, J.; Zhou, M.; Sang, Y.; Jiang, Y.; Liu, L. The diagnostic role of a short screening tool—The distress thermometer: A meta-analysis. Support. *Care Cancer* **2014**, *22*, 1741–1755. [CrossRef] [PubMed]
37. Donovan, K.A.; Grassi, L.; McGinty, H.L.; Jacobsen, P.B. Validation of the distress thermometer worldwide: State of the science. *Psychooncology* **2014**, *23*, 241–250. [CrossRef]
38. Moro, C.; Brunelli, C.; Miccinesi, G.; Fallai, M.; Morino, P.; Piazza, M.; Labianca, R.; Ripamonti, C. Edmonton Symptom Assessment Scale (ESAS): Italian validation in two palliative care settings. *Support. Care Cancer* **2006**, *14*, 30–37. [CrossRef]
39. Battaglia, Y.; Zerbinati, L.; Piazza, G.; Martino, E.; Provenzano, M.; Esposito, P.; Massarenti, S.; Andreucci, M.; Storari, A.; Grassi, L. Screening Performance of Edmonton Symptom Assessment System in Kidney Transplant Recipients. *J. Clin. Med.* **2020**, *9*, 995. [CrossRef]
40. Lucas, A.R.; Bass, M.B.; Rothrock, N.E.; O'Connor, M.L.; Sorkin, M.R.; Nawyn, J.; Albinali, F.; Wagner, L.I. Development of an eHealth System to Capture and Analyze Patient Sensor and Self-Report Data: Mixed-Methods Assessment of Potential Applications to Improve Cancer Care Delivery. *JMIR Med. Inform.* **2018**, *6*, e46. [CrossRef]
41. Grassi, L.; Costantini, A.; Kissane, D.; Brunetti, S.; Caruso, R.; Piazza, G.; Marchetti, P.; Sabato, S.; Nanni, M.G. The factor structure and use of the Demoralization Scale (DS-IT) in Italian cancer patients. *Psychooncology* **2017**, *26*, 1965–1971. [CrossRef]
42. Lancichinetti, A.; Kivelä, M.; Saramäki, J.; Fortunato, S. Characterizing the community structure of complex networks. *PLoS ONE* **2010**, *5*, e11976. [CrossRef]
43. Friedman, J.; Hastie, T.; Tibshirani, R. Sparse inverse covariance estimation with the graphical lasso. *Biostatistics* **2008**, *9*, 432–441. [CrossRef]
44. Lauritzen, S. *Graphical Models*; Oxford University Press: Oxford, UK, 1996.
45. Epskamp, S.; Cramer, A.O.; Waldorp, L.J.; Schmittmann, V.D.; Borsboom, D. qgraph: Network visualizations of relationships in psychometric data. *J. Stat. Softw.* **2012**, *48*, 1–18. [CrossRef]
46. Pons, P.; Latapy, M. Computing communities in large networks using random walks. *J. Graph Algorithms Appl.* **2006**, *10*, 191. [CrossRef]
47. Epskamp, S.; Fried, E.I. A tutorial on regularized partial correlation networks. *Psychol. Methods.* **2018**, *23*, 617–634. [CrossRef] [PubMed]
48. Christensen, A.P.; Gross, G.M.; Golino, H.F.; Silvia, P.J.; Kwapil, T.R. Exploratory Graph Analysis of the Multidimensional Schizotypy Scale. *Schizophr. Res.* **2019**, *206*, 43–51. [CrossRef] [PubMed]
49. Fonseca-Pedrero, E. Network analysis: A new way of understanding psychopathology? *Rev. Psiquiatr. Salud. Ment.* **2017**, *10*, 206. (In English); (In Spanish) [CrossRef] [PubMed]
50. Látos, M.; Devecsery, A.; Lázár, G.; Horváth, Z.; Szederkényi, E.; Szenohradszky, P.; Csabai, M. The role of body image integrity and posttraumatic growth in kidney transplantation: A 3-year longitudinal study. *Health Psychol. Open* **2015**, *2*, 2055102915581214. [CrossRef]
51. Esparza, T.; Martínez, T.; Leibovich de Figueroa, N. Longitudinal study of posttraumatic growth and quality of life in women's breast cancer survivors. *Psicooncología* **2015**, *12*, 303–314.
52. Occhipinti, S.; Chambers, S.K.; Lepore, S.; Aitken, J.; Dunn, J. A longitudinal study of post-traumatic growth and psychological distress in colorectal cancer survivor. *PLoS ONE* **2015**, *10*, e0139119. [CrossRef]
53. Barskova, T.; Oesterreich, R. Post-traumatic growth in people living with a serious medical condition and its relations to physical and mental health: A systematic review. *Disabil Rehabil.* **2009**, *31*, 1709–1733. [CrossRef]
54. Baseotto, M.C.; Morris, P.G.; Gillespie, D.C.; Trevethan, C.T. Post-traumatic growth and value-directed living after acquired brain injury. *Neuropsychol. Rehabil.* **2020**, *27*, 1–20. [CrossRef] [PubMed]
55. Radstaak, M.; Hüning, L.; Bohlmeijer, E.T. Well-Being Therapy as Rehabilitation Therapy for Posttraumatic Stress Disorder Symptoms: A Randomized Controlled Trial. *J. Trauma. Stress* **2020**, *33*, 813–823. [CrossRef] [PubMed]
56. Joralemon, D. Organ wars: The battle for body parts. *Med. Anthropol. Q.* **1995**, *9*, 335–356. [CrossRef] [PubMed]
57. Helmes, E.; McNeill, P.D.; Holden, R.R.; Jackson, C. The construct of alexithymia: Associations with defense mechanisms. *J. Clin. Psychol.* **2008**, *64*, 318–331. [CrossRef]
58. Lumley, M.A.; Neely, L.C.; Burger, A.J. The assessment of alexithymia in medical settings: Implications for understanding and treating health problems. *J. Pers. Assess.* **2007**, *89*, 230–246. [CrossRef]
59. Bagby, R.M.; Parker, J.D.; Taylor, G.J. The twenty-item Toronto Alexithymia Scale-I. Item selection and cross-validation of the factor structure. *J. Psychosom. Res.* **1994**, *38*, 23–32. [CrossRef]
60. Anand-Kumar, V.; Kung, M.; Painter, L.; Broadbent, E. Impact of organ transplantation in heart, lung and liver recipients: Assessment of positive life changes. *Psychol. Health* **2014**, *29*, 687–697. [CrossRef] [PubMed]

61. Goetzmann, L.; Lieberherr, M.; Krombholz, L.; Ambühl, P.; Boehler, A.; Noll, G.; Muellhaupt, B.; Wagner, R.; Buddeberg, C.; Klaghofer, R. Subjective experiences following organ transplantation—A qualitative study of 120 heart, lung, liver, and kidney recipients. *Z. Psychosom. Med. Psychother.* **2010**, *56*, 268–282.
62. Goetzmann, L.; Ruegg, L.; Stamm, M.; Ambühl, P.; Boehler, A.; Halter, J.; Muellhaupt, B.; Noll, G.; Schanz, U.; Wagner-Huber, R.; et al. Psychosocial profiles after transplantation: A 24-month follow-up of heart, lung, liver, kidney and allogeneic bone-marrow patients. *Transplantation* **2008**, *86*, 662–668. [CrossRef] [PubMed]
63. Cann, A.; Calhoun, L.G.; Tedeschi, R.G.; Taku, K.; Vishnevsky, T.; Triplett, K.N.; Danhauer, S.C. A short form of the Posttraumatic Growth Inventory. *Anxiety Stress Coping* **2010**, *23*, 127–137. [CrossRef]
64. Kaur, N.; Porter, B.; LeardMann, C.A.; Tobin, L.E.; Lemus, H.; Luxton, D.D. Millennium Cohort Study Team. Evaluation of a modified version of the Posttraumatic Growth Inventory-Short Form. *BMC Med. Res. Methodol.* **2017**, *17*, 69. [CrossRef] [PubMed]
65. Battaglia, Y.; Cojocaru, E.; Fiorini, F.; Granata, A.; Esposito, P.; Russo, L.; Bortoluzzi, A.; Storari, A.; Russo, D. Vitamin D in kidney transplant recipients. *Clin. Nephrol.* **2020**, *93*, 57–64. [CrossRef] [PubMed]
66. Jamilian, H.; Amirani, E.; Milajerdi, A.; Kolahdooz, F.; Mirzaei, H.; Zaroudi, M.; Ghaderi, A.; Asemi, Z. The effects of vitamin D supplementation on mental health, and biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: A systematic review and meta-analysis of randomized controlled trials. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2019**, *94*, 109651. [CrossRef]
67. Aucella, F.; Battaglia, Y.; Bellizzi, V.; Bolignano, D.; Capitanini, A.; Cupisti, A. Physical exercise programs in CKD: Lights, shades and perspectives. *J. Nephrol.* **2015**, *28*, 143–150. [CrossRef] [PubMed]
68. Belvederi Murri, M.; Folesani, F.; Zerbinati, L.; Nanni, M.G.; Ounalli, H.; Caruso, R.; Grassi, L. Physical Activity Promotes Health and Reduces Cardiovascular Mortality in Depressed Populations: A Literature Overview. *Int. J. Environ. Res. Public Health* **2020**, *17*, 5545. [CrossRef] [PubMed]
69. Morgado, F.F.D.R.; do Vale, W.S.; Lopes, C.S.; Maranhão Neto, G.A.; Lattari, E.; Mediano, M.F.F.; Rostila, M.; Griep, R.H.; Machado, S.; Penna, T.A.; et al. Psychosocial determinants of physical activity among workers: An integrative review. *Rev. Bras. Med. Trab.* **2021**, *18*, 472–487. [CrossRef]
70. Aucella, F.; Gesuete, A.; Battaglia, Y. A "Nephrological" Approach to Physical Activity. *Kidney Blood Press. Res.* **2014**, *39*, 189–196. [CrossRef]

Journal of
Clinical Medicine

MDPI

Review

Antibody-Mediated Rejection and Recurrent Primary Disease: Two Main Obstacles in Abdominal Kidney, Liver, and Pancreas Transplants

Tsukasa Nakamura [1,*] and Takayuki Shirouzu [2]

[1] Department of Organ Transplantation and General Surgery, Kyoto Prefectural University of Medicine, Kajii-cho 465, Kamigyo-ku, Kyoto 602-8566, Japan
[2] Molecular Diagnositcs Division, Wakunaga Pharmaceutical Co., Ltd., 13-4 Arakicho, shinjyuku-ku, Tokyo 160-0007, Japan; shirouzu_t@wakunaga.co.jp
* Correspondence: tsukasa@koto.kpu-m.ac.jp; Tel.: +81-752-515-532

Abstract: The advances in acute phase care have firmly established the practice of organ transplantation in the last several decades. Then, the next issues that loom large in the field of transplantation include antibody-mediated rejection (ABMR) and recurrent primary disease. Acute ABMR is a daunting hurdle in the performance of organ transplantation. The recent progress in desensitization and preoperative monitoring of donor-specific antibodies enables us to increase positive outcomes. However, chronic active ABMR is one of the most significant problems we currently face. On the other hand, recurrent primary disease is problematic for many recipients. Notably, some recipients, unfortunately, lost their vital organs due to this recurrence. Although some progress has been achieved in these two areas, many other factors remain largely obscure. In this review, these two topics will be discussed in light of recent discoveries.

Keywords: antibody-mediated rejection; recurrent primary disease; renal transplantation; liver transplantation; pancreas transplantation

Citation: Nakamura, T.; Shirouzu, T. Antibody-Mediated Rejection and Recurrent Primary Disease: Two Main Obstacles in Abdominal Kidney, Liver, and Pancreas Transplants. *J. Clin. Med.* **2021**, *10*, 5417. https://doi.org/10.3390/jcm10225417

Academic Editor: Charat Thongprayoon

Received: 10 October 2021
Accepted: 18 November 2021
Published: 19 November 2021

1. Introduction

From the late 20th century to the beginning of the 21st century, significant progress has been achieved in acute phase care for transplant patients. These advances firmly place organ transplantation into firmly established therapeutic procedures for organ failure patients. However, the better outcomes in the acute phase become, the more other issues are exposed. Firstly, donor specific anti-human leukocyte antigen (HLA) antibodies (DSA), resulting in chronic antibody-mediated rejection (ABMR), are recognized as major obstacles that we have yet to conquer. Organ transplantation is haunted by DSA unless we change graft sources or develop a new technology. Secondly, controlling the recurrence of the primary disease will continue to be a major issue in many diseases as long as we continue to use live organs and not machines.

The primary purpose of this review is to deepen the understanding of these two issues and to improve graft survival and patient survival after the acute phase of transplants.

2. Methods

We have written this review by focusing on two major issues: ABMR due to de novo DSA (dnDSA) and recurrence of primary disease. In preparing this review, English-language abstracts cited in PubMed were selected. Citations were chosen based on their relevance to each section. In Section 3, articles related with dnDSA, not preforming DSA, were selected. As recent dnDSA studies in kidney transplantation are summarized in a table, the most recent randomized trials or prospective cohort studies using representative drugs were selected as much as possible. Since the number of related studies is scarce in

liver and pancreas transplants, we selected studies that included therapeutic approaches regardless of study design. In Section 4, we sought studies of a relatively large scale in order to show reliable recurrence data and also basic mechanisms as to why the primary disease recurs, if available.

3. An Overview of Antibody-Mediated Rejection

Rejection after organ transplantation is roughly divided into cell-mediated and antibody-related (humoral) immune mechanisms. Originally, T cell-mediated rejections (TCMRs) and ABMR against the ABO blood group were recognized as major barriers. In the 1970s, the introduction of cyclosporin, followed by tacrolimus, mycophenolate mofetil, anti-CD25 antibodies (Abs), and thymoglobulin, etc., dramatically reduced the incidence of severe acute TCMR. Furthermore, plasmapheresis and anti-CD20 Abs, which are recognized as desensitization, also brought better outcomes in ABO incompatible organ transplantation. Conversely, the issues regarding DSA are still disputable and seem not to be reasonably addressed. Therefore, discussions regarding ABMR and DSA are more frequently observed recently. This possibly reflects that the direction of researcher's interests is shifting to ABMR due to DSA, especially for chronic types as our interests shift to long-term outcomes [1]. Nevertheless, it is also true that close correlations between TCMR and the onset of DSA inducing ABMR are becoming apparent. Thus, the effects of TCMR are being reassessed from perspective of long-range consequences. Therefore, the discussion only between DSA and ABMR seems to be simplistic and could be enriched by a broader view, which adds TCMR. In this section, we will review chronic ABMR, TCMR, and related topics in kidney, liver, and pancreas transplants.

3.1. Kidney Transplantation

Kidney transplantation was the first successful organ transplantation and is the most frequently performed. Therefore, a large amount of knowledge about rejection has been gained, and this has improved outcomes so far in this field. However, ABMR due to DSA remains a major barrier to achieving a good prognosis in kidney transplants. In recent years, the impact of preformed DSA on ABMR has become smaller, but the impact of newly produced DSA (de novo DSA (dnDSA)) on ABMR is still significant. dnDSA could be detected at the stage of performing a biopsy by observing mild renal dysfunction or at the stage of protocol biopsy performed for an asymptomatic recipient. Patients who produced dnDSA have been reported to demonstrate worse graft survival rates than recipients without dnDSA [1].

Given the fact that the consequences of dnDSA production are significant, it is reasonable to believe that prevention and early detection are crucial for adequate management. Monitoring tests for anti-HLA Abs with an immobilized single allele of purified HLA are available for the detection of dnDSA [2]. Recently, it has been reported that the graft immunocomplex capture fluorescent analysis (ICFA) method using transplanted tissue pieces obtained by allograft biopsies is also effective for the early detection of DSA production [3]. Pathological diagnosis is widely used as a gold standard in diagnosing ABMR based on the Banff Classification, which was initially reported in 1993 with the aim of creating an internationally unified standard for kidney transplant pathology [4]. The Banff classification has been updated every two years and is a standard that reflects the current situation and helps clinicians in making diagnoses. In addition to introducing chronic active ABMR in 2001 [5], the concept of chronic TCMR was partially introduced in the 2005 Banff Classification update, and the 2017 Banff classification update recently introduced the features of chronic active TCMR. Chronic active TCMR can be considered as a major factor resulting in interstitial fibrosis and tubular atrophy (IFTA). IFTA is also associated with poor long-term outcomes. Moreover, considering inflammation in IFTA areas may supply better outcomes [6].

It must be admitted that the development of dnDSA is closely related to T cell activities. Activated B-lymphocytes linage cells start to produce dnDSA as a consequence of

the interaction between B cells and CD4 T cells through direct, indirect, and semidirect pathways [2]. Thus, TCMR theoretically appears to have an important role in subsequent ABMR, although limited evidence exists. In a recent prospective study, the reported incidence of TCMR in the first year after transplant was around twice as high as in recipients who developed dnDSA compared with patients without dnDSA. The presence of dnDSA was associated with the severity of TCMR and subsequent graft loss. Furthermore, patients with dnDSA, accompanied by more severe tubulointerstitial inflammation, were more prone to recurrent TCMR [7]. Another study demonstrated that recipients with dnDSA accompanied by a prior history of TCMR showed inferior graft outcomes [8]. Taken together, these studies suggest that dnDSA-related ABMR is preceded by T cell involvement and also that TCMR affects graft outcomes through ABMR.

The risks of dnDSA development may vary according to an individual's immunological background. The rates of dnDSA development have been reported as being around 10% at 12 months after transplant under standard immunosuppressants [9,10]. However, recipients with high immunological risks (highly sensitized patients with high-titer of preformed DSA) are more susceptible to this particular development than those with low-risk, although preformed DSA became undetectable at 12 months after surgery with appropriate preparations [11]. In terms of immunosuppression, it is true, theoretically, that the levels of calcineurin inhibitors (CNI), mTOR inhibitors [12], antimetabolites [13], steroid, and several other monoclonal and polyclonal antibodies are related to the occurrence of dnDSA. Among them, CNI is one of the main immunosuppressants used to control dnDSA. High tacrolimus variability (instability) may well result in dnDSA production in pediatric recipients [14]. It can be admitted that this study has highlighted the importance of medication adherence. Furthermore, complete withdrawal from CNI by using mTOR inhibitors seems to favor dnDSA development [12,15]. Regarding steroid administration, this still plays an important role in controlling ABMR due to dnDSA [16]. On the other hand, there was a study that examined the effects of steroid withdrawal at 7 days post-transplant, and no negative effects on dnDSA were observed [17]. However, the latter study applied thymoglobulin induction for all recipients, which might alter immunological reactions. Therefore, the CNI and steroid-free strategies could involve immunological risks, although these approaches are attractive from the persepctive of medication side effects. It can be argued that the minimization of CNI by using mTOR inhibitors does prevent CNI toxicity. However, mTOR inhibitors without CNI or complete withdrawal from steroid administration pose a higher risk of DSA for recipients with standard induction therapy.

A variety of therapeutic approaches have been implemented, owing to the recent development of synthetic antibodies, in addition to orthodox therapeutic modalities. Several institutions applied steroid pulse, plasmapheresis, and intravenous immunoglobulin therapy (IVIG), combined with anti-CD 20 Abs administration for dnDSA mediated ABMR [18]. Other than these conventional therapies, several monoclonal Abs including eculizumab (anti-C5), tocilizumab, and clazakizumab (anti-interleukin 6) have recently been introduced in this field, although a proteasome inhibitor (bortezomib) failed to show therapeutic effects [19]. A pilot study to investigate the role of complement by using eculizumab showed a slight stabilization of renal function due to a terminal complement inhibitor, albeit with an underpowered design (treatment group $n = 10$) [20]. Choi et al. reported 80% graft survival at 6-year post-introduction in tocilizumab-treated patients for whom IVIG and rituximab, with or without plasma exchange, did not succeed [21]. Several other investigations of tocilizumab and clazakizumab followed and showed a significant reduction in dnDSA [22] and a suppression of eGFR decline, respectively [23]. However, these studies indicate that a certain population of patients did not show the efficacy of these treatments. Thus, patient selection for dnDSA treatment would be another key issue in this field. On the other hand, belatacept-based immunosuppression clearly prevented the development of dnDSA, compared with an ordinal cyclosporin-based treatment regimen [24]. These results remind us of the importance of suppressing dnDSA development. Furthermore, in addition to the

introduction of novel medications, it is also pivotal to seek optimal regimens with existing therapeutic modalities for dnDSA mediated ABMR.

3.2. Liver Transplantation

Despite the significant effects on graft survival of ABO blood type-related ABMR, the role of dnDSA in liver transplant remains largely unknown. There are many studies that demonstrate serum dnDSA (s-DSA) and graft outcomes. These studies demonstrated that the existence of s-DSA is not necessarily related with poor outcomes [25], which is contrary to the facts in kidney transplant. In other words, it is not wise to assess ABMR of liver allografts only by s-DSA. In fact, clinically, the Banff working group advocates diagnostic criteria that consist of (1) pathological findings, (2) positivity of DSA, (3) C4d positivity, and (4) excluding other causative factors [26]. These studies, regarding dnDSA, possibly include s-DSA positive but intra-graft DSA (g-DSA) negative cases (i.e., the circumstance where allografts are free from damages due to s-DSA). It is important to distinguish these cases clinically, although limited availability of DSA examination hinders routine investigations of g-DSA. In fact, our recent study showed that g-DSA was closely associated with graft rejection and could be a more reliable indicator for the graft outcomes [27]. On the other hand, other research studies suggest that DSA clearance by Kupffer cells [28], release of HLA from liver in soluble form [29], allografts size [30], and liver regenerative abilities prevent harming liver allografts [31]. Thus, believing the information regarding s-DSA only without questioning may misconstrue the truth of what happens in liver allografts.

With the minimization of immunosuppressants, it is often observed that liver transplant recipients also developed dnDSA in serum, with the tendency of this belonging to class II rather than class I [25,32]. In terms of the relation between dnDSA and TCMR, the prevalence of dnDSA was clearly higher in recipients who experienced TCMR, albeit without a correlation with frequency and severity [33]. With regard to the effects of dnDSA development, the correlation between dnDSA and more severe fibrosis was observed [34,35]. These studies also demonstrated that rejection signs existed more frequently in the dnDSA positive group. Interestingly, s-DSA positivity in subclinical TCMR tended to be associated with more severe inflammation and fibrosis, while s-DSA negative subclinical TCMR resulted in no histological rejection, supported by gene transcriptional evidence [36]. In a relatively large study, the relation between dnDSA and fibrosis can also be observed by looking at 61 dnDSA positive patients among a total of 749 liver transplant recipients, albeit without pathological insights and HLA–C/DP information [37]. In this retrospective study, the most frequent target of HLA was the DQ locus (85%, 52 out of 61 patients). Cyclosporin usage rather than tacrolimus and low CNI levels (tacrolimus < 3 ng/mL, cyclosporin < 75 ng/mL in the first year) were detected as risk factors of developing dnDSA. On the other hand, high model for end-stage liver disease (MELD) scores and advanced recipient age (>60) functioned as protective factors. These findings appear to be consistent with immunological theories, i.e., deteriorating or elderly patients may exhibit milder immunological reactions.

Given the fact that the existence of dnDSA does not necessarily indicate the onset of ABMR, it is still unclear whether we should make interventions against the status of positive dnDSA. Conversely, it is better to seek remedies if ABMR is confirmed based on the Banff criteria. To address ABMR due to dnDSA, several therapeutic strategies have been applied so far. Simple reinforcement of immunosuppressants appears to be the first choice. Several cases showed a reduction in s-DSA MFI [32]. In addition, several institutions seem to apply other therapies for ABMR just as in renal transplant: anti-CD 20 Abs, IVIG, and plasmapheresis [38,39]. However, it seems to be rare to reverse the severe fibrotic changes after the onset of chronic ABMR. Thus, at present, it is of vital importance to prevent the development of dnDSA and subsequent ABMR.

3.3. Pancreas Transplantation

Due to improvements in surgical techniques and immunosuppression protocols, the graft survival of pancreas transplant has greatly improved. Similarly to other organs, with the pancreas, it is true that our attention has been shifting from these acute issues to ABMR. However, the association between dnDSA and graft outcomes is not well understood. Several studies have demonstrated the negative impact of ABMR on pancreas graft survival. However, many of these studies were limited by retrospective styles, small sample size, or lack of histological assessment. Among them, de Kort et al. initially investigated 27 pancreas transplant patients according to their s-DSA and biopsy findings. Graft survival largely depended on s-DSA positivity accompanied by complement activation (C4d positivity) [40]. Furthermore, they revealed that ABMR played a significant role in early graft loss < 1 year after transplant, especially in the setting of thrombotic cases [41]. Without histological assessment, a larger study consisting of 167 pancreas transplant recipients showed that about 15% (26/167) recipients developed s-DSA during a nine-year follow-up and resulted in significantly inferior outcomes: graft survival dropped around 30% and 20% from recipients without anti HLA Abs and DSA, respectively [42].

Regarding the class of DSA, a large part of dnDSA belonged to class II [43], especially DQ [44]. These results are also consistent with findings of kidney and liver transplants. Considering the recent developments in HLA matching, a more sophisticated approach should also become possible in the field of pancreas transplant. This approach demonstrated that the development of dnDSA after pancreas transplant was associated with the number of predicted indirectly recognizable HLA epitopes (PIRCHE) II [45]. Practically, it seems to be difficult to consider all information regarding HLA prior to deceased organ transplant. Nonetheless, it would be plausible to cater tailored immunosuppression based on their immunological status, provided that details of donors' HLA information are supplied.

Although there is no research focusing on the optimal treatment for ABMR in pancreas transplant, the ABMR of pancreas grafts seems also to be managed by additional anti-humoral therapies with reinforcement of standard immunosuppressants [40,42,43]. However, it is controversial to initiate preemptive treatments for dnDSA positive cases without ABMR signs. Uva et al. added Belatacept to the maintenance of immunosuppressants for selected patients, although they denied universal preemptive interventions [46].

Overall, ABMR in pancreas transplant remains relatively unclear compared to other fields. Given the fact that many pancreas transplants have been conducted together with kidney transplants, research on ABMR in pancreas transplant would progress with the knowledge of kidney transplants. Indeed, there are criteria for ABMR of pancreas grafts [47]. Due to the hesitancy regarding pancreas graft biopsies, it must be admitted that an accurate assessment of rejection in pancreas graft is limited on several occasions. In addition to pancreas biopsies, there is a different approach: Duodenal graft biopsies have been taken [48]. This procedure may additionally shed light on pancreas graft ABMR according to intestinal transplantation and could improve outcomes. Limited knowledge of pancreas ABMR demands large prospective cohorts in the future.

Recent studies regarding chronic ABMR due to dnDSA in each field are summarized in Table 1.

Table 1. Summary of recent studies in chronic ABMR due to dnDSA.

Renal Transplant	Study Design, Number of ABMR Patients (Number of Treated Patients)	Treatment	Median (or Mean) Post-Transplant/Post-Treatment Follow-Up Period (Months)	Major Outcomes	Reference
	RCT, 25(12)	Rituximab + IVIG vs. placebo	(118)/12	Treatment group eGFR decline was slightly smaller but not significant.	Moreso 2018 [49]
	RCT, 20(10)	Eculizumab vs. control	N/A/6	Slight stabilization was noted in renal function while on treatment.	Kulkarni 2017 [20]
	Prospective, 36(36)	Tocilizumab	N/A/39.1	Significant reduction in DSA and stabilization of renal function.	Choi 2017 [21]
	RCT, 20	Clazakizumab vs. placebo	10.6 (4.4–16.2) years post-op to inclusion in the trial/52 weeks	The mean eGFR decline during treatment was notably slower.	Doberer 2020 [23]
	RCT, 44(21)	Bortezomib vs. placebo	N/A/24	No significant improvement in GFR decline in the treatment group despite significant toxicity.	Eskandary 2018 [19]
	Retrospective, 123 (108 at least steroid + IVIG)	Steroid pulse+IVIG(+rituximab, PP, thymoglobulin)	9.5 (2.7–20.3)/4.3 (0–8.8) from diagnosis of ABMR	The combination of steroid pulse and IVIG demonstrated a reduced risk of graft loss.	Redfield 2016 [50]
	RCT, all patients: 660 (219/226/215)	Belatacept more intense vs. Belatacept less intense vs. cyclosporine treated	Up to 7 years follow-up from randomization	Belatacept-based immunosuppression effectively suppressed DSA production.	Bray 2018 [24]
Liver Transplant					
	Retrospective, 9(9) (dnDSA + acute ABMR patients)	Steroid, IVIG, PP, rituximab, ATG, retransplantation	44(13–66)/36(3–65)	Seven out of nine recipients demonstrated stable liver enzyme tests.	Del Bello 2015 [38]
	Retrospective, 4(3 pediatric)	IVIG	N/A/N/A	IVIG had minimal effects on MFI of DSA.	Guerra 2017 [51]
	Retrospective, 9(9, pediatric 4, adult 5)	Rituximab + α(IVIG, Bortezomib)	104(17–245)/60(5–65)	The administration of rituximab for chronic ABMR may be feasible.	Sakamoto 2021 [39]

Table 1. *Cont.*

Renal Transplant	Study Design, Number of ABMR Patients (Number of Treated Patients)	Treatment	Median (or Mean) Post-Transplant/Post-Treatment Follow-Up Period (Months)	Major Outcomes	Reference
PancreasTransplant					
	* Retrospective, various treatments, 9(4)	(nonstandard treatment for 4 patients) ATG, IVIG, PP, alemtuzumab, pancreatectomy	21.7 (range 0.1–169.5) months/N/A	Three patients received pharmacological treatments and 4 out of 9 patients lost their graft.	de Kort 2010 [40]
	* Retrospective, various treatments, 4(4)	Steroids, IVIG, PP	55.2/N/A	A quarter had graft failure approximately 2 years after treatment.	Parajuli 2019 [43]
	* Retrospective, various treatments, 8(N/A)	Steroid pulse, IVIG, PP(five sessions), Belatacept	N/A/N/A	Beyond the scope of this study to discuss the optimal treatment.	Uva 2020 [46]

* Not focused on treatments; ABMR: antibody-mediated rejection; dnDSA: de novo donor specific anti-HLA antibodies; DSA: donor specific anti-HLA antibodies; IVIG: intravenous immunoglobulin therapy;
PP: plasmapheresis; RCT: randomized controlled trial.

4. An Overview of Recurrent Primary Disease

Recurrent primary disease plays an important role in determining graft outcomes in almost all fields of organ transplantation. Discussing the recurrence of primary disease always requires a clear definition of the recurrence. Described here are several reports regarding recurrences of primary diseases in the different fields. It is important, however, to keep in mind that misleading reports can exist due to an unclear definition of recurrence. Thus, in this review, the forms of recurrences are determined as follows for the sake of clarity: pathological recurrence (PR), mild-to-moderate clinical recurrence (mCR), and severe clinical recurrence (sCR) that results in end-stage organ failure.

4.1. Kidney Transplantation

There are many renal transplant recipients whose primary diseases are uncertain. In these cases, nephritis after transplantation cannot be recognized as recurrent or de novo nephritis precisely. However, recurrences of primary diseases certainly cause negative effects on graft survival rates. The management of recurrences should be considered seriously.

4.1.1. IgA nephropathy

Recurrent IgA nephropathy (IgAN) is a crucial topic in renal transplant recipients with primary IgA nephropathy because a reported recurrence rate seems to be around 30% [52,53] at 10 years after transplant and as high as 50% in biopsied patients [54,55]. Although many of these recurrent incidents are characterized by only PR with a benign clinical course, around 10% of these cases over the course of 10 years were associated with the aggressive deterioration of renal function: sCR, resulting in graft loss. Thus, the control of IgAN recurrence remains an unmet need in the field of renal transplantation. Several factors may affect the pathogenesis of IgAN recurrence, such as low levels of immunosuppression, especially steroid avoidance [56] or HLA mismatch [57].

Cumulative data have suggested that circulating under-galactosylated or galactose-deficient (Gd) IgA1 and the subsequent generation of anti-GdIgA1 IgG (αIgA) play central roles in the pathogenesis of IgAN [58]. Suzuki et al. [59] reported that the levels of serum αIgA were closely associated with disease activity in native IgAN. As a progression of αIgA research, Julian and his colleagues [60] revealed glomerular deposition of αIgA in native IgAN by means of the extraction of biopsy specimens, although not having enough tissue prevented individual analysis. In terms of IgAN recurrence after renal transplantation, Berthelot et al. followed 60 IgAN transplant patients, concluding that high serum GdIgA1 resulted in IgAN recurrence. Julian's group also demonstrated a similar result that serum normalized αIgA was an independent risk factor for IgAN recurrence [61]. In research described above, GdIgA1 and αIgA were measured by the conventional ELISA method. By using the ICFA method, we measured αIgA (serum/intragraft) and GdIgA1/αIgA immunocomplexes (intragraft) to investigate whether a causal relationship exists between αIgA and IgAN recurrence. In this report, the IgAN recurrence group demonstrated significantly higher serum αIgA levels at the time of recurrence confirmation. The IgAN recurrence group also exhibited higher intragraft αIgA and relatively higher ICs than those of a non-IgAN recurrence group [62]. According to these reports, therefore, it is reasonable to believe that GdIgA1 and αIgA are key molecules in IgAN recurrence and important targets to prevent recurrence. Nevertheless, there is no concrete evidence regarding therapeutics that target GdIgA1 and αIgA at this moment.

Several studies suggest strategies as a part of prophylaxis to suppress IgAN recurrence, although no established induction therapy exists. As a prevention of IgAN recurrence, the Jikei group adopted elective tonsillectomy 1 year after renal transplantation and examined the relationship between tonsillectomy and serum GdIgA1 and GdIgA1 deposition in tonsils and kidneys [63]. They revealed that elective tonsillectomy reduced the rates of IgAN recurrence, coupled with decreased immunoreactivities of GdIgA1. This report supports an interesting tonsil–kidney circulation based on GdIgA1.

Since HLA mismatch was suggested as a risk factor for the recurrence, there may exist a difference in outcomes between related or unrelated donor transplants [64]. Rodas et al. reported that full mismatches in HLA-B mitigated the recurrence of IgAN by examining 86 transplants, which included 38 living donor transplants [57].

Next, our concern is therapeutic modalities available in IgAN recurrence. Although there is no specific guideline for renal transplant recipients, many institutions basically follow native IgAN treatments consisting of steroid pulse [65], rituximab, tonsillectomy [63], and elimination of other exacerbating factors. A simple observation strategy often takes place for many PR cases, especially in elderly recipients, combined with lifestyle guidance. Either way, the current situation demands large prospective cohorts to establish a certain treatment strategy for IgAN recurrence.

4.1.2. Focal Segmental Glomerulosclerosis

FSGS is one of the main indications for renal transplant in relatively younger populations and recurs with a variety of rates from 10 to 60% within a couple of years after transplantation [66,67]. Due to its humoral-type pathogenesis, immediate recurrence is common and can occur within 24 h after transplant. As a typical example of clinical recurrence (CR), massive proteinuria occurs immediately following reperfusion. At this initial point, fusion of the podocyte foot processes is the only finding by electron microscopic investigation. This progresses to a typical focal segmental sclerosis within a few months, which can be observed under an optical microscope. Under the current medical settings, a recurrence of FSGS significantly deteriorates graft survival and demonstrates around 30–60% survival rate at 5 to 10 years after transplant [68–70].

We would like to examine a typical example that suggests that pathogenesis of FSGS is based on a circulating factor [71]. An sCR-deteriorated allograft in a FSGS recipient can regain its function in a different non-FSGS recipient after re-transplantation. Furthermore, plasma obtained from FSGS patients can reproduce FSGS phenomena in rats [72]. Subsequently, intensive study has revealed that the soluble urokinase-type plasminogen activator receptor (suPAR) is involved in the onset of FSGS [73]. Podocyte effacement is also closely related to serum suPAR levels, while suPAR levels reflect the response to therapy [74]. Therefore, it is reasonable to believe that reduction in suPAR results in a low CR rate.

Considering the possibility of immediate deterioration, it is reasonable to carry out prophylaxis measurements prior to transplant. Several approaches have been attempted as induction therapies, and these approaches can be roughly divided into B-cell depletion and apheresis. Although there is a report of these approaches having no preventive effects [75], the effectiveness of plasma exchange or apheresis is advocated by many institutions. On the other hand, B-cell depletion is often accomplished by anti-CD20 Abs. Prior to transplant, B-cell depletion followed by a series of plasma exchange or apheresis is one of the induction therapies that can be used to mitigate recurrence. These effects could be explained by the reduction in circulating factors, including suPAR. As a donor selection, a living related donor may have disadvantages in terms of recurrence. This study demonstrated a 6.6% increase in recurrence rates at 10 years after renal transplant in a living related donor compared to an unrelated donor [76].

Current therapeutics for CR consist of reinforcement of immunosuppressants, including steroid pulse, rituximab, and plasma exchange [77]. Although suPAR levels rebound relatively quickly, it has been reported that CR status was stabilized following these therapies [78]. Further investigation into suPAR and other humoral factors may provide more sophisticated therapeutic approaches for FSGS.

4.1.3. Membranoproliferative Glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is evidenced by the thickening of capillary walls and diffuse mesangial cell proliferation under a light microscope. This disease can be divided into primary (unknown reason) and secondary (known reason) MPGN. Previously, MPGN had also been split into three distinct types based on patho-

logical findings: sub-endothelial and mesangial immune deposits (type I, most common, mainly secondary MPGN); dense-deposit disease associated with the deposition of complement C3 without immunoglobulin deposition (type II); and a mixture of sub-epithelial and sub-endothelial immune deposits (type III, subtype of type I) [79]. Recently, this categorization has been replaced by a pathogenesis-based classification: (1) alternative complement pathway activation and (2) immunoglobulin-related type. C3 glomerulopathy belongs to classification (1) and is determined where glomerulonephritis is accompanied only by C3 deposition without C1q or C4 deposition. C3 glomerulopathy has two forms: dense-deposit diseases and another C3-deposited glomerulopathy, which were previously categorized in type I and III [80,81].

Recurrence rates at 11.8%, 15.6%, and 18.9% at 5, 10, and 15 years, respectively, after renal transplantation have been reported [70]. More prominently, C3 glomerulopathy exhibits higher than 80% recurrence rates in a small case series (19 patients) [82]. About 50 to 70% high rates of graft failure within 5 years after recurrence were observed in MPGN recurrent cases, especially in dense-deposit disease [70,82]. Thus, it is pivotal to explain these serious outcomes after recurrence for renal transplant candidates in advance. Furthermore, we should seek circumstances where recurrence is less likely to occur. An Israeli group reported that 19% recipients of MPGN type I recurred over a 118 $\pm$ 61 months follow-up period, and HLA B49 and DR4 were considered to be risk alleles. This research also suggests that an unrelated donor is preferred for transplant due to MPGN, based on the finding that higher rates (25%) of living related to donor renal transplant recipients recurred MPGN rather than to unrelated donor transplant (0%), although another study denied the participation of donor category in recurrent MPGN [76]. In addition, recurrent MPGN, again, clearly showed worse graft survival [83]. Although an optimal induction therapy has not been determined, a B-cell targeted immunosuppressive approach appears to be logical.

The strategies for recurrence of MPGN are also similar to those for the primary disease, which consist of plasma exchange, rituximab, steroid pulse, or eculizumab [84]. Nevertheless, given the significant rates of recurrence and graft failure, it is crucial to inform patients of the outcomes of renal transplantation and to establish effective therapeutic strategies.

4.1.4. Membranous Nephropathy

Membranous nephropathy (MN) occurs as a pure glomerular-specific autoimmune disease, as well as a secondary disease due to a systemic condition such as infections, malignant disorders, autoimmune diseases, etc. This entity is induced by immunocomplex deposition in the sub-epithelial area of the glomerular basement membrane [85]. The primary cause seems to be a development of Abs against podocytes. The major Abs that have been identified include anti-phospholipase A2 receptor antibodies (PLA2R) [86] and thrombospondin type-1 domain-containing 7A antibodies (THSD7A) [87].

Recurrence rates at 10% (5-year), 16% (10-year), and 18% (15-year) were observed, which are similar to those of MPGN discussed above. The recurrence rates could be higher (up to 40%) when including PR found by a protocol biopsy [88]. PR is likely to occur within 1 year after transplant and has a possibility of recurring as early as 2 weeks post-transplant. The secondary MN displayed relatively low rates of recurrence, provided that the primary diseases were well controlled. The recurring MN could result in notable graft failure rates of up to 60% [70]. A lack of consistency in the graft failure rates in recurring cases would indicate that the studied population was not heterogenous.

Regarding recurrence and autoantibody levels, Kattal et al. investigated 26 MN recipients according to their anti-PLA2R levels and revealed that anti-PLA2R levels were 83% and 42% of the positive and negative predictive values, respectively, for recurrent MN after transplant [89]. In addition, anti-THSD7A also could induced a recurrence of MN, as evidenced in a case report accompanying an investigation into a murine MN model [90].

Therefore, autoantibody levels may enable us to stratify recipients into appropriate recurrent risk groups.

In order to prevent recurrence, many researchers may seek to perform treatments with induction therapy prior to transplant. However, at present, there appears to be no reliable induction therapy for meaningfully suppressing recurrence. Furthermore, it is still controversial whether unrelated donors are advantageous to recurrent MN [76,91].

The management of recurring MN, again, follows primary MN treatments consisting of treatments for nephrotic syndrome and decreased renal function.

Features of IgAN, FSGS, MPGN, and MN with overall recurrence rates are described in Table 2.

Table 2. Overall recurrence rate, other characteristics, available prophylaxis, and treatments in glomerulonephritis after kidney transplantation.

	IgA Nephropathy	Focal Segmental Glomerulosclerosis	Membranoproliferative Glomerulonephritis	Membranous Nephropathy
Recurrence Rate	About 30%/50–120 months (28.6%/121 ± 69 months [52], 34.9%/median 49 (range 4–213) months [53])	Differ widely between reports (10.4%/median 6.1 years (follow-up) [66], 46.7%/2.2 ± 1.8 years [92], 57.6%/median 1.25 (1 day to 30 months) months [67])	Differ widely between reports (11.8%, 15.6%, and 18.9% at 5, 10, and 15 years [70], 84.2%/76 months follow-up (C3 glomerulopathy) [82])	Differ widely between reports (10%, 16%, and 18% at 5, 10, and 15 years [70], 11.4%/median 3.6 (1.0–4.7) years [93], 44%/13.6 months [88])
Graft loss due to clinical recurrence (%)	10.8% at 10 years [53], 21.4%/130.8 ± 10.6 months follow up periods [94], 58% at 5 years [70]	Differ widely between reports (43% at 5 years [70], 39%/median 5 years [69], 9%/median 29.5 months [67])	About 50–70%/~5 years (56.3%/median 42 months (C3 glomerulopathy) [82], 70% at 5 years [70])	About 50–60%/~5 years (47.4% allograft loss/median 3.6 (1.0–4.7) years [93], 59% at 5 years [70])
Pathogenesis	Galactose-deficient IgA1, anti- galactose-deficient IgA1 IgG, immunocomplex	Circulating permeability factors, such as suPAR	Alternative complement pathway activation or immunoglobulin deposition	Anti-phospholipase A2 receptor, or thrombospondin type-1 domain-containing 7A antibodies, etc.
Risk factors of recurrence based on donor type/factors	HLA match, related donor	Related donor	Related donor (controversial)	Related donor (controversial)
Prophylaxis (Induction Therapy)	Tonsilectomy	Plasma exchange, apheresis, rituximab	Plasma exchange, rituximab	N/A
Treatments	Steroid pulse, rituximab, tonsilectomy	Plasma exchange, apheresis, rituximab	plasma exchange, rituximab, steroid pulse, or eculizumab	Steroid pulse, Rituximab

4.1.5. Lupus Nephritis

Generally, lupus nephritis is diagnosed in various rates up to 70% of patients with systemic lupus erythematosus (SLE) [95]. Typically, the onset of nephritis is observed within 3 to 5 years after SLE diagnosis [96]. Pathologically, nuclear antigens, especially DNA and anti–nuclear/DNA complement-binding IgG, seem to play important roles in the onset of lupus nephritis [97].

CR after renal transplantation can be less than 5% [98]. It can be argued that pathological activity decreases at the time of renal failure and the introduction of immunosuppression after transplant also suppresses disease activity. A large study for lupus nephritis indicated equivalent outcomes relative to non-lupus nephritis renal transplant recipients [99]. Deegens et al. reported that one patient out of 23 recipients exhibited lupus nephritis, albeit with no biopsy evidence due to coagulopathy [100]. However, it is true that the recurrence rate largely relies on the executing rates of biopsy procedures. In fact, PR increased by up to 50% when less aggressive types of histological changes—class II, III, etc.—are included [101]. Taken together, PR seems to be relatively common, but does not affect overall outcomes. Therefore, it is reasonable to perform renal transplant for lupus-related renal failure.

Regarding induction therapy, there is no standard recommendation at present. However, it is recommended that renal transplant should be performed after the introduction of dialysis therapy for several months, with the dosage of prednisolone decreased to less than 10 mg/day [102]. From this perspective, preemptive renal transplant may not be feasible, especially in cases with rapid progression to renal failure. When considering preemptive transplant, disease activities assessed by anti-nuclear Abs and anti-double strand DNA Abs, CH50, C3, etc., should be carefully reviewed.

For CR cases, treatment plans consist of steroid pulse, increasing the dose of mycophenolate mofetil or substituting cyclophosphamide for mycophenolate mofetil, and anti-CD20 Abs [103].

4.1.6. Anti-Neutrophil Cytoplasmic Autoantibody or Anti-Glomerular Basement Membrane Antibody Positive Rapidly Progressive Glomerulonephritis

Both anti-neutrophil cytoplasmic autoantibodies (ANCA) and anti-glomerular basement membrane (GBM) antibody-positive rapidly progressive glomerulonephritis (RPGN) are categorized as acute progressions to renal failure accompanied with hematuria, proteinuria, and anemia. Clinical RPGN comprises a variety of diseases such as part of IgAN, thrombotic microangiopathy, acute interstitial nephritis, etc. This section features ANCA-related nephritis and anti-GBM-Abs-related nephritis [104].

Around 10% recurrence rates have been reported for ANCA-related nephritis after transplant, and one-third of these resulted in graft loss over the first five years [105]. Although ANCA titers are utilized to assess disease activity, several studies indicate that the levels of ANCA could not be relied upon to assess recurrence [106,107]. Nonetheless, the activity of primary disease is considered important for controlling recurrence. Thus, it is sensible to wait for renal transplant at least one year after the activity becomes under control [108].

On the other hand, the recent recurrence rates for anti-GBM-Abs-related nephritis seem to be less than 5%, which is lower than that of ANCA-related nephritis [109,110]. Interestingly, the step for waiting for remission is similar, but a decrease in anti-GBM Abs for at least 12 months consecutive is also required for safe renal transplant [111].

It is feasible to perform renal transplants for these two RPGN-induced renal failures when indicated due to the relative lower rates of recurrence.

Clinically important information regarding lupus nephritis, anti-ANCA, and anti-GBM RPGN is summarized in Table 3.

Table 3. Summary of recurrence rate and graft survival in patients with recurrence in lupus/ANCA/anti-GBM nephritis following kidney transplantation.

	Lupus Nephritis	ANCA Related Nephritis	Anti-GBM Abs Related Nephritis
Recurrence Rate	Vary between reports due to the frequency of biopsies, 30%/6.8 ± 4.9 (range, 3 months-20 years) [101], 4.3%/74.2 ± 72.2 months [100]	2.8% per patient year, 10%/the first 5 years post-op [105], 4.7%/median 5.5 years [106]	3.9%/median 6.4 years [109], 2.7% [110]
Graft loss due to clinical recurrence (%)	2%/6.8–4.9 (range, 3 months-20 years) [101], 1/31(3%) graft losses among 80 lupus transplant was caused by recurrence [112]	Four out of 11 recurrent cases lost theirgrafts within 5 years of transplantation [105], 2.8%/median 5.5 years [106]	3.9%/median 6.4 years [109], 0.9% [110]
Pathogenesis	Type III allergy	Neutrophil activation due to proteinase 3/myeloperoxidase-ANCA etc. [113]	Anti-GBM Abs(type II allergy)
Recommendation	Better to perform kidney transplant after introduction of dialysis therapy for several months, and being able to reduce prednisolone <10 mg/day	Wait for renal transplant at least 12 months after the disease activity becomes under control.	Confirm a decrease in anti-GBM Abs for at least consecutive 12 months

Abs: antibodies; ANCA: anti-neutrophil cytoplasmic autoantibodies; GBM: glomerular basement membrane.

4.1.7. Amyloidosis and Mimickers

Amyloidosis is a systemic disease and a relatively rare entity for renal transplantation. Amyloid deposition is observed under the electron microscopy at around 5–12 nm fibrils, which are β-pleated sheets [114]. In addition, a positive Congo red stain and an apple-green birefringence with polarized light are often used for confirmation [115]. Mass spectrometry became a useful tool for distinguishing the following subtypes [116].

AL (fibrils due to immunoglobulin light chain clonal production); AA (serum amyloid A congregation, secondary amyloidosis); ATTR (hereditary amyloidosis, genetic mutation of misfolding-prone protein (mainly transthyretin)); and ATTRwt (wild-type transthyretin misfolding). Responsible proteins of ATTR include apolipoprotein A-I, A-II, lysozyme, fibrinogen, and cystatin C, etc., in addition to transthyretin [117,118].

Although amyloidosis negatively impacted the patients' graft survival, the overall outcomes were equivalent with diabetes mellitus (DM) or elderly (>65 years) recipients after renal transplantation. Around 15% recurrence rates had been reported, depending on the subtype of amyloidosis, and significantly affected patients' survival. Regarding AL amyloidosis, a complete hematologic response seems to be key in achieving better survival [119] and acceptable outcomes (median duration to graft loss: 10.4 years), whilst patients with partial or no response demonstrated inferior outcomes (5.5 years) [120]. Apolipoprotein A-I and lysozyme amyloidosis showed better graft survival, with 13.1 years at median [119]. These studies suggest that renal transplant outcomes largely rely on the type of primary amyloidosis. Therefore, at least, it is reasonable to evaluate patients carefully and manage the primary amyloidosis appropriately in order to mitigate the risk of recurrence when considering renal transplant for amyloidosis.

On the other hand, as a mimicker, fibrillary glomerulonephritis (FGN) shares several common characteristics both clinically and histologically: nephrotic or nephrotic syndrome, deposits of 12–24 nm fibrils, and occasionally positive Congo red stain (4%). FGN is characterized by DnaJ homolog subfamily B member 9 as an auto-antigen [121] and possibly recurs after transplant with the rate at around 20% [122]. Thus, it is pivotal to make a correct diagnosis in confusing cases by using mass spectrometry.

In addition to recipients diagnosed with amyloidosis, it is essential to keep renal amyloidosis in mind in cases of new-onset proteinuria or nephrotic syndrome following renal transplantation for an unknown primary disease, since a routine pathological screening may not identify the early signs of recurrent amyloidosis.

4.2. Liver Transplantation

After liver transplantation, a variety of medical problems might occur, such as renal damage, new onset of DM, etc., in addition to rejection and infection. Furthermore, primary disease recurrences are another significant issue. There are at least three major mechanisms by which a primary disease recurs in liver grafts. The first category is a recurrence in the same manner as the primary disease, for example, primary biliary cholangitis or primary sclerosing cholangitis. Secondly, a recurrence of hepatitis due to a virus that is responsible for the primary hepatitis belongs to this category. Third, this category contains recurrences of tumors in liver graft. It can be recognized as primary tumor metastases to liver grafts.

4.2.1. Viral Hepatitis

Although viral hepatitis B and C have been the leading reasons for adult liver transplants [123], a recent rapid increase in nonalcoholic steatohepatitis has rearranged this trend [124].

Regarding hepatitis B virus (HBV) reactivation following transplant, there are two main distinctive situations. The first instance is HBV hepatitis in HBsAg (hepatitis B surface antigen)-positive recipients, whereas HBV transfer from a donor who is HBsAg-negative/hepatitis B core antibodies (anti-HBc Abs)-positive is another example. From the perspective of recurrence, former conditions will be discussed here.

For recipients with HBV infection, inappropriate prophylaxis results in HBV reinfection of liver grafts. Following the removal of the infected liver, the reinfection is established with HBV remaining in the recipient's blood stream. This trend seems to be more apparent in recipients with preoperative HBV-DNA positive than HBV-DNA and hepatitis B e antigen (HBeAg) double-negative cases. Eighty-three percent of preoperative HBV-DNA positive recipients had serums that became HBsAg-positive after surgery. Nevertheless, HBV-DNA and HBeAg double-negative cases also showed an HBsAg resurgence in 58% of recipients [125]. Therefore, HBV exists in recipients, albeit with negative results for HBV-DNA. It is well known that the outcomes of HBV reinfection have significant negative effects on liver grafts: rapid progression to cirrhosis [126]. As prophylaxis strategies, initially, hepatitis B immunoglobulin or lamivudine administration had been implemented. However, higher than 30% reactivation was confirmed despite prophylaxis [125,127]. Thereafter, it was proved that hepatitis B immunoglobulin and lamivudine coadministration was effective enough to suppress reactivation, with a rate of 0–10% [128,129]. Consequently, it became a standard practice to apply preoperative lamivudine treatment, intraoperative hepatitis B immunoglobulin IV, and postoperative coadministration.

Although hepatitis C virus (HCV) has spread worldwide, the introduction of direct-acting antivirals (DAA) has completely changed its management after liver transplantation [130]. In addition, DAA provides an opportunity to expand a donor pool to the HCV-positive population [131]. As a natural course of liver transplant for HCV, the rates of HCV-recurrent infection are significantly high and typically occur soon after surgery, while only 5% of recipients may escape from recurrence. In many cases, HCV RNA becomes detectable two to four weeks after transplant. Subsequently, many recipients show histological chronic hepatitis [132,133]. Before the introduction of DAA, it was believed that these factors might affect the overall outcomes of liver transplants. Surprisingly, these negative influences may be limited to slight-to-moderate inferior outcomes in 5-year patient survival rates [134]. Conversely, several studies have reported that HCV recurrence possibly caused serious outcomes, such as fibrosing cholestatic hepatitis [135], immediate progression to cirrhosis [136], or deteriorated patient survival [137].

Before the emergence of DAA, several treatment strategies were reported. From the 1990s, interferon therapy commenced [138]. Ribavirin was added in the 2000s [139] and was later improved by Peg-interferon [140]. However, the sustained virological response (SVR) after transplant remained only around 10–40%, which was far below acceptable rates [141,142]. In 2011, telaprevir was approved as a first-generation proteinase inhibitor and established as a triple-drug treatment [143]. The second generation simeprevir, emerged in 2013, and the regimen further improved [144].

Next, in 2014, the interferon-free DAAs, asunaprevir, and daclatasvir were developed with successfully high SVRs [145]. Conversely, it is true that candidates for liver transplant may have belonged to a category where preoperative treatment was ineffective or impossible due to refractory mutated HCV or patients' other conditions. Thus, SVRs after transplant were inevitably lower than those of native HCV patients [146].

Considering these serious consequences, the effective DAA introduction was significant, especially after the emergence of sofosbuvir and ledipasvir. As the SVRs had reached nearly 100% both in native HCV and liver transplant patients [147,148], DAA introduction after liver transplant became a standard therapy. Furthermore, for recipients with renal failure, cirrhosis, or prior DAA failure, the effectiveness of glecaprevir and pibrentasvir has been reported [149].

4.2.2. Malignant Tumor

Liver transplantation is indicated for end-stage liver disease with tumors or unresectable malignant liver tumors under certain conditions. Hepatocellular carcinoma (HCC) is a representative tumor that is most frequently indicated for liver transplantation. Originally, it is well known that the outcomes of liver transplantation for HCC without staging were inferior to those of other primary diseases without HCC [150]. This is primarily

because HCC recurs in transplanted liver grafts in immunocompromised patients. Thus, liver transplantation should proceed in circumstances where HCC is not disseminated to the outside of the liver. To overcome the recurrence of HCC, the Milan criteria was clinically introduced worldwide [151]. However, a certain patient group demonstrated the equivalent outcomes, albeit with deviation from the Milan criteria. The criteria were then modestly expanded to the University of California, San Francisco (UCSF) criteria, which showed a 75% survival rate in 5 years [152]. The Kyoto group also expanded the eligibility for liver transplantation with an excellent survival rate of 82% and a low recurrence rate of 7% at 5-years post-transplant [153]. It is noteworthy that the Kyoto group not only applied tumor size and numbers, but also serum des-gamma-carboxy prothrombin levels as an assessment for tumor activities. Recently, there have been further efforts to establish several other safer models, such as the hazard associated with liver transplantation for hepatocellular carcinoma which incorporates a dynamic α–fetoprotein response [154].

Early stage unresectable cholangiocarcinoma is also considered as an indication for liver transplantation. Cholangiocarcinoma is the second most common liver cancer and pathophysiologically divided into two different groups: extrahepatic cholangiocarcinoma and intrahepatic cholangiocarcinoma. Among extrahepatic cholangiocarcinoma, perihilar cholangiocarcinoma may be an indication for liver transplantation, whilst liver transplantation is not indicated for distal cholangiocarcinoma given its location [155]. LT can only be a valid treatment plan if it can promise a better survival rate compared to liver resection. In order to achieve this, the Mayo Clinic protocol that was originally reported in 2000 has been supported. In summary, liver transplantation is performed following neoadjuvant chemoradiotherapy, which consists of extra-beam and transcatheter radiation therapy and intravenous 5-FU administration [156]. Based on experience, elevated CA19-9, encased portal vein, and non-radical resection were identified as predictors of recurrence following liver transplantation [157]. On the other hand, several initial studies regarding intrahepatic cholangiocarcinoma seem to be difficult to interpret since the pathological conditions of recipients were not sufficient. However, recent studies show that liver transplantation for very early intrahepatic cholangiocarcinoma—a single tumor measuring less than 2 cm—promises acceptable outcomes, with a 73% 5-year patient survival rate [158]. In summary, these results were obtained from incidental pathological findings in recipients with cirrhosis or initial misinterpretation as HCC. Thus, initial diagnosis such as very early intrahepatic cholangiocarcinoma should first be considered for liver resection. However, if liver resection is not an option, as with portal hypertension, etc., liver transplantation might be the last resort.

4.2.3. Primary Biliary Cholangitis

Primary biliary cholangitis (PBC) is categorized as a cholestatic liver disease accompanied by autoimmune features. These features comprise anti-mitochondrial Abs (AMA) positivity (>90% of patients), targeting anti-E2 domain of pyruvate dehydrogenase complex Abs [159], and meaningful overlap with other autoimmune disorders, such as Sjogren's syndrome and thyroiditis, etc. [160]. Thus, PBC is recognized as part of a systemic autoimmune condition. Histologically, PBC is characterized by a chronic destructive form of nonsuppurative granulomatous lesions with or without lymphocytes-infiltrate cholangitis in small-sized and medium-sized biliary trees [161].

With the introduction of ursodeoxycholic acid (UDCA) in the early stages of PBC, the resulting rates of liver transplant or death improved to 6% and 22% at 10 and 20 years, respectively, after the onset of PBC [162]. The outcomes of liver transplant are generally good, with around 80% and 70% survival rates reported at 5 and 10 years, respectively [163,164]. Interestingly, similar outcomes have been reported with deceased and living liver transplant donors [165]. The rates of recurrent PBC appear to vary depending on whether recipients received prophylactic UDCA administration and the frequency of liver biopsies. Recurrence rates at 10% to 20% have been reported in recipients with prophylaxis during an approximately 10-year follow-up period [166,167], whereas patients without

prophylaxis have demonstrated greater than 30% recurrence rates over the same time period [168,169]. These studies support that UDCA is effective for the majority of patients with recurrence, but there were no significant improvements in histological changes and patients' survival [169].

Diagnosis of recurrent PBC is largely reliant on histological findings, since only around 10% of recurrent PBC patients demonstrate classic symptoms of PBC, i.e., pruritis, jaundice, xerostomia, or keratoconjunctivitis sicca, etc. In order to standardize recurrent PBC, pathological features combined with the existence of AMA and elevated IgM are often adopted clinically. Pathological features include the following four findings: 1. epithelioid granulomas called florid lesions, 2. lymphoplasmacytic infiltration, 3. lymphocytes aggregation, and 4. bile duct injury. Definite recurrent PBC is defined by having all 3–4 pathological features, while conditions meeting 2/4 criteria are considered as probable recurrent PBC [170].

Citing definite risk factors for recurrence of PBC is still controversial, although several studies suggest that immunosuppressants, HLA alleles, HLA mismatch, recipient/donor age, and gender play a role, which appear to be feasible from the perspective of the behavior of autoimmune nature. Manousou et al. reported that therapy with cyclosporin and azathioprine in combination had preventive effects on the recurrence of PBC, although cyclosporin and tacrolimus alone had minimal influence [168]. Egawa et al. also suggest that there is superiority in conversion to cyclosporin from tacrolimus within 1 year to decrease the risk of recurrence, albeit with a disadvantage of cyclosporin as a primary calcineurin inhibitor [171]. This research enumerated several other risk factors: serum IgM 554 mg/dL or higher and donor-recipient sex mismatch. Recent meta-analysis from six retrospective studies showed tacrolimus inferiority and UDCA protective effects for the recurrence of PBC [172].

Regarding disease activity of recurrent PBC, it basically exhibits an indolent style that barely requires re-transplantation and tends not to affect long-term outcomes. A Japanese multicenter study showed that PBC recurrence rarely became an indication for re-transplantation after analyzing seven re-transplant cases from 516 liver transplant recipients of PBC [173], while Charatcharoenwitthaya et al. reported that two out of thirty-eight recurrent cases required re-transplantation [169]. Taken together, it can be argued that PBC recurrence seldom results in graft loss.

4.2.4. Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is characterized by both intra-hepatic and extra-hepatic multiple or diffuse bile duct stenoses, which result in a chronic cholestatic conditions associated with cirrhosis. Pathogenesis of PSC remains unclear, but, as with other multifactorial diseases, genetic factors along with environmental factors may play a role in its onset. Notable findings include a strong association of up to 80% with inflammatory bowel disease, especially ulcerative colitis [174]. In addition, PSC increases the risk of primary liver cancer, especially cholangiocarcinoma, by up to 1500 times compared with the general population and increases the annual morbidity rate by 0.5% [175]. At present, liver transplantation is the only established therapy for PSC. The overall survival rates without liver transplant in PSC patients were 78 and 60% at 10 and 20 years, respectively, following the onset of PSC [176].

The outcomes of liver transplantation for PSC differ from relatively poor to acceptable graft survival rates. From the European Liver Transplant Registry, 80, 69, and 57% graft survival rates at 1, 5, and 10 years, respectively, were reported [163], while U.S. data showed 86.5, 78, and 71.5% for the same time periods [164]. Although several discrepancies exist, the outcomes are generally worse than those for liver transplants for other cholestatic diseases. It can be argued that recurrent PSC in transplanted livers is one of the reasons for the worse outcomes.

Periductal concentric fibrosis (onion skin fibrosis) accompanied with lymphocyte infiltration is a well-known histological finding. However, this typical feature is not often

observed in PSC clinically. In order to diagnose PSC, cholangiography is considered the most important step. A beaded or "pruned tree" appearance and band-like strictures are characteristic features of PSC. Recurrent PSC is determined in PSC recipients who demonstrate these cholangiography findings or pathologies, with the exception of cases of hepatic artery thrombosis or other similar conditions [177]. Pathological features are considered particularly important in the diagnosis. Recurrence rates of around 20% have been reported, especially when less than a median of 5 years has passed since transplant [178–180].

The discrepancies in the reported incidences of recurrence may reveal some risk factors, although several risk factors have been reported from various institutions. These risk factors are considered to be possible issues for grafts, recipients–donor relations and recipients. Regarding liver grafts, marginal or extended donor criteria grafts may exhibit a higher incidence of recurrence [179]. Meaningful insights can also be obtained from living donor liver transplants. Notably, related (parent/child) pairs may show higher recurrence rates, and this tendency is clearer if recipients are followed-up longer: The hazard ratio is 3.12 (>12 months follow-up) [181]. Another study suggested higher recurrence rates in living donor liver transplants, compared to deceased donor transplant [182]. However, a multi-center cohort study denied the impact of donor type, albeit without collecting adequate HLA data [183].

In relation to recipient issues, several factors have been discussed, such as inflammatory bowel disease, rejection/immunosuppressants, younger age, and high MELD scores, etc. Of these, topics regarding inflammatory bowel disease are often discussed. Given the "leaky gut" hypothesis, unchanged bowel bacterial flora or substances from those after transplant may evoke the same pathological condition in transplanted liver grafts [184]. Thus, pretransplant colectomy with a remission of inflammatory bowel disease may confer PSC pathogenesis-free circumstances. Rejection, especially a refractory one, also imposes a higher risk of recurrence that may be explained by the fact that PSC and rejection share the same immunological pathways [185]. Taken together, several institutions seem to apply the following as clinically acceptable strategies: (1) avoidance of a first-degree relative as a donor and reliance on a deceased donor, if trusting the results that showed living donor inferiority, and (2) minimal withdrawal of immunosuppression.

Recurrent PSC tends to demonstrate CR style and demonstrates inferior outcomes (graft loss) that require re-transplantation with high probabilities (30–70%) compared to recurrent PBC or autoimmune hepatitis [181,183]. These results suggest that recurrent PSC progresses vehemently to graft loss. As with native PSC, no standard therapy has yet been established for recurrent PSC except for re-transplantation, although biliary tract drainage can be attempted in several cases as rescue therapy.

4.2.5. Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is an inflammatory, basically progressive, autoimmune disease that mainly affects middle-aged women (but possibly all ages and both genders) due to uncertain causes. The disease progression seems to rely on environmental triggers and genetic factors [186]. Although no disease-specific markers have been identified, several autoantibodies have been recognized in AIH: anti-nuclear, anti-smooth muscle, anti-liver kidney microsome, and anti-liver cytosol antigen type I. Based on these serological markers, AIH can be separated into type I (anti-nuclear or anti-smooth muscle Abs, or both) and II (anti-liver kidney microsome Abs) AIH [187]. In addition, the existence of type III AIH (anti-soluble liver antigens Abs) has been advocated [188], although the characteristic of this entity overlaps with type I AIH [187]. AIH is also characterized by mildly elevated serum IgG levels and interface hepatitis or plasma-lymphocytic infiltration. In terms of treatment, the first recommendation is corticosteroids followed by azathioprine and mycophenolate mofetil in that order [189]. If appropriate therapies are provided, the long-term outcome of AIH generally provides sufficient life expectancy. However, compared to other chronic liver diseases, the lack of these interventions results in relatively rapid progression to cirrhosis and liver failure, which requires liver transplant [190].

The recurrence of AIH after liver transplantation was initially reported with the rate of 26%, including PR to CR, by the Pittsburgh group [191]. Several other studies reported similar results of around 30 to 40% recurrence rates [192,193], including ambiguous cases. According to a recent systematic review, 8–12% and 36–68% PR to CR have been reported in one and five years, respectively, after liver transplant [194]. PR, with an indolent clinical course, may make up a large part of the recurrence. Although there is no firm consensus regarding recurrence, the diagnosis of recurrence largely relies on clinical manifestations: abnormal liver function tests, positive autoantibody status, high gamma globulinemia, and pathological findings (lymphoplasmacytic infiltration to portal area, central perivenulitis, interface hepatitis, and foci of necrosis) without evidence of endothelialitis and ductulitis, with the exclusion of rejection and viral infection. Notably, it is important to pay attention to the existence of TCMR, because the frequency of TCMR is generally higher in AIH, which may influence the diagnosis of recurrence [195].

Genetic factors may also play roles in the recurrence of AIH. Several studies over the last two decades detected HLA-DR isotype involvement. Two initial reports suggested that HLA-DR3 positivity in recipients has a negative impact on recurrence [192], especially in HLA–DR3 negative allografts [191]. A recent study also described how mismatches on both HLA-DR alleles results in a significant risk of recurrence, especially for patients with single-agent immunosuppression. This study also showed that racial factors might play a role in developing a recurrence [196]. It is also true that the high activity of primary AIH before transplant influences the rates of recurrence. In a multivariate analysis, the degree of inflammatory activity and high IgG levels were recognized as risk factors for recurrence [197]. Information regarding recurrence rates based on the type of AIH seems to be limited [198].

Treatment strategies for recurrence are based mainly on primary AIH and consist of steroid, azathioprine, and mycophenolate mofetil. In recipients with risk factors, a certain level of immunosuppression is required, although a steroid-minimization approach is often applied in the liver transplant field. The long-term outcome of recurrence may show slight disadvantages in graft survival rates [199], but there appears to be no significant difference between non-recurrence and recurrence populations [196].

These three autoimmune liver diseases are summarized in Table 4 from the perspective of liver transplantation.

Table 4. Overall graft and patient survival and other key information in autoimmune liver diseases after liver transplantation.

	Primary Biliary Cholangitis	Primary Sclerosing Cholangitis	Autoimmune Hepatitis
Patient Survival after liver transplantation	About 80–90%, and 70–80% at 5 and 10 years (86% and 76% at 5 and 10 years [200], 90% and 79% at 5 and 10 years [169], 80% and 71% at 5 and 10 years [163], 84.4% and 79% at 5 and 10 years [164])	About 80–90%, and 70–80% at 5 and 10 years (78% and 70% at 5 and 10 years [163], 87.4% and 83.2% at 5 and 10 years [164], 89% and 79% at 5 and 10 years [201])	About 75% at 5 years (76–78% at 5 years [197])
Patient Survival in the recurrent group	About 95% and 80–90% at 5 and 10 years (96% and 83% at 5 and 10 years [200], 88.5%/10.1 ± 4.3 years (follow-up period) [169])	About 80%, and 50% at 5 and 10 years (84% and 56% at 5 and 10 years [201])	About 75% at 5 years (76% at 5 years [197])
Recurrence Rate	About 10% and 20–30% at 5 and 10 years(9.6% and 20.6% at 5 and 10 years [171], 13% and 29% at 5 and 10 years [200])	About 10–20% and 10–30% at 5 and 10 years (13% at 5 years [202], 14.3% at 9 years [201], 18.1% and 36% at 5 and 10 years [178], 23%/median 4.6 years [179])	About 10–20%, and 30% at 5 and 10 years (18%, and 32% at 5 and 10 years [197], 25%/15 ± 2 months (follow-up period) [203])

Table 4. *Cont.*

	Primary Biliary Cholangitis	Primary Sclerosing Cholangitis	Autoimmune Hepatitis
Pathological vs. clinical recurrence	Pathological recurrence predominant	High clinical recurrence rates (30–70%)	Pathological recurrence predominant
Prophylaxis	Ursodeoxycholic acid	N/A	N/A
Treatments	Ursodeoxycholic acid	N/A	Steroid, Azathioprine, mycophenolate mofetil
Risk factors based on donor type/factors	Gender mismatch	A first degree relative donor	HLA-DR locus mismatching, recipient DR3+/donor DR3-

4.3. Pancreas Transplantation

Although the target diseases for pancreas transplantation may have a narrow range, recurrent primary DM could have a large negative impact on graft survival. Pancreas transplant is basically indicated for patients with type 1 insulin dependent DM or after total pancreatectomy. However, several studies, discussed below, showed that type 2 DM patients without significant insulin resistant can also become candidates for pancreas transplant with acceptable outcomes. In order to control primary outcomes, DM is a key for achieving excellent outcomes. In this section, we describe type 1 DM, which may recur after transplant.

Type 1 Diabetes Mellitus

Type 1 DM can be classified as an autoimmune disease. Pancreas-islet-related autoantibodies are frequently identified. Although up to 20 or more different autoantibodies have been reported, islet cell Abs (ICA), insulin autoantibody (IAA), glutamic acid decarboxylase 65 (GAD65) antibody, insulinoma-associated protein-2 (IA-2) antibody, and zinc transporter 8 (ZnT8) autoantibody have been considered as clinically important autoantibodies [204]. ICA was originally discovered by Bottazzo et al. in 1974 [205]. Measuring ICA is the gold-standard method for diagnosing and predicting type 1 DM, primarily due to its high sensitivity and specificity. However, it is unreasonable to use this in the clinical setting, as it involves a complicated procedure. Furthermore, GAD65 and IA-2 have been identified as the mainly corresponding antigens of ICA [206]. Therefore, alternatively, anti-GAD65 and IA-2 Abs seem to be substituted clinically for ICA.

The overall rate of developing type 1 DM after pancreas transplantation is relatively low, provided that effective immunosuppression is introduced [207]. However, as a typical example, recurrence occurred in twins or HLA-identical siblings who underwent living-related pancreas transplantation with minimal effects of immunosuppression [208]. These studies point to the importance of common HLA sharing and immunosuppression. The existence of HLA-DR3 and HLA-DR4 in the recipient's allele is particularly considered as a risk factor. HLA-DR allele sharing also seems to be an unfavorable factor regarding recurrence [209]. Conversely, even though conventional immunosuppression was introduced for HLA-mismatched pancreas transplantation, type 1 DM recurrence was observed. It has been reported that autoantibody positivity is related to poor glucose tolerance, although this study did not include histologic examination and discards the possibilities of other causes [210].

Thus, it is reasonable to believe that a certain population is vulnerable in nature to the recurrence of an autoimmune disease. By employing a progression of immunosuppressive medications, the Miami group reported a reduction in the recurrence rate in type 1 DM. This effect may be due to induction therapy, such as anti-CD25 antibody or thymoglobulin, rather than maintenance immunosuppression [209]. However, the administration of 3–4 medications in combination may additionally suppress recurrence.

5. Feature Perspective and Concluding Remarks

ABMR and a recurrence of primary disease are looming subjects that hinder the achievement of excellent long-term graft survival. It has been considered that ABMR is prominent in renal transplant rather than liver and pancreas transplants. Nevertheless, as a development of immunological assessments, the identification of ABMR in liver and pancreas transplants has become more apparent than before. Due to the fact that there seems to be common immunological reactions in these different organs, integrated approaches from different fields could accelerate the understanding of ABMR. Furthermore, knowing the nature of the primary disease would also facilitate tailoring immunosuppression to mitigate the risk of recurrence.

Lowering immunosuppression under a certain threshold could trigger the onset of these two distinctive conditions. Thus, it would be ideal to find more precise thresholds for each case by monitoring the recipients' immunological status. Regarding induction therapies, a single induction at pre-transplant or peri-transplant may be unfeasible with respect to maintaining the condition free from both ABMR and recurrence in the long term, because the recovery of immunity occurs over the course of post-transplant. However, more specific tolerance induction or complete remission from the primary disease could alleviate the risk of both entities or realize a better condition. ABMR and recurrence of primary diseases have their own preferences regarding donor immunological backgrounds, which would be contrary to each other. Donor selection could be arranged by estimating the advantages and disadvantages. Adjustment of these factors could result in improved outcomes in organ transplantation.

Author Contributions: Conceptualization, T.N.; writing—original draft preparation, T.N. and T.S.; writing—review and editing, T.N. and T.S.; funding acquisition, T.N. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: The APC was funded by JSPS KAKENHI Grant Number 19K18066.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ABMR	antibody-mediated rejection
Abs	antibodies
AIH	autoimmune hepatitis
AMA	anti-mitochondrial antibodies
ANCA	anti-neutrophil cytoplasmic autoantibodies
anti-HBc Abs	anti-hepatitis B core antibodies
CNI	calcineurin inhibitors
CR	clinical recurrence
DAA	direct-acting antivirals
DM	diabetes mellitus
dnDSA	de novo donor specific anti-HLA antibodies
DSA	donor specific anti-HLA antibodies
FGN	fibrillary glomerulonephritis
FSGS	focal segmental glomerulosclerosis
g-DSA	intra-graft DSA
GAD65	glutamic acid decarboxylase 65
GBM	anti-glomerular basement membrane
GdIgA1	galactose-deficient IgA1
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus

HLA	human leukocyte antigen
IAA	insulin autoantibody
IA-2	insulinoma-associated protein-2
ICA	islet cell antibodies
IFTA	interstitial fibrosis and tubular atrophy
IgAN	IgA nephropathy
IVIG	intravenous immunoglobulin therapy
mCR	mild-to-moderate clinical recurrence
MELD	model for end-stage liver disease
MN	membranous nephropathy
MPGN	membranoproliferative glomerulonephritis
mTOR	mammalian target of rapamycin
PBC	primary biliary cholangitis
PLA2R	anti-phospholipase A2 receptor antibodies
PR	pathological recurrence
PSC	primary sclerosing cholangitis
RPGN	rapidly progressive glomerulonephritis
s-DSA	serum DSA
sCR	severe clinical recurrence
SLE	systemic lupus erythematosus
suPAR	soluble urokinase type plasminogen activator receptor
SVR	sustained virological response
TCMR	T cell-mediated rejection
THSD7A	thrombospondin type-1 domain-containing 7A antibodies
UDCA	ursodeoxycholic acid
ZnT8	zinc transporter 8
αIgA	anti-GdIgA1 IgG

References

1. Wiebe, C.; Gibson, I.W.; Blydt-Hansen, T.D.; Karpinski, M.; Ho, J.; Storsley, L.J.; Goldberg, A.; Birk, P.E.; Rush, D.N.; Nickerson, P.W. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am. J. Transplant.* **2012**, *12*, 1157–1167. [CrossRef] [PubMed]
2. Nakamura, T.; Shirouzu, T.; Nakata, K.; Yoshimura, N.; Ushigome, H. The Role of Major Histocompatibility Complex in Organ Transplantation-Donor Specific Anti-Major Histocompatibility Complex Antibodies Analysis Goes to the Next Stage. *Int. J. Mol. Sci.* **2019**, *20*, 4544. [CrossRef] [PubMed]
3. Nakamura, T.; Ushigome, H.; Watabe, K.; Imanishi, Y.; Masuda, K.; Matsuyama, T.; Harada, S.; Koshino, K.; Iida, T.; Nobori, S.; et al. Graft Immunocomplex Capture Fluorescence Analysis to Detect Donor-Specific Antibodies and HLA Antigen Complexes in the Allograft. *Immunol. Investig.* **2017**, *46*, 295–304. [CrossRef]
4. Solez, K.; Axelsen, R.A.; Benediktsson, H.; Burdick, J.F.; Cohen, A.H.; Colvin, R.B.; Croker, B.P.; Droz, D.; Dunnill, M.S.; Halloran, P.F.; et al. International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int.* **1993**, *44*, 411–422. [CrossRef] [PubMed]
5. Racusen, L.C.; Colvin, R.B.; Solez, K.; Mihatsch, M.J.; Halloran, P.F.; Campbell, P.M.; Cecka, M.J.; Cosyns, J.P.; Demetris, A.J.; Fishbein, M.C.; et al. Antibody-mediated rejection criteria—An addition to the Banff 97 classification of renal allograft rejection. *Am. J. Transplant.* **2003**, *3*, 708–714. [CrossRef] [PubMed]
6. Mannon, R.B.; Matas, A.J.; Grande, J.; Leduc, R.; Connett, J.; Kasiske, B.; Cecka, J.M.; Gaston, R.S.; Cosio, F.; Gourishankar, S.; et al. Inflammation in areas of tubular atrophy in kidney allograft biopsies: A potent predictor of allograft failure. *Am. J. Transplant.* **2010**, *10*, 2066–2073. [CrossRef]
7. Cherukuri, A.; Mehta, R.; Sharma, A.; Sood, P.; Zeevi, A.; Tevar, A.D.; Rothstein, D.M.; Hariharan, S. Post-transplant donor specific antibody is associated with poor kidney transplant outcomes only when combined with both T-cell-mediated rejection and non-adherence. *Kidney Int.* **2019**, *96*, 202–213. [CrossRef] [PubMed]
8. Devos, J.M.; Gaber, A.O.; Teeter, L.D.; Graviss, E.A.; Patel, S.J.; Land, G.A.; Moore, L.W.; Knight, R.J. Intermediate-term graft loss after renal transplantation is associated with both donor-specific antibody and acute rejection. *Transplantation* **2014**, *97*, 534–540. [CrossRef] [PubMed]
9. Heilman, R.L.; Nijim, A.; Desmarteau, Y.M.; Khamash, H.; Pando, M.J.; Smith, M.L.; Chakkera, H.A.; Huskey, J.; Valdez, R.; Reddy, K.S. De novo donor-specific human leukocyte antigen antibodies early after kidney transplantation. *Transplantation* **2014**, *98*, 1310–1315. [CrossRef] [PubMed]
10. Everly, M.J.; Rebellato, L.M.; Haisch, C.E.; Ozawa, M.; Parker, K.; Briley, K.P.; Catrou, P.G.; Bolin, P.; Kendrick, W.T.; Kendrick, S.A.; et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation* **2013**, *95*, 410–417. [CrossRef] [PubMed]

11. Wan, S.S.; Chadban, S.J.; Watson, N.; Wyburn, K. Development and outcomes of de novo donor-specific antibodies in low, moderate, and high immunological risk kidney transplant recipients. *Am. J. Transplant.* **2020**, *20*, 1351–1364. [CrossRef] [PubMed]
12. De Fijter, J.W.; Holdaas, H.; Øyen, O.; Sanders, J.S.; Sundar, S.; Bemelman, F.J.; Sommerer, C.; Pascual, J.; Avihingsanon, Y.; Pongskul, C.; et al. Early Conversion From Calcineurin Inhibitor- to Everolimus-Based Therapy Following Kidney Transplantation: Results of the Randomized ELEVATE Trial. *Am. J. Transplant.* **2017**, *17*, 1853–1867. [CrossRef] [PubMed]
13. Filler, G.; Todorova, E.K.; Bax, K.; Alvarez-Elías, A.C.; Huang, S.H.; Kobrzynski, M.C. Minimum mycophenolic acid levels are associated with donor-specific antibody formation. *Pediatr. Transplant.* **2016**, *20*, 34–38. [CrossRef]
14. Solomon, S.; Colovai, A.; Del Rio, M.; Hayde, N. Tacrolimus variability is associated with de novo donor-specific antibody development in pediatric renal transplant recipients. *Pediatr. Nephrol.* **2020**, *35*, 261–270. [CrossRef] [PubMed]
15. Croze, L.E.; Tetaz, R.; Roustit, M.; Malvezzi, P.; Janbon, B.; Jouve, T.; Pinel, N.; Masson, D.; Quesada, J.L.; Bayle, F.; et al. Conversion to mammalian target of rapamycin inhibitors increases risk of de novo donor-specific antibodies. *Transpl. Int.* **2014**, *27*, 775–783. [CrossRef]
16. Bertrand, D.; Gatault, P.; Jauréguy, M.; Garrouste, C.; Sayegh, J.; Bouvier, N.; Caillard, S.; Lanfranco, L.; Galinier, A.; Laurent, C.; et al. Protocol Biopsies in Patients With Subclinical De Novo Donor-specific Antibodies After Kidney Transplantation: A Multicentric Study. *Transplantation* **2020**, *104*, 1726–1737. [CrossRef] [PubMed]
17. Delgado, J.C.; Fuller, A.; Ozawa, M.; Smith, L.; Terasaki, P.I.; Shihab, F.S.; Eckels, D.D. No occurrence of de novo HLA antibodies in patients with early corticosteroid withdrawal in a 5-year prospective randomized study. *Transplantation* **2009**, *87*, 546–548. [CrossRef] [PubMed]
18. Nakamura, T.; Ushigome, H.; Shirouzu, T.; Yoshimura, N. *Donor Specific anti-HLA Antibodies in Organ Transplantation—Transition from Serum DSA to Intra-Graft DSA*, November 5th 2018 ed.; Mahdi, B.M., Ed.; Intech: London, UK, 2019; pp. 19–41.
19. Eskandary, F.; Regele, H.; Baumann, L.; Bond, G.; Kozakowski, N.; Wahrmann, M.; Hidalgo, L.G.; Haslacher, H.; Kaltenecker, C.C.; Aretin, M.B.; et al. A Randomized Trial of Bortezomib in Late Antibody-Mediated Kidney Transplant Rejection. *J. Am. Soc. Nephrol. JASN* **2018**, *29*, 591–605. [CrossRef] [PubMed]
20. Kulkarni, S.; Kirkiles-Smith, N.C.; Deng, Y.H.; Formica, R.N.; Moeckel, G.; Broecker, V.; Bow, L.; Tomlin, R.; Pober, J.S. Eculizumab Therapy for Chronic Antibody-Mediated Injury in Kidney Transplant Recipients: A Pilot Randomized Controlled Trial. *Am. J. Transplant* **2017**, *17*, 682–691. [CrossRef]
21. Choi, J.; Aubert, O.; Vo, A.; Loupy, A.; Haas, M.; Puliyanda, D.; Kim, I.; Louie, S.; Kang, A.; Peng, A.; et al. Assessment of Tocilizumab (Anti-Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients. *Am. J. Transplant.* **2017**, *17*, 2381–2389. [CrossRef] [PubMed]
22. Shin, B.H.; Everly, M.J.; Zhang, H.; Choi, J.; Vo, A.; Zhang, X.; Huang, E.; Jordan, S.C.; Toyoda, M. Impact of Tocilizumab (Anti-IL-6R) Treatment on Immunoglobulins and Anti-HLA Antibodies in Kidney Transplant Patients With Chronic Antibody-mediated Rejection. *Transplantation* **2020**, *104*, 856–863. [CrossRef]
23. Doberer, K.; Duerr, M.; Halloran, P.F.; Eskandary, F.; Budde, K.; Regele, H.; Reeve, J.; Borski, A.; Kozakowski, N.; Reindl-Schwaighofer, R.; et al. A Randomized Clinical Trial of Anti-IL-6 Antibody Clazakizumab in Late Antibody-Mediated Kidney Transplant Rejection. *J. Am. Soc. Nephrol. JASN* **2021**, *32*, 708–722. [CrossRef] [PubMed]
24. Bray, R.A.; Gebel, H.M.; Townsend, R.; Roberts, M.E.; Polinsky, M.; Yang, L.; Meier-Kriesche, H.U.; Larsen, C.P. De novo donor-specific antibodies in belatacept-treated vs cyclosporine-treated kidney-transplant recipients: Post hoc analyses of the randomized phase III BENEFIT and BENEFIT-EXT studies. *Am. J. Transplant.* **2018**, *18*, 1783–1789. [CrossRef] [PubMed]
25. Vandevoorde, K.; Ducreux, S.; Bosch, A.; Guillaud, O.; Hervieu, V.; Chambon-Augoyard, C.; Poinsot, D.; André, P.; Scoazec, J.Y.; Robinson, P.; et al. Prevalence, Risk Factors, and Impact of Donor-Specific Alloantibodies After Adult Liver Transplantation. *Liver Transplant.* **2018**, *24*, 1091–1100. [CrossRef] [PubMed]
26. Demetris, A.J.; Bellamy, C.; Hubscher, S.G.; O'Leary, J.; Randhawa, P.S.; Feng, S.; Neil, D.; Colvin, R.B.; McCaughan, G.; Fung, J.J.; et al. 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *Am. J. Transplant.* **2016**, *16*, 2816–2835. [CrossRef]
27. Nakamura, T.; Shirouzu, T.; Kawai, S.; Sugimoto, R.; Harada, S.; Yoshikawa, M.; Nobori, S.; Ushigome, H. Intra–Liver Allograft C3d–Binding Donor Specific anti–HLA Antibodies Predict Rejection after Liver Transplantation. *Transplant. Proc.* **2022**, in press.
28. Gugenheim, J.; Le Thai, B.; Rouger, P.; Gigou, M.; Gane, P.; Vial, M.C.; Charpentier, B.; Reynes, M.; Bismuth, H. Relationship between the liver and lymphocytotoxic alloantibodies in inbred rats. Specific absorption by nonparenchymal liver cells. *Transplantation* **1988**, *45*, 474–478. [CrossRef] [PubMed]
29. Chauhan, B.; Phelan, D.L.; Marsh, J.W.; Mohanakumar, T. Characterization of antiidiotypic antibodies to donor HLA that develop after liver transplantation. *Transplantation* **1993**, *56*, 443–448. [CrossRef]
30. Astarcioglu, I.; Cursio, R.; Reynes, M.; Gugenheim, J. Increased risk of antibody-mediated rejection of reduced-size liver allografts. *J. Surg. Res.* **1999**, *87*, 258–262. [CrossRef] [PubMed]
31. Liu, X.; Xu, J.; Brenner, D.A.; Kisseleva, T. Reversibility of Liver Fibrosis and Inactivation of Fibrogenic Myofibroblasts. *Curr. Pathobiol. Rep.* **2013**, *1*, 209–214. [CrossRef] [PubMed]
32. Feng, S.; Bucuvalas, J.C.; Mazariegos, G.V.; Magee, J.C.; Sanchez-Fueyo, A.; Spain, K.M.; Lesniak, A.; Kanaparthi, S.; Perito, E.; Venkat, V.L.; et al. Efficacy and Safety of Immunosuppression Withdrawal in Pediatric Liver Transplant Recipients: Moving Toward Personalized Management. *Hepatology* **2021**, *73*, 1985–2004. [CrossRef] [PubMed]

33. Schluckebier, D.; Cousin, V.L.; Petit, L.M.; Belli, D.; Wildhaber, B.; Rougemont, A.L.; Villard, J.; Ferrari-Lacraz, S.; McLin, V.A. Preformed and de novo DSA are associated with T-cell-mediated rejection in pediatric liver transplant recipients requiring clinically indicated liver biopsy. *Pediatr. Transplant.* **2020**, *24*, e13611. [CrossRef] [PubMed]
34. Miyagawa-Hayashino, A.; Yoshizawa, A.; Uchida, Y.; Egawa, H.; Yurugi, K.; Masuda, S.; Minamiguchi, S.; Maekawa, T.; Uemoto, S.; Haga, H. Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts. *Liver Transplant.* **2012**, *18*, 1333–1342. [CrossRef] [PubMed]
35. Del Bello, A.; Congy-Jolivet, N.; Muscari, F.; Lavayssière, L.; Esposito, L.; Cardeau-Desangles, I.; Guitard, J.; Dörr, G.; Suc, B.; Duffas, J.P.; et al. Prevalence, incidence and risk factors for donor-specific anti-HLA antibodies in maintenance liver transplant patients. *Am. J. Transplant.* **2014**, *14*, 867–875. [CrossRef] [PubMed]
36. Höfer, A.; Jonigk, D.; Hartleben, B.; Verboom, M.; Hallensleben, M.; Hübscher, S.G.; Manns, M.P.; Jaeckel, E.; Taubert, R. DSA Are Associated With More Graft Injury, More Fibrosis, and Upregulation of Rejection-associated Transcripts in Subclinical Rejection. *Transplantation* **2020**, *104*, 551–561. [CrossRef] [PubMed]
37. Kaneku, H.; O'Leary, J.G.; Banuelos, N.; Jennings, L.W.; Susskind, B.M.; Klintmalm, G.B.; Terasaki, P.I. De novo donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. *Am. J. Transplant.* **2013**, *13*, 1541–1548. [CrossRef] [PubMed]
38. Del Bello, A.; Congy-Jolivet, N.; Danjoux, M.; Muscari, F.; Lavayssière, L.; Esposito, L.; Cardeau-Desangles, I.; Guitard, J.; Dörr, G.; Milongo, D.; et al. De novo donor-specific anti-HLA antibodies mediated rejection in liver-transplant patients. *Transpl. Int.* **2015**, *28*, 1371–1382. [CrossRef] [PubMed]
39. Sakamoto, S.; Akamatsu, N.; Hasegawa, K.; Ohdan, H.; Nakagawa, K.; Egawa, H. The efficacy of rituximab treatment for antibody-mediated rejection in liver transplantation: A retrospective Japanese nationwide study. *Hepatol. Res.* **2021**, *51*, 990–999. [CrossRef]
40. De Kort, H.; Munivenkatappa, R.B.; Berger, S.P.; Eikmans, M.; van der Wal, A.; de Koning, E.J.; van Kooten, C.; de Heer, E.; Barth, R.N.; Bruijn, J.A.; et al. Pancreas allograft biopsies with positive c4d staining and anti-donor antibodies related to worse outcome for patients. *Am. J. Transpl.* **2010**, *10*, 1660–1667. [CrossRef] [PubMed]
41. De Kort, H.; Mallat, M.J.; van Kooten, C.; de Heer, E.; Brand-Schaaf, S.H.; van der Wal, A.M.; Roufosse, C.; Roelen, D.L.; Bruijn, J.A.; Claas, F.H.; et al. Diagnosis of early pancreas graft failure via antibody-mediated rejection: Single-center experience with 256 pancreas transplantations. *Am. J. Transpl.* **2014**, *14*, 936–942. [CrossRef]
42. Cantarovich, D.; De Amicis, S.; Akl, A.; Devys, A.; Vistoli, F.; Karam, G.; Soulillou, J.P. Posttransplant donor-specific anti-HLA antibodies negatively impact pancreas transplantation outcome. *Am. J. Transpl.* **2011**, *11*, 2737–2746. [CrossRef] [PubMed]
43. Parajuli, S.; Alagusundaramoorthy, S.; Aziz, F.; Garg, N.; Redfield, R.R.; Sollinger, H.; Kaufman, D.; Djamali, A.; Odorico, J.; Mandelbrot, D. Outcomes of Pancreas Transplant Recipients with De Novo Donor-specific Antibodies. *Transplantation* **2019**, *103*, 435–440. [CrossRef]
44. Ladowski, J.M.; Mullins, H.; Romine, M.; Kloda, D.; Young, C.; Hauptfeld-Dolejsek, V.; Houp, J.; Locke, J. Eplet mismatch scores and de novo donor-specific antibody development in simultaneous pancreas-kidney transplantation. *Hum. Immunol.* **2021**, *82*, 139–146. [CrossRef] [PubMed]
45. Chaigne, B.; Geneugelijk, K.; Bédat, B.; Ahmed, M.A.; Hönger, G.; De Seigneux, S.; Demuylder-Mischler, S.; Berney, T.; Spierings, E.; Ferrari-Lacraz, S.; et al. Immunogenicity of Anti-HLA Antibodies in Pancreas and Islet Transplantation. *Cell Transpl.* **2016**, *25*, 2041–2050. [CrossRef] [PubMed]
46. Uva, P.D.; Quevedo, A.; Roses, J.; Toniolo, M.F.; Pilotti, R.; Chuluyan, E.; Casadei, D.H. Anti-Hla donor-specific antibody monitoring in pancreas transplantation: Role of protocol biopsies. *Clin. Transpl.* **2020**, *34*, e13998. [CrossRef] [PubMed]
47. Drachenberg, C.B.; Torrealba, J.R.; Nankivell, B.J.; Rangel, E.B.; Bajema, I.M.; Kim, D.U.; Arend, L.; Bracamonte, E.R.; Bromberg, J.S.; Bruijn, J.A.; et al. Guidelines for the diagnosis of antibody-mediated rejection in pancreas allografts-updated Banff grading schema. *Am. J. Transpl.* **2011**, *11*, 1792–1802. [CrossRef] [PubMed]
48. Gunther Brockmann, J.; Butt, A.; AlHussaini, H.F.; AlMana, H.; AlSaad, K.; Al-Awwami, M.; Clemens Broering, D.; Ali, T. Protocol Duodenal Graft Biopsies Aid Pancreas Graft Surveillance. *Transplantation* **2019**, *103*, 622–629. [CrossRef]
49. Moreso, F.; Crespo, M.; Ruiz, J.C.; Torres, A.; Gutierrez-Dalmau, A.; Osuna, A.; Perello, M.; Pascual, J.; Torres, I.B.; Redondo-Pachon, D.; et al. Treatment of chronic antibody mediated rejection with intravenous immunoglobulins and rituximab: A multicenter, prospective, randomized, double-blind clinical trial. *Am. J. Transplant.* **2018**, *18*, 927–935. [CrossRef]
50. Redfield, R.R.; Ellis, T.M.; Zhong, W.; Scalea, J.R.; Zens, T.J.; Mandelbrot, D.; Muth, B.L.; Panzer, S.; Samaniego, M.; Kaufman, D.B.; et al. Current outcomes of chronic active antibody mediated rejection—A large single center retrospective review using the updated BANFF 2013 criteria. *Hum. Immunol.* **2016**, *77*, 346–352. [CrossRef]
51. Guerra, M.R.; Naini, B.V.; Scapa, J.V.; Reed, E.F.; Busuttil, R.W.; Cheng, E.Y.; Farmer, D.G.; Vargas, J.H.; Venick, R.S.; McDiarmid, S.V.; et al. Obliterative portal venopathy: A histopathologic finding associated with chronic antibody-mediated rejection in pediatric liver allografts. *Pediatr. Transplant.* **2018**, *22*, e13124. [CrossRef] [PubMed]
52. Kawabe, M.; Yamamoto, I.; Komatsuzaki, Y.; Yamakawa, T.; Katsumata, H.; Katsuma, A.; Mafune, A.; Nakada, Y.; Kobayashi, A.; Tanno, Y.; et al. Recurrence and graft loss after renal transplantation in adults with IgA vasculitis. *Clin. Exp. Nephrol.* **2017**, *21*, 714–720. [CrossRef]
53. Ponticelli, C.; Traversi, L.; Feliciani, A.; Cesana, B.M.; Banfi, G.; Tarantino, A. Kidney transplantation in patients with IgA mesangial glomerulonephritis. *Kidney Int.* **2001**, *60*, 1948–1954. [CrossRef]

54. Di Vico, M.C.; Messina, M.; Fop, F.; Barreca, A.; Segoloni, G.P.; Biancone, L. Recurrent IgA nephropathy after renal transplantation and steroid withdrawal. *Clin. Transplant.* **2018**, *32*, e13207. [CrossRef] [PubMed]
55. Mousson, C.; Charon-Barra, C.; Funes de la Vega, M.; Tanter, Y.; Justrabo, E.; Martin, L.; Rifle, G. Recurrence of IgA nephropathy with crescents in kidney transplants. *Transplant. Proc.* **2007**, *39*, 2595–2596. [CrossRef]
56. Clayton, P.; McDonald, S.; Chadban, S. Steroids and recurrent IgA nephropathy after kidney transplantation. *Am. J. Transplant.* **2011**, *11*, 1645–1649. [CrossRef] [PubMed]
57. Rodas, L.M.; Ruiz-Ortiz, E.; Garcia-Herrera, A.; Pereira, A.; Blasco, M.; Ventura-Aguiar, P.; Viñas Gomis, O.; Egri, N.; De Sousa, E.; Palou, E.; et al. IgA Nephropathy Recurrence after Kidney Transplantation: Role of Recipient Age and Human Leukocyte Antigen-B Mismatch. *Am. J. Nephrol.* **2020**, *51*, 357–365. [CrossRef]
58. Suzuki, H.; Fan, R.; Zhang, Z.; Brown, R.; Hall, S.; Julian, B.A.; Chatham, W.W.; Suzuki, Y.; Wyatt, R.J.; Moldoveanu, Z.; et al. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J. Clin. Investig.* **2009**, *119*, 1668–1677. [CrossRef] [PubMed]
59. Suzuki, Y.; Matsuzaki, K.; Suzuki, H.; Okazaki, K.; Yanagawa, H.; Ieiri, N.; Sato, M.; Sato, T.; Taguma, Y.; Matsuoka, J.; et al. Serum levels of galactose-deficient immunoglobulin (Ig) A1 and related immune complex are associated with disease activity of IgA nephropathy. *Clin. Exp. Nephrol.* **2014**, *18*, 770–777. [CrossRef]
60. Rizk, D.V.; Saha, M.K.; Hall, S.; Novak, L.; Brown, R.; Huang, Z.Q.; Fatima, H.; Julian, B.A.; Novak, J. Glomerular Immunodeposits of Patients with IgA Nephropathy Are Enriched for IgG Autoantibodies Specific for Galactose-Deficient IgA1. *J. Am. Soc. Nephrol. JASN* **2019**, *30*, 2017–2026. [CrossRef] [PubMed]
61. Berthoux, F.; Suzuki, H.; Mohey, H.; Maillard, N.; Mariat, C.; Novak, J.; Julian, B.A. Prognostic Value of Serum Biomarkers of Autoimmunity for Recurrence of IgA Nephropathy after Kidney Transplantation. *J. Am. Soc. Nephrol. JASN* **2017**, *28*, 1943–1950. [CrossRef]
62. Nakamura, T.; Shirouzu, T.; Harada, S.; Sugimoto, R.; Nobori, S.; Yoshikawa, M.; Ushigome, H.; Kawai, S. The Abundance of Anti-Galactose-deficient IgA1 Autoantibodies Results in Glomerular Deposition and IgA Nephropathy Recurrence after Renal Transplantation. *Transplantation* **2021**, *105*. in press. [CrossRef]
63. Kawabe, M.; Yamamoto, I.; Yamakawa, T.; Katsumata, H.; Isaka, N.; Katsuma, A.; Nakada, Y.; Kobayashi, A.; Koike, K.; Ueda, H.; et al. Association Between Galactose-Deficient IgA1 Derived From the Tonsils and Recurrence of IgA Nephropathy in Patients Who Underwent Kidney Transplantation. *Front. Immunol.* **2020**, *11*, 2068. [CrossRef]
64. Okumi, M.; Okada, D.; Unagami, K.; Kakuta, Y.; Iizuka, J.; Takagi, T.; Shirakawa, H.; Omoto, K.; Ishida, H.; Tanabe, K. Higher immunoglobulin A nephropathy recurrence in related-donor kidney transplants: The Japan Academic Consortium of Kidney Transplantation study. *Int. J. Urol.* **2019**, *26*, 903–909. [CrossRef]
65. Messina, M.; di Vico, M.C.; Ariaudo, C.; Mazzucco, G.; Fop, F.; Segoloni, G.P.; Biancone, L. Treatment protocol with pulse and oral steroids for IgA Nephropathy after kidney transplantation. *J. Nephrol.* **2016**, *29*, 575–583. [CrossRef]
66. Jiang, S.H.; Kennard, A.L.; Walters, G.D. Recurrent glomerulonephritis following renal transplantation and impact on graft survival. *BMC Nephrol.* **2018**, *19*, 344. [CrossRef]
67. Alasfar, S.; Matar, D.; Montgomery, R.A.; Desai, N.; Lonze, B.; Vujjini, V.; Estrella, M.M.; Manllo Dieck, J.; Khneizer, G.; Sever, S.; et al. Rituximab and Therapeutic Plasma Exchange in Recurrent Focal Segmental Glomerulosclerosis Postkidney Transplantation. *Transplantation* **2018**, *102*, e115–e120. [CrossRef]
68. Francis, A.; Trnka, P.; McTaggart, S.J. Long-Term Outcome of Kidney Transplantation in Recipients with Focal Segmental Glomerulosclerosis. *Clin. J. Am. Soc. Nephrol. CJASN* **2016**, *11*, 2041–2046. [CrossRef]
69. Uffing, A.; Pérez-Sáez, M.J.; Mazzali, M.; Manfro, R.C.; Bauer, A.C.; de Sottomaior Drumond, F.; O'Shaughnessy, M.M.; Cheng, X.S.; Chin, K.K.; Ventura, C.G.; et al. Recurrence of FSGS after Kidney Transplantation in Adults. *Clin. J. Am. Soc. Nephrol. CJASN* **2020**, *15*, 247–256. [CrossRef]
70. Allen, P.J.; Chadban, S.J.; Craig, J.C.; Lim, W.H.; Allen, R.D.M.; Clayton, P.A.; Teixeira-Pinto, A.; Wong, G. Recurrent glomerulonephritis after kidney transplantation: Risk factors and allograft outcomes. *Kidney Int.* **2017**, *92*, 461–469. [CrossRef]
71. Gallon, L.; Leventhal, J.; Skaro, A.; Kanwar, Y.; Alvarado, A. Resolution of recurrent focal segmental glomerulosclerosis after retransplantation. *N. Engl. J. Med.* **2012**, *366*, 1648–1649. [CrossRef]
72. Sharma, M.; Sharma, R.; Reddy, S.R.; McCarthy, E.T.; Savin, V.J. Proteinuria after injection of human focal segmental glomerulosclerosis factor. *Transplantation* **2002**, *73*, 366–372. [CrossRef] [PubMed]
73. Wei, C.; El Hindi, S.; Li, J.; Fornoni, A.; Goes, N.; Sageshima, J.; Maiguel, D.; Karumanchi, S.A.; Yap, H.K.; Saleem, M.; et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat. Med.* **2011**, *17*, 952–960. [CrossRef]
74. Alachkar, N.; Wei, C.; Arend, L.J.; Jackson, A.M.; Racusen, L.C.; Fornoni, A.; Burke, G.; Rabb, H.; Kakkad, K.; Reiser, J.; et al. Podocyte effacement closely links to suPAR levels at time of posttransplantation focal segmental glomerulosclerosis occurrence and improves with therapy. *Transplantation* **2013**, *96*, 649–656. [CrossRef]
75. Verghese, P.S.; Rheault, M.N.; Jackson, S.; Matas, A.J.; Chinnakotla, S.; Chavers, B. The effect of peri-transplant plasmapheresis in the prevention of recurrent FSGS. *Pediatr. Transplant.* **2018**, *22*, e13154. [CrossRef]
76. Kennard, A.L.; Jiang, S.H.; Walters, G.D. Increased glomerulonephritis recurrence after living related donation. *BMC Nephrol.* **2017**, *18*, 25. [CrossRef]

77. Lanaret, C.; Anglicheau, D.; Audard, V.; Büchler, M.; Caillard, S.; Couzi, L.; Malvezzi, P.; Mesnard, L.; Bertrand, D.; Martinez, F.; et al. Rituximab for recurrence of primary focal segmental glomerulosclerosis after kidney transplantation: Results of a nationwide study. *Am. J. Transplant.* **2021**, *21*, 3021–3033. [CrossRef] [PubMed]
78. Staeck, O.; Slowinski, T.; Lieker, I.; Wu, K.; Rudolph, B.; Schmidt, D.; Brakemeier, S.; Neumayer, H.H.; Wei, C.; Reiser, J.; et al. Recurrent Primary Focal Segmental Glomerulosclerosis Managed With Intensified Plasma Exchange and Concomitant Monitoring of Soluble Urokinase-Type Plasminogen Activator Receptor-Mediated Podocyte β3-integrin Activation. *Transplantation* **2015**, *99*, 2593–2597. [CrossRef]
79. Sethi, S.; Nester, C.M.; Smith, R.J. Membranoproliferative glomerulonephritis and C3 glomerulopathy: Resolving the confusion. *Kidney Int.* **2012**, *81*, 434–441. [CrossRef]
80. Fakhouri, F.; Le Quintrec, M.; Frémeaux-Bacchi, V. Practical management of C3 glomerulopathy and Ig-mediated MPGN: Facts and uncertainties. *Kidney Int.* **2020**, *98*, 1135–1148. [CrossRef]
81. Sethi, S.; Fervenza, F.C. Membranoproliferative glomerulonephritis: Pathogenetic heterogeneity and proposal for a new classification. *Semin. Nephrol.* **2011**, *31*, 341–348. [CrossRef]
82. Regunathan-Shenk, R.; Avasare, R.S.; Ahn, W.; Canetta, P.A.; Cohen, D.J.; Appel, G.B.; Bomback, A.S. Kidney Transplantation in C3 Glomerulopathy: A Case Series. *Am. J. Kidney Dis.* **2019**, *73*, 316–323. [CrossRef]
83. Green, H.; Rahamimov, R.; Rozen-Zvi, B.; Pertzov, B.; Tobar, A.; Lichtenberg, S.; Gafter, U.; Mor, E. Recurrent membranoproliferative glomerulonephritis type I after kidney transplantation: A 17-year single-center experience. *Transplantation* **2015**, *99*, 1172–1177. [CrossRef]
84. Gonzalez Suarez, M.L.; Thongprayoon, C.; Hansrivijit, P.; Kovvuru, K.; Kanduri, S.R.; Aeddula, N.R.; Pivovarova, A.I.; Chewcharat, A.; Bathini, T.; Mao, M.A.; et al. Treatment of C3 Glomerulopathy in Adult Kidney Transplant Recipients: A Systematic Review. *Med. Sci.* **2020**, *8*, 44. [CrossRef] [PubMed]
85. Moszczuk, B.; Kiryluk, K.; Pączek, L.; Mucha, K. Membranous Nephropathy: From Research Bench to Personalized Care. *J. Clin. Med.* **2021**, *10*, 1205. [CrossRef]
86. Beck, L.H., Jr.; Bonegio, R.G.; Lambeau, G.; Beck, D.M.; Powell, D.W.; Cummins, T.D.; Klein, J.B.; Salant, D.J. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N. Engl. J. Med.* **2009**, *361*, 11–21. [CrossRef]
87. Tomas, N.M.; Beck, L.H., Jr.; Meyer-Schwesinger, C.; Seitz-Polski, B.; Ma, H.; Zahner, G.; Dolla, G.; Hoxha, E.; Helmchen, U.; Dabert-Gay, A.S.; et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N. Engl. J. Med.* **2014**, *371*, 2277–2287. [CrossRef]
88. Sprangers, B.; Lefkowitz, G.I.; Cohen, S.D.; Stokes, M.B.; Valeri, A.; Appel, G.B.; Kunis, C.L. Beneficial effect of rituximab in the treatment of recurrent idiopathic membranous nephropathy after kidney transplantation. *Clin. J. Am. Soc. Nephrol. CJASN* **2010**, *5*, 790–797. [CrossRef]
89. Kattah, A.; Ayalon, R.; Beck, L.H., Jr.; Sethi, S.; Sandor, D.G.; Cosio, F.G.; Gandhi, M.J.; Lorenz, E.C.; Salant, D.J.; Fervenza, F.C. Anti-phospholipase A_2 receptor antibodies in recurrent membranous nephropathy. *Am. J. Transplant.* **2015**, *15*, 1349–1359. [CrossRef] [PubMed]
90. Tomas, N.M.; Hoxha, E.; Reinicke, A.T.; Fester, L.; Helmchen, U.; Gerth, J.; Bachmann, F.; Budde, K.; Koch-Nolte, F.; Zahner, G.; et al. Autoantibodies against thrombospondin type 1 domain-containing 7A induce membranous nephropathy. *J. Clin. Investig.* **2016**, *126*, 2519–2532. [CrossRef] [PubMed]
91. Batal, I.; Vasilescu, E.R.; Dadhania, D.M.; Adel, A.A.; Husain, S.A.; Avasare, R.; Serban, G.; Santoriello, D.; Khairallah, P.; Patel, A.; et al. Association of HLA Typing and Alloimmunity With Posttransplantation Membranous Nephropathy: A Multicenter Case Series. *Am. J. Kidney Dis.* **2020**, *76*, 374–383. [CrossRef] [PubMed]
92. Hickson, L.J.; Gera, M.; Amer, H.; Iqbal, C.W.; Moore, T.B.; Milliner, D.S.; Cosio, F.G.; Larson, T.S.; Stegall, M.D.; Ishitani, M.B.; et al. Kidney transplantation for primary focal segmental glomerulosclerosis: Outcomes and response to therapy for recurrence. *Transplantation* **2009**, *87*, 1232–1239. [CrossRef]
93. Yang, W.L.; Bose, B.; Zhang, L.; McStea, M.; Cho, Y.; Fahim, M.; Hawley, C.M.; Pascoe, E.M.; Johnson, D.W. Long-term outcomes of patients with end-stage kidney disease due to membranous nephropathy: A cohort study using the Australia and New Zealand Dialysis and Transplant Registry. *PLoS ONE* **2019**, *14*, e0221531. [CrossRef] [PubMed]
94. Choy, B.Y.; Chan, T.M.; Lo, S.K.; Lo, W.K.; Lai, K.N. Renal transplantation in patients with primary immunoglobulin A nephropathy. *Nephrol. Dial. Transplant.* **2003**, *18*, 2399–2404. [CrossRef]
95. Tamirou, F.; Houssiau, F.A. Management of Lupus Nephritis. *J. Clin. Med.* **2021**, *10*, 670. [CrossRef] [PubMed]
96. Seligman, V.A.; Lum, R.F.; Olson, J.L.; Li, H.; Criswell, L.A. Demographic differences in the development of lupus nephritis: A retrospective analysis. *Am. J. Med.* **2002**, *112*, 726–729. [CrossRef]
97. Hoffman, I.E.; Peene, I.; Meheus, L.; Huizinga, T.W.; Cebecauer, L.; Isenberg, D.; De Bosschere, K.; Hulstaert, F.; Veys, E.M.; De Keyser, F. Specific antinuclear antibodies are associated with clinical features in systemic lupus erythematosus. *Ann. Rheum. Dis.* **2004**, *63*, 1155–1158. [CrossRef]
98. Contreras, G.; Mattiazzi, A.; Guerra, G.; Ortega, L.M.; Tozman, E.C.; Li, H.; Tamariz, L.; Carvalho, C.; Kupin, W.; Ladino, M.; et al. Recurrence of lupus nephritis after kidney transplantation. *J. Am. Soc. Nephrol. JASN* **2010**, *21*, 1200–1207. [CrossRef] [PubMed]
99. Bunnapradist, S.; Chung, P.; Peng, A.; Hong, A.; Chung, P.; Lee, B.; Fukami, S.; Takemoto, S.K.; Singh, A.K. Outcomes of renal transplantation for recipients with lupus nephritis: Analysis of the Organ Procurement and Transplantation Network database. *Transplantation* **2006**, *82*, 612–618. [CrossRef]

100. Deegens, J.K.; Artz, M.A.; Hoitsma, A.J.; Wetzels, J.F. Outcome of renal transplantation in patients with systemic lupus erythematosus. *Transpl. Int.* **2003**, *16*, 411–418. [CrossRef]
101. Goral, S.; Ynares, C.; Shappell, S.B.; Snyder, S.; Feurer, I.D.; Kazancioglu, R.; Fogo, A.B.; Helderman, J.H. Recurrent lupus nephritis in renal transplant recipients revisited: It is not rare. *Transplantation* **2003**, *75*, 651–656. [CrossRef]
102. Ponticelli, C.; Moroni, G. Renal transplantation in lupus nephritis. *Lupus* **2005**, *14*, 95–98. [CrossRef]
103. Rovin, B.H.; Furie, R.; Latinis, K.; Looney, R.J.; Fervenza, F.C.; Sanchez-Guerrero, J.; Maciuca, R.; Zhang, D.; Garg, J.P.; Brunetta, P.; et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: The Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum.* **2012**, *64*, 1215–1226. [CrossRef] [PubMed]
104. Moroni, G.; Ponticelli, C. Rapidly progressive crescentic glomerulonephritis: Early treatment is a must. *Autoimmun. Rev.* **2014**, *13*, 723–729. [CrossRef] [PubMed]
105. Göçeroğlu, A.; Rahmattulla, C.; Berden, A.E.; Reinders, M.E.; Wolterbeek, R.; Steenbergen, E.J.; Hilbrands, L.B.; Noorlander, I.; Berger, S.P.; Peutz-Kootstra, C.J.; et al. The Dutch Transplantation in Vasculitis (DUTRAVAS) Study: Outcome of Renal Transplantation in Antineutrophil Cytoplasmic Antibody-associated Glomerulonephritis. *Transplantation* **2016**, *100*, 916–924. [CrossRef] [PubMed]
106. Little, M.A.; Hassan, B.; Jacques, S.; Game, D.; Salisbury, E.; Courtney, A.E.; Brown, C.; Salama, A.D.; Harper, L. Renal transplantation in systemic vasculitis: When is it safe? *Nephrol. Dial. Transplant.* **2009**, *24*, 3219–3225. [CrossRef]
107. Nachman, P.H.; Segelmark, M.; Westman, K.; Hogan, S.L.; Satterly, K.K.; Jennette, J.C.; Falk, R. Recurrent ANCA-associated small vessel vasculitis after transplantation: A pooled analysis. *Kidney Int.* **1999**, *56*, 1544–1550. [CrossRef] [PubMed]
108. Kluth, D.C.; Rees, A.J. Anti-glomerular basement membrane disease. *J. Am. Soc. Nephrol. JASN* **1999**, *10*, 2446–2453. [CrossRef]
109. Singh, T.; Kharadjian, T.B.; Astor, B.C.; Panzer, S.E. Long-term outcomes in kidney transplant recipients with end-stage kidney disease due to anti-glomerular basement membrane disease. *Clin. Transplant.* **2021**, *35*, e14179. [CrossRef]
110. Tang, W.; McDonald, S.P.; Hawley, C.M.; Badve, S.V.; Boudville, N.C.; Brown, F.G.; Clayton, P.A.; Campbell, S.B.; de Zoysa, J.R.; Johnson, D.W. Anti-glomerular basement membrane antibody disease is an uncommon cause of end-stage renal disease. *Kidney Int.* **2013**, *83*, 503–510. [CrossRef]
111. Netzer, K.O.; Merkel, F.; Weber, M. Goodpasture syndrome and end-stage renal failure–to transplant or not to transplant? *Nephrol. Dial. Transplant.* **1998**, *13*, 1346–1348. [CrossRef]
112. Lochhead, K.M.; Pirsch, J.D.; D'Alessandro, A.M.; Knechtle, S.J.; Kalayoglu, M.; Sollinger, H.W.; Belzer, F.O. Risk factors for renal allograft loss in patients with systemic lupus erythematosus. *Kidney Int.* **1996**, *49*, 512–517. [CrossRef] [PubMed]
113. Jennette, J.C.; Nachman, P.H. ANCA Glomerulonephritis and Vasculitis. *Clin. J. Am. Soc. Nephrol. CJASN* **2017**, *12*, 1680–1691. [CrossRef]
114. Ronco, P.M.; Aucouturier, P. The molecular bases of plasma cell dyscrasia-related renal diseases. *Nephrol. Dial. Transplant.* **1999**, *14* (Suppl. S1), 4–8. [CrossRef]
115. Röcken, C.; Sletten, K. Amyloid in surgical pathology. *Virchows Arch.* **2003**, *443*, 3–16. [CrossRef]
116. Vrana, J.A.; Gamez, J.D.; Madden, B.J.; Theis, J.D.; Bergen, H.R., 3rd; Dogan, A. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood* **2009**, *114*, 4957–4959. [CrossRef] [PubMed]
117. Abe, R.; Katoh, N.; Takahashi, Y.; Takasone, K.; Yoshinaga, T.; Yazaki, M.; Kametani, F.; Sekijima, Y. Distribution of amyloidosis subtypes based on tissue biopsy site—Consecutive analysis of 729 patients at a single amyloidosis center in Japan. *Pathol. Int.* **2021**, *71*, 70–79. [CrossRef]
118. Westermark, P.; Benson, M.D.; Buxbaum, J.N.; Cohen, A.S.; Frangione, B.; Ikeda, S.; Masters, C.L.; Merlini, G.; Saraiva, M.J.; Sipe, J.D. Amyloid: Toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. *Amyloid* **2005**, *12*, 1–4. [CrossRef] [PubMed]
119. Pinney, J.H.; Lachmann, H.J.; Sattianayagam, P.T.; Gibbs, S.D.; Wechalekar, A.D.; Venner, C.P.; Whelan, C.J.; Gilbertson, J.A.; Rowczenio, D.; Hawkins, P.N.; et al. Renal transplantation in systemic amyloidosis-importance of amyloid fibril type and precursor protein abundance. *Am. J. Transplant.* **2013**, *13*, 433–441. [CrossRef]
120. Angel-Korman, A.; Stern, L.; Sarosiek, S.; Sloan, J.M.; Doros, G.; Sanchorawala, V.; Havasi, A. Long-term outcome of kidney transplantation in AL amyloidosis. *Kidney Int.* **2019**, *95*, 405–411. [CrossRef]
121. Andeen, N.K.; Yang, H.Y.; Dai, D.F.; MacCoss, M.J.; Smith, K.D. DnaJ Homolog Subfamily B Member 9 Is a Putative Autoantigen in Fibrillary GN. *J. Am. Soc. Nephrol. JASN* **2018**, *29*, 231–239. [CrossRef]
122. El Ters, M.; Bobart, S.A.; Cornell, L.D.; Leung, N.; Bentall, A.; Sethi, S.; Fidler, M.; Grande, J.; Hernandez, L.H.; Cosio, F.G.; et al. Recurrence of DNAJB9-Positive Fibrillary Glomerulonephritis After Kidney Transplantation: A Case Series. *Am. J. Kidney Dis.* **2020**, *76*, 500–510. [CrossRef] [PubMed]
123. Kim, W.R.; Terrault, N.A.; Pedersen, R.A.; Therneau, T.M.; Edwards, E.; Hindman, A.A.; Brosgart, C.L. Trends in waiting list registration for liver transplantation for viral hepatitis in the United States. *Gastroenterology* **2009**, *137*, 1680–1686. [CrossRef]
124. Younossi, Z.M.; Stepanova, M.; Ong, J.; Trimble, G.; AlQahtani, S.; Younossi, I.; Ahmed, A.; Racila, A.; Henry, L. Nonalcoholic Steatohepatitis Is the Most Rapidly Increasing Indication for Liver Transplantation in the United States. *Clin. Gastroenterol. Hepatol.* **2021**, *19*, 580–589.e5. [CrossRef] [PubMed]
125. Samuel, D.; Muller, R.; Alexander, G.; Fassati, L.; Ducot, B.; Benhamou, J.P.; Bismuth, H. Liver transplantation in European patients with the hepatitis B surface antigen. *N. Engl. J. Med.* **1993**, *329*, 1842–1847. [CrossRef]

126. Todo, S.; Demetris, A.J.; Van Thiel, D.; Teperman, L.; Fung, J.J.; Starzl, T.E. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. *Hepatology* **1991**, *13*, 619–626.
127. Mutimer, D.; Pillay, D.; Dragon, E.; Tang, H.; Ahmed, M.; O'Donnell, K.; Shaw, J.; Burroughs, N.; Rand, D.; Cane, P.; et al. High pre-treatment serum hepatitis B virus titre predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. *J. Hepatol.* **1999**, *30*, 715–721. [CrossRef]
128. Markowitz, J.S.; Martin, P.; Conrad, A.J.; Markmann, J.F.; Seu, P.; Yersiz, H.; Goss, J.A.; Schmidt, P.; Pakrasi, A.; Artinian, L.; et al. Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. *Hepatology* **1998**, *28*, 585–589. [CrossRef]
129. Degertekin, B.; Lok, A.S. What is the optimal regimen for preventing hepatitis B recurrence after liver transplantation? *Nat. Clin. Pract. Gastroenterol. Hepatol.* **2009**, *6*, 68–69. [CrossRef] [PubMed]
130. Cotter, T.G.; Paul, S.; Sandıkçı, B.; Couri, T.; Bodzin, A.S.; Little, E.C.; Sundaram, V.; Charlton, M. Improved Graft Survival After Liver Transplantation for Recipients With Hepatitis C Virus in the Direct-Acting Antiviral Era. *Liver Transplant.* **2019**, *25*, 598–609. [CrossRef] [PubMed]
131. Bethea, E.; Arvind, A.; Gustafson, J.; Andersson, K.; Pratt, D.; Bhan, I.; Thiim, M.; Corey, K.; Bloom, P.; Markmann, J.; et al. Immediate administration of antiviral therapy after transplantation of hepatitis C-infected livers into uninfected recipients: Implications for therapeutic planning. *Am. J. Transplant.* **2020**, *20*, 1619–1628. [CrossRef]
132. Bizollon, T.; Ducerf, C.; Trepo, C.; Mutimer, D. Hepatitis C virus recurrence after liver transplantation. *Gut* **1999**, *44*, 575–578. [CrossRef] [PubMed]
133. Teixeira, R.; Pastacaldi, S.; Papatheodoridis, G.V.; Burroughs, A.K. Recurrent hepatitis C after liver transplantation. *J. Med. Virol.* **2000**, *61*, 443–454. [CrossRef]
134. Ghobrial, R.M.; Farmer, D.G.; Baquerizo, A.; Colquhoun, S.; Rosen, H.R.; Yersiz, H.; Markmann, J.F.; Drazan, K.E.; Holt, C.; Imagawa, D.; et al. Orthotopic liver transplantation for hepatitis C: Outcome, effect of immunosuppression, and causes of retransplantation during an 8-year single-center experience. *Ann. Surg.* **1999**, *229*, 824–831, discussion 831–823. [CrossRef]
135. Verna, E.C.; Abdelmessih, R.; Salomao, M.A.; Lefkowitch, J.; Moreira, R.K.; Brown, R.S., Jr. Cholestatic hepatitis C following liver transplantation: An outcome-based histological definition, clinical predictors, and prognosis. *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.* **2013**, *19*, 78–88. [CrossRef]
136. Neumann, U.P.; Berg, T.; Bahra, M.; Seehofer, D.; Langrehr, J.M.; Neuhaus, R.; Radke, C.; Neuhaus, P. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J. Hepatol.* **2004**, *41*, 830–836. [CrossRef]
137. Mutimer, D.J.; Gunson, B.; Chen, J.; Berenguer, J.; Neuhaus, P.; Castaing, D.; Garcia-Valdecasas, J.C.; Salizzoni, M.; Moreno, G.E.; Mirza, D. Impact of donor age and year of transplantation on graft and patient survival following liver transplantation for hepatitis C virus. *Transplantation* **2006**, *81*, 7–14. [CrossRef]
138. Omata, M.; Yokosuka, O.; Takano, S.; Kato, N.; Hosoda, K.; Imazeki, F.; Tada, M.; Ito, Y.; Ohto, M. Resolution of acute hepatitis C after therapy with natural beta interferon. *Lancet* **1991**, *338*, 914–915. [CrossRef]
139. Berg, T.; Hoffmann, R.M.; Teuber, G.; Leifeld, L.; Lafrenz, M.; Baumgarten, R.; Spengler, U.; Zeuzem, S.; Pape, G.R.; Hopf, U. Efficacy of a short-term ribavirin plus interferon alpha combination therapy followed by interferon alpha alone in previously untreated patients with chronic hepatitis C: A randomized multicenter trial. *Liver* **2000**, *20*, 427–436. [CrossRef] [PubMed]
140. Carrat, F.; Bani-Sadr, F.; Pol, S.; Rosenthal, E.; Lunel-Fabiani, F.; Benzekri, A.; Morand, P.; Goujard, C.; Pialoux, G.; Piroth, L.; et al. Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: A randomized controlled trial. *JAMA* **2004**, *292*, 2839–2848. [CrossRef] [PubMed]
141. Terrault, N.A. Prophylactic and preemptive therapies for hepatitis C virus-infected patients undergoing liver transplantation. *Liver Transplant.* **2003**, *9*, S95–S100. [CrossRef]
142. Bzowej, N.; Nelson, D.R.; Terrault, N.A.; Everson, G.T.; Teng, L.L.; Prabhakar, A.; Charlton, M.R. PHOENIX: A randomized controlled trial of peginterferon alfa-2a plus ribavirin as a prophylactic treatment after liver transplantation for hepatitis C virus. *Liver Transplant.* **2011**, *17*, 528–538. [CrossRef] [PubMed]
143. Kumada, H.; Toyota, J.; Okanoue, T.; Chayama, K.; Tsubouchi, H.; Hayashi, N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J. Hepatol.* **2012**, *56*, 78–84. [CrossRef] [PubMed]
144. Fried, M.W.; Buti, M.; Dore, G.J.; Flisiak, R.; Ferenci, P.; Jacobson, I.; Marcellin, P.; Manns, M.; Nikitin, I.; Poordad, F.; et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: The randomized PILLAR study. *Hepatology* **2013**, *58*, 1918–1929. [CrossRef] [PubMed]
145. Manns, M.; Pol, S.; Jacobson, I.M.; Marcellin, P.; Gordon, S.C.; Peng, C.Y.; Chang, T.T.; Everson, G.T.; Heo, J.; Gerken, G.; et al. All-oral daclatasvir plus asunaprevir for hepatitis C virus genotype 1b: A multinational, phase 3, multicohort study. *Lancet* **2014**, *384*, 1597–1605. [CrossRef]
146. Ikegami, T.; Ueda, Y.; Akamatsu, N.; Ishiyama, K.; Goto, R.; Soyama, A.; Kuramitsu, K.; Honda, M.; Shinoda, M.; Yoshizumi, T.; et al. Asunaprevir and daclatasvir for recurrent hepatitis C after liver transplantation: A Japanese multicenter experience. *Clin. Transplant.* **2017**, *31*, e13109. [CrossRef] [PubMed]
147. Bourlière, M.; Bronowicki, J.P.; de Ledinghen, V.; Hézode, C.; Zoulim, F.; Mathurin, P.; Tran, A.; Larrey, D.G.; Ratziu, V.; Alric, L.; et al. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: A randomised, double-blind, phase 2 trial (SIRIUS). *Lancet. Infect. Dis.* **2015**, *15*, 397–404. [CrossRef]

148. Elfeki, M.A.; Abou Mrad, R.; Modaresi Esfeh, J.; Zein, N.N.; Eghtesad, B.; Zervos, X.; Hanouneh, I.A.; O'Shea, R.; Carey, W.D.; Alkhouri, N. Sofosbuvir/Ledipasvir Without Ribavirin Achieved High Sustained Virologic Response for Hepatitis C Recurrence After Liver Transplantation: Two-Center Experience. *Transplantation* **2017**, *101*, 996–1000. [CrossRef]
149. Ueda, Y.; Kobayashi, T.; Ikegami, T.; Miuma, S.; Mizuno, S.; Akamatsu, N.; Takaki, A.; Ishigami, M.; Takatsuki, M.; Sugawara, Y.; et al. Efficacy and safety of glecaprevir and pibrentasvir treatment for 8 or 12 weeks in patients with recurrent hepatitis C after liver transplantation: A Japanese multicenter experience. *J. Gastroenterol.* **2019**, *54*, 660–666. [CrossRef] [PubMed]
150. Figueras, J.; Jaurrieta, E.; Valls, C.; Benasco, C.; Rafecas, A.; Xiol, X.; Fabregat, J.; Casanovas, T.; Torras, J.; Baliellas, C.; et al. Survival after liver transplantation in cirrhotic patients with and without hepatocellular carcinoma: A comparative study. *Hepatology* **1997**, *25*, 1485–1489. [CrossRef]
151. Mazzaferro, V.; Regalia, E.; Doci, R.; Andreola, S.; Pulvirenti, A.; Bozzetti, F.; Montalto, F.; Ammatuna, M.; Morabito, A.; Gennari, L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med.* **1996**, *334*, 693–699. [CrossRef] [PubMed]
152. Yao, F.Y.; Ferrell, L.; Bass, N.M.; Watson, J.J.; Bacchetti, P.; Venook, A.; Ascher, N.L.; Roberts, J.P. Liver transplantation for hepatocellular carcinoma: Expansion of the tumor size limits does not adversely impact survival. *Hepatology* **2001**, *33*, 1394–1403. [CrossRef] [PubMed]
153. Kaido, T.; Ogawa, K.; Mori, A.; Fujimoto, Y.; Ito, T.; Tomiyama, K.; Takada, Y.; Uemoto, S. Usefulness of the Kyoto criteria as expanded selection criteria for liver transplantation for hepatocellular carcinoma. *Surgery* **2013**, *154*, 1053–1060. [CrossRef]
154. Halazun, K.J.; Rosenblatt, R.E.; Mehta, N.; Lai, Q.; Hajifathalian, K.; Gorgen, A.; Brar, G.; Sasaki, K.; Doyle, M.B.M.; Tabrizian, P.; et al. Dynamic α-Fetoprotein Response and Outcomes After Liver Transplant for Hepatocellular Carcinoma. *JAMA Surg.* **2021**, *156*, 559–567. [CrossRef] [PubMed]
155. Razumilava, N.; Gores, G.J. Cholangiocarcinoma. *Lancet* **2014**, *383*, 2168–2179. [CrossRef]
156. De Vreede, I.; Steers, J.L.; Burch, P.A.; Rosen, C.B.; Gunderson, L.L.; Haddock, M.G.; Burgart, L.; Gores, G.J. Prolonged disease-free survival after orthotopic liver transplantation plus adjuvant chemoirradiation for cholangiocarcinoma. *Liver Transplant.* **2000**, *6*, 309–316. [CrossRef] [PubMed]
157. Darwish Murad, S.; Kim, W.R.; Therneau, T.; Gores, G.J.; Rosen, C.B.; Martenson, J.A.; Alberts, S.R.; Heimbach, J.K. Predictors of pretransplant dropout and posttransplant recurrence in patients with perihilar cholangiocarcinoma. *Hepatology* **2012**, *56*, 972–981. [CrossRef]
158. Sapisochin, G.; Rodríguez de Lope, C.; Gastaca, M.; Ortiz de Urbina, J.; Suarez, M.A.; Santoyo, J.; Castroagudín, J.F.; Varo, E.; López-Andujar, R.; Palacios, F.; et al. "Very early" intrahepatic cholangiocarcinoma in cirrhotic patients: Should liver transplantation be reconsidered in these patients? *Am. J. Transplant.* **2014**, *14*, 660–667. [CrossRef]
159. Jones, D.E. Autoantigens in primary biliary cirrhosis. *J. Clin. Pathol.* **2000**, *53*, 813–821. [CrossRef]
160. Chalifoux, S.L.; Konyn, P.G.; Choi, G.; Saab, S. Extrahepatic Manifestations of Primary Biliary Cholangitis. *Gut Liver* **2017**, *11*, 771–780. [CrossRef]
161. Nakanuma, Y. Distribution of B lymphocytes in nonsuppurative cholangitis in primary biliary cirrhosis. *Hepatology* **1993**, *18*, 570–575. [CrossRef]
162. Corpechot, C.; Carrat, F.; Bahr, A.; Chrétien, Y.; Poupon, R.E.; Poupon, R. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology* **2005**, *128*, 297–303. [CrossRef]
163. Adam, R.; Karam, V.; Delvart, V.; O'Grady, J.; Mirza, D.; Klempnauer, J.; Castaing, D.; Neuhaus, P.; Jamieson, N.; Salizzoni, M.; et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J. Hepatol.* **2012**, *57*, 675–688. [CrossRef] [PubMed]
164. Singal, A.K.; Guturu, P.; Hmoud, B.; Kuo, Y.F.; Salameh, H.; Wiesner, R.H. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* **2013**, *95*, 755–760. [CrossRef] [PubMed]
165. Kashyap, R.; Safadjou, S.; Chen, R.; Mantry, P.; Sharma, R.; Patil, V.; Maloo, M.; Ryan, C.; Marroquin, C.; Barry, C.; et al. Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases–an analysis of the UNOS database. *J. Gastrointest. Surg.* **2010**, *14*, 1362–1369. [CrossRef] [PubMed]
166. Bosch, A.; Dumortier, J.; Maucort-Boulch, D.; Scoazec, J.Y.; Wendum, D.; Conti, F.; Morard, I.; Rubbia-Brandt, L.; Terris, B.; Radenne, S.; et al. Preventive administration of UDCA after liver transplantation for primary biliary cirrhosis is associated with a lower risk of disease recurrence. *J. Hepatol.* **2015**, *63*, 1449–1458. [CrossRef] [PubMed]
167. Kogiso, T.; Egawa, H.; Teramukai, S.; Taniai, M.; Hashimoto, E.; Tokushige, K.; Sakisaka, S.; Sakabayashi, S.; Yamamoto, M.; Umeshita, K.; et al. Risk factors for recurrence of primary biliary cholangitis after liver transplantation in female patients: A Japanese multicenter retrospective study. *Hepatol. Commun.* **2017**, *1*, 394–405. [CrossRef]
168. Manousou, P.; Arvaniti, V.; Tsochatzis, E.; Isgro, G.; Jones, K.; Shirling, G.; Dhillon, A.P.; O'Beirne, J.; Patch, D.; Burroughs, A.K. Primary biliary cirrhosis after liver transplantation: Influence of immunosuppression and human leukocyte antigen locus disparity. *Liver Transplant.* **2010**, *16*, 64–73. [CrossRef] [PubMed]
169. Charatcharoenwitthaya, P.; Pimentel, S.; Talwalkar, J.A.; Enders, F.T.; Lindor, K.D.; Krom, R.A.; Wiesner, R.H. Long-term survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. *Liver Transplant.* **2007**, *13*, 1236–1245. [CrossRef]
170. Neuberger, J. Recurrent primary biliary cirrhosis. *Liver Transplant.* **2003**, *9*, 539–546. [CrossRef] [PubMed]

171. Egawa, H.; Sakisaka, S.; Teramukai, S.; Sakabayashi, S.; Yamamoto, M.; Umeshita, K.; Uemoto, S. Long-Term Outcomes of Living-Donor Liver Transplantation for Primary Biliary Cirrhosis: A Japanese Multicenter Study. *Am. J. Transplant.* **2016**, *16*, 1248–1257. [CrossRef]
172. Li, X.; Peng, J.; Ouyang, R.; Yang, Y.; Yu, C.; Lin, H. Risk factors for recurrent primary biliary cirrhosis after liver transplantation: A systematic review and meta-analysis. *Dig. Liver Dis.* **2021**, *53*, 309–317. [CrossRef]
173. Egawa, H.; Nakanuma, Y.; Maehara, Y.; Uemoto, S.; Eguchi, S.; Sato, Y.; Shirabe, K.; Takatsuki, M.; Mori, A.; Yamamoto, M.; et al. Disease recurrence plays a minor role as a cause for retransplantation after living-donor liver transplantation for primary biliary cirrhosis: A multicenter study in Japan. *Hepatol. Res.* **2013**, *43*, 502–507. [CrossRef]
174. Chapman, R.; Fevery, J.; Kalloo, A.; Nagorney, D.M.; Boberg, K.M.; Shneider, B.; Gores, G.J. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* **2010**, *51*, 660–678. [CrossRef]
175. Burak, K.; Angulo, P.; Pasha, T.M.; Egan, K.; Petz, J.; Lindor, K.D. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am. J. Gastroenterol.* **2004**, *99*, 523–526. [CrossRef]
176. Boonstra, K.; Weersma, R.K.; van Erpecum, K.J.; Rauws, E.A.; Spanier, B.W.; Poen, A.C.; van Nieuwkerk, K.M.; Drenth, J.P.; Witteman, B.J.; Tuynman, H.A.; et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* **2013**, *58*, 2045–2055. [CrossRef]
177. Graziadei, I.W.; Wiesner, R.H.; Marotta, P.J.; Porayko, M.K.; Hay, J.E.; Charlton, M.R.; Poterucha, J.J.; Rosen, C.B.; Gores, G.J.; LaRusso, N.F.; et al. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* **1999**, *30*, 1121–1127. [CrossRef] [PubMed]
178. Hildebrand, T.; Pannicke, N.; Dechene, A.; Gotthardt, D.N.; Kirchner, G.; Reiter, F.P.; Sterneck, M.; Herzer, K.; Lenzen, H.; Rupp, C.; et al. Biliary strictures and recurrence after liver transplantation for primary sclerosing cholangitis: A retrospective multicenter analysis. *Liver Transplant.* **2016**, *22*, 42–52. [CrossRef] [PubMed]
179. Alabraba, E.; Nightingale, P.; Gunson, B.; Hubscher, S.; Olliff, S.; Mirza, D.; Neuberger, J. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. *Liver Transplant.* **2009**, *15*, 330–340. [CrossRef] [PubMed]
180. Campsen, J.; Zimmerman, M.A.; Trotter, J.F.; Wachs, M.; Bak, T.; Steinberg, T.; Kam, I. Clinically recurrent primary sclerosing cholangitis following liver transplantation: A time course. *Liver Transplant.* **2008**, *14*, 181–185. [CrossRef]
181. Egawa, H.; Ueda, Y.; Ichida, T.; Teramukai, S.; Nakanuma, Y.; Onishi, S.; Tsubouchi, H. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation in Japanese registry. *Am. J. Transplant* **2011**, *11*, 518–527. [CrossRef] [PubMed]
182. Kashyap, R.; Mantry, P.; Sharma, R.; Maloo, M.K.; Safadjou, S.; Qi, Y.; Jain, A.; Maliakkal, B.; Ryan, C.; Orloff, M. Comparative analysis of outcomes in living and deceased donor liver transplants for primary sclerosing cholangitis. *J. Gastrointest. Surg.* **2009**, *13*, 1480–1486. [CrossRef]
183. Gordon, F.D.; Goldberg, D.S.; Goodrich, N.P.; Lok, A.S.; Verna, E.C.; Selzner, N.; Stravitz, R.T.; Merion, R.M. Recurrent primary sclerosing cholangitis in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study: Comparison of risk factors between living and deceased donor recipients. *Liver Transplant.* **2016**, *22*, 1214–1222. [CrossRef]
184. Dhillon, A.K.; Kummen, M.; Trøseid, M.; Åkra, S.; Liaskou, E.; Moum, B.; Vesterhus, M.; Karlsen, T.H.; Seljeflot, I.; Hov, J.R. Circulating markers of gut barrier function associated with disease severity in primary sclerosing cholangitis. *Liver Int.* **2019**, *39*, 371–381. [CrossRef]
185. Martinez, M.; Perito, E.R.; Valentino, P.; Mack, C.L.; Aumar, M.; Broderick, A.; Draijer, L.G.; Fagundes, E.D.T.; Furuya, K.N.; Gupta, N.; et al. Recurrence of Primary Sclerosing Cholangitis after Liver Transplant in Children: An International Observational Study. *Hepatology* **2021**, *74*, 2047–2057. [CrossRef] [PubMed]
186. Krawitt, E.L. Autoimmune hepatitis. *N. Engl. J. Med.* **2006**, *354*, 54–66. [CrossRef] [PubMed]
187. EASL Clinical Practice Guidelines: Autoimmune hepatitis. *J. Hepatol.* **2015**, *63*, 971–1004. [CrossRef]
188. Manns, M.; Gerken, G.; Kyriatsoulis, A.; Staritz, M.; Meyer zum Büschenfelde, K.H. Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. *Lancet* **1987**, *1*, 292–294. [CrossRef]
189. Tanaka, A. Autoimmune Hepatitis: 2019 Update. *Gut Liver* **2020**, *14*, 430–438. [CrossRef] [PubMed]
190. Czaja, A.J. Rapidity of treatment response and outcome in type 1 autoimmune hepatitis. *J. Hepatol.* **2009**, *51*, 161–167. [CrossRef]
191. Wright, H.L.; Bou-Abboud, C.F.; Hassanein, T.; Block, G.D.; Demetris, A.J.; Starzl, T.E.; Van Thiel, D.H. Disease recurrence and rejection following liver transplantation for autoimmune chronic active liver disease. *Transplantation* **1992**, *53*, 136–139. [CrossRef] [PubMed]
192. Narumi, S.; Hakamada, K.; Sasaki, M.; Freise, C.E.; Stock, P.G.; Roberts, J.P.; Ascher, N.L. Liver transplantation for autoimmune hepatitis: Rejection and recurrence. *Transplant. Proc.* **1999**, *31*, 1955–1956. [CrossRef]
193. Milkiewicz, P.; Hubscher, S.G.; Skiba, G.; Hathaway, M.; Elias, E. Recurrence of autoimmune hepatitis after liver transplantation. *Transplantation* **1999**, *68*, 253–256. [CrossRef] [PubMed]
194. Montano-Loza, A.J.; Bhanji, R.A.; Wasilenko, S.; Mason, A.L. Systematic review: Recurrent autoimmune liver diseases after liver transplantation. *Aliment. Pharm.* **2017**, *45*, 485–500. [CrossRef] [PubMed]
195. Milkiewicz, P.; Gunson, B.; Saksena, S.; Hathaway, M.; Hubscher, S.G.; Elias, E. Increased incidence of chronic rejection in adult patients transplanted for autoimmune hepatitis: Assessment of risk factors. *Transplantation* **2000**, *70*, 477–480. [CrossRef] [PubMed]

196. McCabe, M.; Rush, N.; Lammert, C.; Patidar, K.R.; Nephew, L.; Saxena, R.; Ekser, B.; Salven, J.; Kubal, C.; Ghabril, M. HLA-DR Mismatch and Black Race Are Associated With Recurrent Autoimmune Hepatitis After Liver Transplantation. *Transpl. Direct* **2021**, *7*, e714. [CrossRef]
197. Montano-Loza, A.J.; Mason, A.L.; Ma, M.; Bastiampillai, R.J.; Bain, V.G.; Tandon, P. Risk factors for recurrence of autoimmune hepatitis after liver transplantation. *Liver Transplant.* **2009**, *15*, 1254–1261. [CrossRef]
198. Cattan, P.; Berney, T.; Conti, F.; Calmus, Y.; Homberg, J.C.; Houssin, D.; Soubrane, O. Outcome of orthotopic liver transplantation in autoimmune hepatitis according to subtypes. *Transpl. Int.* **2002**, *15*, 34–38. [CrossRef]
199. De Quadros Onofrio, F.; Neong, E.; Adebayo, D.; Kollmann, D.; Adeyi, O.A.; Fischer, S.; Hirschfield, G.M.; Hansen, B.E.; Bhat, M.; Galvin, Z.; et al. Single-Center North American Experience of Liver Transplantation in Autoimmune Hepatitis: Infrequent Indication but Good Outcomes for Patients. *J. Can. Assoc. Gastroenterol.* **2021**, *4*, 137–144. [CrossRef] [PubMed]
200. Montano-Loza, A.J.; Wasilenko, S.; Bintner, J.; Mason, A.L. Cyclosporine A protects against primary biliary cirrhosis recurrence after liver transplantation. *Am. J. Transplant.* **2010**, *10*, 852–858. [CrossRef]
201. Ravikumar, R.; Tsochatzis, E.; Jose, S.; Allison, M.; Athale, A.; Creamer, F.; Gunson, B.; Iyer, V.; Madanur, M.; Manas, D.; et al. Risk factors for recurrent primary sclerosing cholangitis after liver transplantation. *J. Hepatol.* **2015**, *63*, 1139–1146. [CrossRef] [PubMed]
202. Cholongitas, E.; Shusang, V.; Papatheodoridis, G.V.; Marelli, L.; Manousou, P.; Rolando, N.; Patch, D.; Rolles, K.; Davidson, B.; Burroughs, A.K. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transplant.* **2008**, *14*, 138–143. [CrossRef]
203. Reich, D.J.; Fiel, I.; Guarrera, J.V.; Emre, S.; Guy, S.R.; Schwartz, M.E.; Miller, C.M.; Sheiner, P.A. Liver transplantation for autoimmune hepatitis. *Hepatology* **2000**, *32*, 693–700. [CrossRef] [PubMed]
204. Sørgjerd, E.P. Type 1 Diabetes-related Autoantibodies in Different Forms of Diabetes. *Curr. Diabetes Rev.* **2019**, *15*, 199–204. [CrossRef]
205. Bottazzo, G.F.; Florin-Christensen, A.; Doniach, D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* **1974**, *2*, 1279–1283. [CrossRef]
206. Månsson, L.; Törn, C.; Landin-Olsson, M. Islet cell antibodies represent autoimmune response against several antigens. *Int. J. Exp. Diabetes Res.* **2001**, *2*, 85–90. [CrossRef] [PubMed]
207. Sutherland, D.E.; Goetz, F.C.; Sibley, R.K. Recurrence of disease in pancreas transplants. *Diabetes* **1989**, *38* (Suppl. S1), 85–87. [CrossRef] [PubMed]
208. Sutherland, D.E.; Sibley, R.; Xu, X.Z.; Michael, A.; Srikanta, A.M.; Taub, F.; Najarian, J.; Goetz, F.C. Twin-to-twin pancreas transplantation: Reversal and reenactment of the pathogenesis of type I diabetes. *Trans. Assoc. Am. Physicians* **1984**, *97*, 80–87. [PubMed]
209. Vendrame, F.; Hopfner, Y.Y.; Diamantopoulos, S.; Virdi, S.K.; Allende, G.; Snowhite, I.V.; Reijonen, H.K.; Chen, L.; Ruiz, P.; Ciancio, G.; et al. Risk Factors for Type 1 Diabetes Recurrence in Immunosuppressed Recipients of Simultaneous Pancreas-Kidney Transplants. *Am. J. Transpl.* **2016**, *16*, 235–245. [CrossRef]
210. Esmatjes, E.; Rodríguez-Villar, C.; Ricart, M.J.; Casamitjana, R.; Martorell, J.; Sabater, L.; Astudillo, E.; Fernández-Cruz, L. Recurrence of immunological markers for type 1 (insulin-dependent) diabetes mellitus in immunosuppressed patients after pancreas transplantation. *Transplantation* **1998**, *66*, 128–131. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Outcomes of Deceased Donor Kidney Transplantation in the Eurotransplant Senior Program with A Focus on Recipients ≥75 Years

Ilias Zompolas [1], Robert Peters [1], Lutz Liefeldt [2], Lukas J. Lehner [2], Klemens Budde [2], Bernhard Ralla [1], Irena Goranova [1], Andreas Maxeiner [1], Markus H. Lerchbaumer [3], Stephan R. Marticorena Garcia [3], Martin Kanne [4], Thorsten Schlomm [1], Matthias R. G. Schulz [1,†] and Frank Friedersdorff [1,4,*,†]

[1] Department of Urology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; ilias.zompolas@charite.de (I.Z.); robert.peters@charite.de (R.P.); bernhard.ralla@charite.de (B.R.); irena.goranova@charite.de (I.G.); andreas.maxeiner@charite.de (A.M.); thorsten.schlomm@charite.de (T.S.); Matthias.schulz2@charite.de (M.R.G.S.)

[2] Department of Nephrology and Internal Intensive Care Medicine, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; lutz.liefeldt@charite.de (L.L.); lukas.lehner@charite.de (L.J.L.); klemens.budde@charite.de (K.B.)

[3] Department of Radiology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; markus.lerchbaumer@charite.de (M.H.L.); stephan.marticorena-garcia@charite.de (S.R.M.G.)

[4] Department of Urology, Evangelisches Krankenhaus Königin Elisabeth Herzberge, 10365 Berlin, Germany; m.kanne@keh-berlin.de

* Correspondence: frank.friedersdorff@charite.de

† Contributed equally.

Citation: Zompolas, I.; Peters, R.; Liefeldt, L.; Lehner, L.J.; Budde, K.; Ralla, B.; Goranova, I.; Maxeiner, A.; Lerchbaumer, M.H.; Marticorena Garcia, S.R.; et al. Outcomes of Deceased Donor Kidney Transplantation in the Eurotransplant Senior Program with A Focus on Recipients ≥75 Years. *J. Clin. Med.* **2021**, *10*, 5633. https://doi.org/10.3390/jcm10235633

Academic Editor: Charat Thongprayoon

Received: 17 October 2021
Accepted: 22 November 2021
Published: 29 November 2021

Abstract: To evaluate the outcomes of kidney transplantations (KTs) in the Eurotransplant Senior Program (ESP) with a focus on the very old, defined as recipients ≥75 years. This retrospective clinical study included 85 patients, who under the ESP protocol underwent deceased donor kidney transplantation from January 2010 to July 2018 at the Charité–Universitätsmedizin Berlin in Germany. Recipients were divided in three age groups, i.e., Group 65–69, Group 70–74, Group ≥75, and compared. Prognostic risk factors for short and long-term outcomes of kidney transplantations were investigated. Graft survival at 1 and 5 years were respectively 90.7% and 68.0% for group 65–69, 88.9% and 76.2% for Group 70–74, and 100% and 71.4% for Group ≥75. Patient survival at 1 and 5 years were respectively 92.9% and 68.0% for Group 65–69, 85.7% and 61.5% for Group 70–74 and 100% and 62.5% for Group ≥75. Serum creatinine did not significantly differ between the three groups, with the exception of serum creatinine at 1 year. Increased recipient age and prolonged time on dialysis correlated with increased occurrence of postoperative complication. An increase in BMI, pretransplant diabetes mellitus and prolonged time on dialysis correlated with the occurrence of delayed graft function (DGF). History of smoking was identified as an independent risk factor for events of rejection. Increased human leukocyte antigen mismatches (HLA-MM) and prolonged cold ischemia time (CIT) correlated with higher rates of intensive care unit (ICU) treatment. This study supports kidney transplantations for the very old. End-stage renal disease (ESRD) patients ≥75 years of age who underwent kidney transplantation experienced comparable results to their younger counterparts. A comprehensive evaluation of ESRD patients with consideration of prognostic risk factor is the most suitable mean of identifying adequate kidney transplant candidates.

Keywords: cold ischemia time; delayed graft function; Eurotransplant Senior Program; end-stage renal disease; intensive care unit; kidney transplantation

1. Introduction

Kidney transplantation is considered the treatment of choice in ESRD, increasing life expectancy and quality of life even for recipients aged ≥65 years [1–4]. The shortage of renal allograft donors combined with an increased demand from an ever-ageing population has led to the use of expanded criteria donor (ECD) kidneys. ECD kidneys, despite being of lower quality than standard criteria donor (SCD) kidneys, minimize waitlisted time for recipients while providing a survival advantage compared wait-listed dialysis patients [3–6]. The Eurotransplant Senior Program implemented in 1999, aimed to optimize the allocation of ECD kidneys from deceased donors aged ≥65 to recipients aged ≥65 based on waiting time and blood type compatibility, disregarding HLA matchmaking while minimizing cold ischemia time. Although good results have been reported in recipients aged ≥65 years, only few studies have focused on the potential benefits of kidney transplantations (KTs) in the very old [3–9]. Studies that evaluated renal allograft recipients over 70 years compared to a waitlisted group or younger counterparts revealed that ≥70-year recipients benefited from the procedure [10–12].

However, no scientific research has explicitly assessed the KTs of patients ≥75 years of age thus far. This age group of patients is destined to become clinically more relevant as the number of people aged 75 to 84 years in the EU is projected to increase by 56.1% from 2019 to 2050 [13].

Primary objective of the present study was to evaluate the outcomes of KTs performed under the ESP protocol and to investigate the age limits in recipients. Secondary objective was to identify prognostic factors influencing the short and long-term outcomes of those transplantations with the prospect to improve the pretransplant evaluation.

2. Patients and Methods

2.1. Study Design

The present retrospective clinical study included 85 patients aged ≥65 years who received a deceased donor kidney transplant from donors ≥65 years allocated through ESP. Recipients were divided into three groups with respect to their age at the time of KT in years as following: Group 65–69, Group 70–74, and Group ≥75. The KTs were conducted by experienced urologic transplant surgeons between January 2010 and July 2018 at the Charité-Universitätsmedizin Berlin. All patients received a renal allograft for the first time and were followed up until death or the end of study (26 May 2020). The immunosuppression protocol after KT was identical for all patients and consisted of tacrolimus, mycophenolate mofetil (MMF) and prednisolone.

This entire analysis was conducted in adherence with the correct scientific research work terms of the Charité Medical University of Berlin, including full anonymization of patient data. All the patients included in the analysis provided written informed consent.

2.2. Data Collection and Outcome Measures

Demographic data, medical history, and postoperative follow-up information were extracted through the electronic database Tbase2. Graft characteristics included donor age, number of HLA-mismatches and cold ischemia time (CIT). Specifics of the operations included the side of transplantation, duration of surgery and warm ischemia time (WIT). Serum creatinine levels and glomerular filtration rate (GFR) were used to estimate the renal function of the patients. Short-term outcomes consisted of inpatient stay, occurrence of postoperative complications, Clavien–Dindo classification, DGF, number of dialysis postoperatively, number of days in the ICU, occurrence of rejection and if ICU treatment was required. Long-term outcomes consisted of serum creatinine levels (mg/dl), graft survival, and patient survival at one, three, and five years, death with functioning graft, and patient mortality at last follow-up.

2.3. Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics, Version 26.0 (Armonk, NY, USA: IBM Corp). Normality of variables was examined with the Kolmogorov-Smirnov test. In order to compare means between groups, the ANOVA test and independent-sample t-test were performed. Fisher's exact test was carried out to analyze nominal variables. Logistic regression analysis was applied to identify independent risk factors influencing the outcomes using the backward elimination method. Regression models controlled for potential confounders including age of recipient and donor, HLA-MM, body mass index (BMI), diabetes mellitus, hypertension, coronary artery disease, tobacco consumption, time on dialysis, CIT, inpatient stay, DGF, ICU treatment, occurrence of rejection and complications. Survival data was assessed with Cox regression analysis, log-rank, and Kaplan–Meier method with the Group 65–69 set as baseline. $p < 0.05$ was considered significant.

3. Results

A total of 85 patients were included in the study with a mean follow-up of 49.72 ± 28.7 months. Demographic data and details regarding the KTs are presented in Tables 1 and 2. Postoperative course following the KT and long-term outcomes are shown Tables 3 and 4.

Table 1. Patient characteristics.

	Groups			
	65–69 years	**70–74 years**	**≥75 years**	***p*-Value**
n	**45**	**28**	**12**	
Gender: male/female	24/16, 53.3%/46.7%	17/11, 60.7%/39.3%	10/2, 83.3%/16.7%	n.s.
Follow-up (months)	46.98 ± 28.6	55.6 ± 30.5	46.25 ± 24.0	n.s.
Age of recipient at time of KT (years)	67.16 ± 1.51	71.86 ± 1.41	77.42 ± 3.30	<0.001
Age of donor at time of KT (years)	71.62 ± 4.38	72.71 ± 5.11	72.92 ± 4.91	n.s.
BMI of recipient (kg/m^2)	27.4 ± 4.6	27.05 ± 4.05	27.14 ± 3.09	n.s.
HLA-mismatches	3.76 ± 1.28	3.71 ± 1.24	3.67 ± 1.16	n.s.
Primary kidney disease				
Vascular/hypertensive disease	5, 11.1%	11, 39.3%	3, 25.0%	n.s.
Glomerulonephritis	13, 28.9%	7, 25.0%	2, 16.7%	n.s.
Diabetic nephropathy	12, 26.7%	4, 14.3%	4, 33.3%	n.s.
Malignancy	2, 4.4%	0	0	n.s.
Genetic/cystic kidneys disease	8, 17.8%	3, 10.7%	2, 16.7%	n.s.
Infection/reflux	1, 2.2%	0	0	n.s.
Systemic disease	1, 2.2%	0	0	n.s.
Autoimmune	0	0	1, 8.3%	n.s.
Various/unknown	3, 6.7%	3, 10.7%	0	n.s.
Pre-existing conditions				
Arterial hypertension	45, 100%	28, 100%	12, 100%	n.s.
Diabetes mellitus	21, 46.7%	11, 39.3%	5, 41.7%	n.s.
Coronary artery disease	16, 35.6%	14, 50%	4, 33.3%	n.s.
Tobacco consumption	17, 37.8%	5, 17.9%	2, 16.7%	n.s.
Previous operations in abdominal region	20, 44.4%	6, 21.4%	9, 75%	n.s.
Dialysis				
Hemodialysis	37, 82.2%	27, 96.4%	11, 91.7%	n.s.
Peritoneal dialysis	8, 17.8%	1, 3.6%	1, 8.3%	n.s.
Time on dialysis (days)	1950 ± 840	1487 ± 461	1418 ± 527	0.008

All values with *n*, percent or mean and standard deviation. n.s = not significant.

Table 2. Surgery details.

	Groups			
	65–69 years	**70–74 years**	**≥75 years**	***p*-Value**
Side of transplantation: fossa iliaca dextra/sinistra	26/19, 57.8%/42.2%	16/12, 57.1%/42.9%	6/6, 50%/50%	n.s.
Operation time (minutes)	203 ± 52.7	202 ± 46.9	235 ± 33.7	n.s.
Cold ischemia time (hours)	10.05 ± 3.78	9.46 ± 3.29	9.11 ± 2.96	n.s.
Warm ischemia time (minutes)	48.1 ± 10.7	52.6 ± 14.5	49.8 ± 10.0	n.s.

All values with *n*, percent, or mean and standard deviation; n.s = not significant.

Table 3. Postoperative course.

	Groups			
	65–69 years	**70–74 years**	**≥75 years**	***p*-Value**
Inpatient stay (days)	22.1 ± 13.0	21.5 ± 15.6	19.3 ± 10.2	n.s.
Occurrence of postoperative complications	12, 26.7%	10, 35.7%	2, 16.7%	n.s.
Clavien–Dindo classification				
Clavien–Dindo 1	7, 15.6%	8, 28.6%	n.a.	
Clavien–Dindo 2	n.a.	n.a.	n.a.	
Clavien–Dindo 3	5, 11.1%	2, 7.1%	2, 16.7%	
Delayed graft function	27, 60%	14, 50%	5, 41.7%	n.s.
Number of dialysis postoperatively	6.44 ± 7.63	2.57 ± 2.10	4.80 ± 3.96	n.s.
ICU required	12, 26.7%	6, 21.4%	3, 25.0%	n.s.
ICU duration (days)	1.58 ± 0.9	4 ± 3.58	3.0 ± 1.73	n.s.
Occurrence of rejection	10, 22.2%	5, 17.9%	1, 8.3%	n.s.
Cause of rejection				
Acute rejection	4, 8.9%	2, 7.1%	1, 8.3%	
Chronic rejection	4, 8.9%	2, 7.1%	0	
Vascular complications	1, 2.2%	0	0	
Tumor	1, 2.2%	0	0	
Infection	0	1, 3.6%	0	

All values with *n*, percent or mean and standard deviation. n.a. = not applicable; n.s = not significant.

The logistic regression analysis controlled for potential confounders. The manifestation of postoperative complications correlated with an increase in age of recipient (regression coefficient B = −0.31, odds ratio Exp(B) = 0.74, $p = 0.049$), the occurrence of DGF (B = −3.70, Exp(B) = 0.25, $p = 0.001$), and an increased time on dialysis (B = −0.002, Exp(B) = 0.998, $p = 0.042$). The event of rejection correlated with a history of smoking (B = −1.392, Exp(B) = 0.249, $p = 0.028$) and DGF (B = −2.145, Exp(B) = 0.117, $p = 0.009$). Requirement of ICU treatment correlated with an increase in HLA-MM (B = −2.633, Exp(B) = 0.72, $p = 0.045$) and an increase in cold ischemia time (B = 1.916, Exp(B) = 6.80, $p = 0.031$). Occurrence of DGF correlated with increase in BMI (B = 0.146, Exp(B) = 1.157, $p = 0.045$), longer period on dialysis (B = 0.01, Exp(B) = 1.001, $p = 0.008$), manifestation of perioperative complications (B = 2.423, Exp(B) = 11.28, $p = 0.001$), and diabetes mellitus (B = 1.586, Exp(B) = 4.88, $p = 0.007$). The occurrence of rejection correlated with graft failure (χ^2 (1, N = 85) = 26.73 $p < 0.001$). There were no significant differences between the three groups after KT regarding serum creatinine, except for creatinine at 1 year (see Figure 1).

Table 4. Long-term outcomes.

	Groups			
	65–69 years	**70–74 years**	**≥75 years**	***p*-Value**
Creatinine levels (mg/dL)				
Preoperatively	6.57 ± 1.86	6.03 ± 1.72	6.95 ± 3.32	n.s.
1-year	1.99 ± 0.93	1.51 ± 0.46	1.79 ± 0.44	0.046
3-year	1.93 ± 0.76	1.91 ± 0.72	2.05 ± 0.68	n.s.
5-year	1.89 ± 0.83	1.82 ± 0.61	2.19 ± 1.13	n.s.
Graft survival				
1-year	90.7%	88.9%	100%	n.s.
3-year	79.4%	80.0%	80.0%	n.s.
5-year	68.0%	76.2%	71.4%	n.s.
Patient survival				
1-year	92.9%	85.7%	100%	n.s.
3-year	79.4%	77.8%	72.7%	n.s.
5-year	68.0%	61.5%	62.5%	n.s.
Patient mortality at last follow-up	11, 24%	12, 42.9%	6, 50%	
Of these: death with functioning graft	8, 72.7% *	7, 58.3% *	4, 66.7% *	
Cause of death				
Cardiovascular	2/4.4%	0	0	
Graft failure	1/2.2%	1/3.6%	0	
Infection/sepsis	3/6.7%	8/28.6%	2/16.7%	
Malignancy	4/8.9%	1/3.6%	2/16.7%	
Traumatic	1/2.2%	2/7.1%	2/16.7%	

All values with *n* (percent) or mean and (standard deviation, SD). $p < 0.05$, * Percentage is the result of *n* of patients with functioning graft divided by *n* of deceased patients at last follow-up; n.s = not significant.

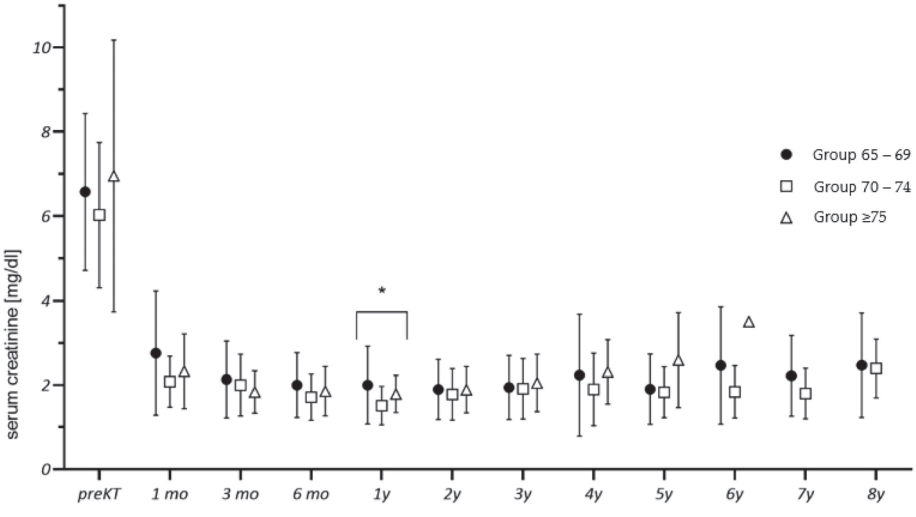


Figure 1. Graft function during follow-up. (* statistically significant difference between the groups).

Figure 1 depicts the creatinine levels of the three age groups up to last follow-up. Figures 2 and 3 illustrate the death-censored graft and patient survival of the three age groups.

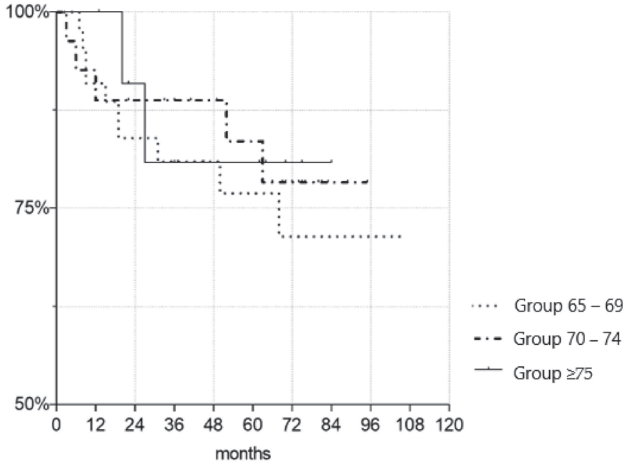


Figure 2. Kaplan–Meier survival plot demonstrating death-censored graft survival. There were no significant differences in the graft survival time between groups ($p = 0.673$ for Gr. 70–74, $p = 0.814$ for Gr. 75+).

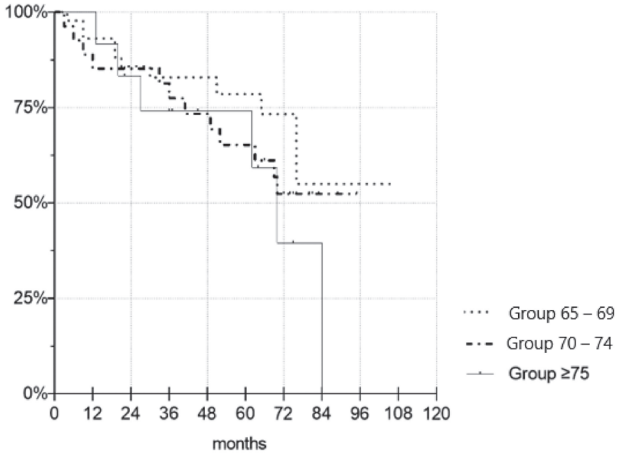


Figure 3. Kaplan–Meier survival plot demonstrating death-censored patient survival. There were no significant differences in the patient survival time between the groups ($p = 0.149$ for Gr. 7074, $p = 0.438$ for Gr. 75+).

4. Discussion

To the best of our knowledge, this is the first study to investigate the clinical outcomes of KT in patients ≥75 years of age. The most important finding is that there were no statistically significant differences in graft and patient survival between the age groups. Recipients aged ≥75 years showed no disadvantages regarding short and long-term outcomes when compared to those aged 65–69 years and 70–74 years. Regarding patient characteristics, pre-existing conditions and ischemia time, no significant differences were established between the three groups except for pre-transplant time on dialysis. Thus, an adequate comparison was possible.

Serum creatinine levels were similar across the three groups up to 5 years of follow-up, suggesting that allograft function was equivalent between the groups. One notable exception was serum creatine levels at 1 year after transplantation, but that difference did not persist.

Overall, recipient and allograft characteristics of this study were similar to those in cohorts examined in recent studies evaluating ESP outcomes [7–9]. Quast et al. conducted a single-center retrospective analysis of 217 KTs with a focus on donor age while Badhe et al. focused on prognostic factors for KTs. Graft and patient survival at 1 and 5 years of Quast, Bahde, and Jacobi et al. were comparable to those in groups 70–74 and ≥75 despite recipients in this study being significantly older (7–9) (see Table 5). These results support KT for ESRD patients ≥75 as biological age does not appear to influence the graft or survival of these patients.

Table 5. Comparison of death-censored graft and patient survival in the Eurotransplant Senior Program.

	Quast(9) ***n* = 217**	**Bahde(7)** ***n* = 89**	**Jacobi(8)** ***n* = 89**	**Our Results** **Group 65–69**	**Group 70–74**	**Group ≥75**
Age of recipients at KT	68.1 ± 3.8	72.2 (70–77)	68.2 ± 3.2	67.16 ± 1.51	71.86 ± 1.41	77.42 ± 3.30
Graft survival						
1-year	76.4%	n.a.	87%	90.7%	88.9%	100%
5-year	57.3%	77%	63%	68.0%	76.2%	71.4%
Patient survival						
1-year	88.2%	n.a	87%	92.9%	85.7%	100%
5-year	71.8%	69.8%	63%	68.0%	61.5%	62.5%

Age of recipient values are given in years and expressed as mean and SD or median and interquartile ranges.

Postoperative complications were common with an overall rate of 28.2% and with increased age, DGF, and time on dialysis identified as independent risk factors. Results by Quast and Bahde showed comparable postoperative complication rates at 23.2% and 22.5%, respectively. Jacobi et al., reported 46% of combined peri- and postoperative complications. Inconsistent definition of postoperative complications limits the accuracy of comparisons that can be made. Therefore, this study encourages the adoption of the more objective Clavien–Dindo classification in surgical literature to improve future evaluations.

Independent risk factors for the development of DGF were pre-transplant diabetes, high BMI, longer time on dialysis, and occurrence of perioperative complications. These results are supported by Badhe et al. who identified BMI ≥ 25 kg/m^2 as a risk factor for DGF and by Parekh et al., who determined pre-transplant diabetes as an independent risk factor in the analysis of 25,523 KTs [7,14]. Previous publications also found that prolonged CIT contributed to a higher incidence of DGF [6,7,11]. However, in this study CIT was kept to a minimum across all groups. This could be the reason that no significant correlation was established between DGF and elongated CIT.

Similarly to previous reports, our analysis identified delayed graft function to meaningfully associated with event of rejection [15,16]. Events of rejection strongly correlated with loss of graft. Preventing such events through adequate selection of transplant candidates and later through well-adjusted immunosuppression is critical.

Nogueira et al. analysis of 997 KT cases found that rejections at 1-year after KT were significantly higher in smokers [17]. This aligns with the results of this study as history of tobacco use correlated with events of rejections. Furthermore, this study established an association of ICU hospitalization with longer CIT and increased HLA-mismatches. It is unclear why HLA-mismatches correlate with higher incidents of ICU hospitalization but not simultaneously with higher incidents of rejection. The current kidney transplant allocation in the ESP with patients over 60 years of age does not take into consideration

the HLA mismatches between donor and recipient. A revised model of kidney allocation that considers for HLA compatibility without compromising CIT can prove beneficial in reducing the need for ICU treatment. A reduction in patients requiring ICU is predominantly of value in the ongoing SARS-CoV-2 pandemic, where ICU availability can swiftly become limited.

The proportion of recipients who died with a functioning graft was 65.5%. This is consistent with the findings of Giessing and Boesmueller et al., who described death as the main cause of graft loss [11,18]. The high proportion of patients dying with a functioning graft suggest that even suboptimal allografts can provide adequate function up to the end of the recipient's life.

The major limitation of this study is its relatively small sample size. Recipients ≥75 years adequate to undergo KT are scarce mainly due to the prolonged waiting time on dialysis. Hence, the assembly of a broader cohort remains challenging. An expansion of the donor pool combined with an increase in kidney donor availability could reduce the waitlisted time and allow for higher rates of transplantation in very old recipients. Additional multicenter studies with bigger cohorts are encouraged to confirm or challenge the results of this study.

5. Conclusions

In conclusion, graft and patient survival of recipients ≥75 years was comparable to Group 65–69 and Group 70–74. Therefore, recipients ≥75 years are appropriate candidates for KT and should not be discriminated with respect to their chronological age. An attentive pre-transplant evaluation with consideration of independent risk factors identified as increased time on dialysis, BMI ≥ 25 kg/m^2, history of smoking, and diabetes mellitus is crucial for transplant outcomes.

Author Contributions: Conceptualization, I.Z., F.F. and M.R.G.S.; methodology, I.Z., F.F., M.R.G.S., L.L. and M.H.L.; software, I.Z. and M.R.G.S.; validation, F.F., R.P., K.B. and L.J.L.; formal analysis, I.Z. and M.R.G.S.; investigation, I.Z.; data curation, I.Z.; writing—original draft preparation, I.Z.; writing—review and editing, all authors; visualization, I.Z., M.R.G.S., M.H.L. and S.R.M.G.; supervision, F.F., B.R., I.G., A.M., M.K. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study, due to its retrospective nature.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data of this study will be in the public archive of Charité-Universitätsmedizin Berlin, Germany.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rebollo, P.; Ortega, F.; Baltar, J.M.; Diaz-Corte, C.; Navascués, R.A.; Naves, M.; Ureña, A.; Badía, X.; Alvarez-Ude, F.; Alvarez-Grande, J. Health-related quality of life (HRQOL) in end stage renal disease (ESRD) patients over 65 years. *Geriatr. Nephrol. Urol.* **1998**, *8*, 85–94. [CrossRef] [PubMed]
2. Laupacis, A.; Keown, P.; Pus, N.; Krueger, H.; Ferguson, B.; Wong, C.; Muirhead, N. A study of the quality of life and cost-utility of renal transplantation. *Kidney Int.* **1996**, *50*, 235–242. [CrossRef] [PubMed]
3. Wolfe, R.A.; Ashby, V.B.; Milford, E.L.; Ojo, A.O.; Ettenger, R.E.; Agodoa, L.Y.C.; Held, P.J.; Port, F.K. Comparison of Mortality in All Patients on Dialy-sis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *N. Engl. J. Med.* **1999**, *341*, 1725–1730. [CrossRef] [PubMed]
4. Medin, C.; Elinder, C.; Hylander, B.; Blom, B.; Wilczek, H. Survival of patients who have been on a waiting list for renal transplan-tation. *Nephrol. Dial. Transplant.* **2000**, *15*, 701–704. [CrossRef] [PubMed]
5. Cohen, B.; Smits, J.M.; Haase, B.; Persijn, G.; Vanrenterghem, Y.; Frei, U. Expanding the donor pool to increase renal transplantation. *Nephrol. Dial. Transplant.* **2005**, *20*, 34–41. [CrossRef] [PubMed]

6. Frei, U.; Noeldeke, J.; Machold-Fabrizii, V.; Arbogast, H.; Margreiter, R.; Fricke, L.; Voiculescu, A.; Kliem, V.; Ebel, H.; Albert, U.; et al. Prospective Age-Matching in Elderly Kidney Transplant Recipients—A 5-Year Analysis of the Eurotransplant Senior Program. *Arab. Archaeol. Epigr.* **2007**, *8*, 50–57. [CrossRef] [PubMed]
7. Bahde, R.; Vowinkel, T.; Unser, J.; Anthoni, C.; Holzen, J.P.; Suwelack, B. Prognostic factors for kidney allograft survival in the Euro-transplant Senior Program. *Ann. Transplant.* **2014**, *19*, 201–209. [PubMed]
8. Jacobi, J.; Beckmann, S.; Heller, K.; Hilgers, K.F.; Apel, H.; Spriewald, B.; Amann, K.U. Deceased Donor Kidney Transplantation in the Euro-transplant Senior Program (ESP): A Single-Center Experience from 2008 to 2013. *Ann. Transplant.* **2016**, *21*, 94–104. [CrossRef] [PubMed]
9. Quast, L.S.; Grzella, S.; Lengenfeld, T.; Pillokeit, N.; Hummels, M.; Zgoura, P.; Westhoff, T.H.; Viebahn, R.; Schenker, P. Outcome of Kidney Transplantation Using Or-gans From Brain-dead Donors Older Than 75 Years. *Transplant. Proc.* **2020**, *52*, 119–126. [CrossRef] [PubMed]
10. Heldal, K.; Hartmann, A.; Grootendorst, D.C.; de Jager, D.J.; Leivestad, T.; Foss, A.; Midtvedt, K. Benefit of kidney transplantation beyond 70 years of age. *Nephrol. Dial. Transplant.* **2010**, *25*, 1680–1687. [CrossRef] [PubMed]
11. Boesmueller, C.; Biebl, M.; Scheidl, S.; Oellinger, R.; Margreiter, C.; Pratschke, J.; Margreiter, R.; Schneeberger, S. Long-Term Outcome in Kidney Transplant Recipients Over 70 Years in the Eurotransplant Senior Kidney Transplant Program: A Single Center Experience. *Transplantation* **2011**, *92*, 210–216. [CrossRef] [PubMed]
12. Rao, P.S.; Merion, R.M.; Ashby, V.B.; Port, F.K.; Wolfe, R.A.; Kayler, L.K. Renal Transplantation in Elderly Patients Older Than 70 Years of Age: Results From the Scientific Registry of Transplant Recipients. *Transplantation* **2007**, *83*, 1069–1074. [CrossRef] [PubMed]
13. European Commission. Statistical Office of the European Union. Ageing Europe: Looking at the Lives of Older People in the EU: 2019 Edition. [Internet]. LU: Publications Office. 2019. Available online: https://data.europa.eu/doi/10.2785/811048 (accessed on 22 May 2021).
14. Parekh, J.; Bostrom, A.; Feng, S. Diabetes Mellitus: A Risk Factor for Delayed Graft Function after Deceased Donor Kidney Transplantation. *Arab. Archaeol. Epigr.* **2010**, *10*, 298–303. [CrossRef] [PubMed]
15. Wu, W.K.; Famure, O.; Li, Y.; Kim, S.J. Delayed graft function and the risk of acute rejection in the modern era of kidney transplan-tation. *Kidney Int.* **2015**, *88*, 851–858. [CrossRef] [PubMed]
16. Weber, S.; Dienemann, T.; Jacobi, J.; Eckardt, K.-U.; Weidemann, A. Delayed graft function is associated with an increased rate of renal allograft rejection: A retrospective single center analysis. *PLoS ONE* **2018**, *13*, e0199445. [CrossRef]
17. Nogueira, J.M.; Haririan, A.; Jacobs, S.C.; Cooper, M.; Weir, M.R. Cigarette Smoking, Kidney Function, and Mortality After Live Donor Kidney Transplant. *Am. J. Kidney Dis.* **2010**, *55*, 907–915. [CrossRef] [PubMed]
18. Giessing, M.; Fuller, T.F.; Friedersdorff, F.; Deger, S.; Wille, A.; Neumayer, H.-H.; Schmidt, D.; Budde, K.; Liefeldt, L. Outcomes of Transplanting Deceased-Donor Kidneys between Elderly Donors and Recipients. *J. Am. Soc. Nephrol.* **2009**, *20*, 37–40. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

The Relationship between Initial Tacrolimus Metabolism Rate and Recipients Body Composition in Kidney Transplantation

Aureliusz Kolonko *, Patrycja Pokora, Natalia Słabiak-Błaż , Beata Czerwieńska, Henryk Karkoszka, Piotr Kuczera, Grzegorz Piecha and Andrzej Więcek

Department of Nephrology, Transplantation and Internal Medicine, Medical University of Silesia, Francuska 20/24, 40-027 Katowice, Poland; pat.pokora@gmail.com (P.P.); nataliablaz@gazeta.pl (N.S.-B.); bczerwienska@op.pl (B.C.); hkarkoszka@poczta.fm (H.K.); p.m.kuczera@gmail.com (P.K.); g.piecha@outlook.com (G.P.); awiecek@sum.edu.pl (A.W.)
* Correspondence: uryniusz@wp.pl; Tel.: +48-322591429

Abstract: There are several premises that the body composition of kidney transplant recipients may play a role in tacrolimus metabolism early after transplantation. The present study aimed at analyzing the relationship between the body composition parameters assessed by bioimpedance analysis (BIA) and initial tacrolimus metabolism. Immediately prior to transplantation, BIA using InBody 770 device was performed in 122 subjects. Tacrolimus concentration-to-dose (C/D) ratio was calculated based on the first blood trough level measurement. There was no difference in phase angle, visceral fat area, lean body mass index (LBMI) and the proportion of lean mass as a percentage of total body mass between the subgroups of slow and fast metabolizers. However, subjects with LBMI $\geq$ median value of 18.7 kg/m^2, despite similar initial tacrolimus dose per kg of body weight, were characterized by a significantly lower tacrolimus C/D ratio (median 1.39 vs. 1.67, respectively; $p < 0.05$) in comparison with the subgroup of lower LBMI. Multivariate regression analysis confirmed that age ($r_{partial} = 0.322$; $p < 0.001$) and LBMI ($r_{partial} = -0.254$; $p < 0.01$) independently influenced the tacrolimus C/D ratio. A LBMI assessed by BIA may influence the tacrolimus metabolism in the early post-transplant period and can be a useful in the optimization of initial tacrolimus dosing.

Keywords: bioimpedance analysis; drug dosing; lean body mass index; pharmacokinetics; tacrolimus C/D ratio

Citation: Kolonko, A.; Pokora, P.; Słabiak-Błaż, N.; Czerwieńska, B.; Karkoszka, H.; Kuczera, P.; Piecha, G.; Więcek, A. The Relationship between Initial Tacrolimus Metabolism Rate and Recipients Body Composition in Kidney Transplantation. *J. Clin. Med.* **2021**, *10*, 5793. https://doi.org/10.3390/jcm10245793

Academic Editor: Wisit Cheungpasitporn

Received: 20 October 2021
Accepted: 9 December 2021
Published: 10 December 2021

1. Introduction

Nowadays, the calcineurin inhibitor tacrolimus is the primary therapeutic option for patients after kidney transplantation. However, due to its substantial intra- and inter-patient variability in metabolism rate the accurate dosing is still challenging [1–3]. Moreover, many external factors also influence the tacrolimus blood trough level, making the frequent drug therapeutic monitoring mandatory [4–6]. As an inappropriately high or low tacrolimus level in the early post-transplant period can result in delayed graft function, acute rejection, diabetes mellitus, serious infections or even thrombotic microangiopathy [7–9], the precise initial dose tailoring is especially important to achieve the first post-transplant tacrolimus trough level within the therapeutic range, i.e., 5–15 ng/mL [10].

To date, several different approaches were proposed in order to optimize the initial tacrolimus dosing, including clinical factors and CYP3A5 genotyping [11–13] as well as computerized dose individualization [14], however not all were successfully validated based on the independent cohort [15]. Taking into account that older and overweight recipients are more prone to develop supratherapeutic first tacrolimus blood levels post-transplant [4,5,16], one could expect that the baseline proportion of fat and lean mass of the recipient may play a role in the post-transplant tacrolimus metabolism [17] and therefore may be another parameter to take into account in the sophisticated process of pre-transplant tacrolimus initial dose calculation. Interestingly, Han et al. reported higher

tacrolimus blood trough and 4-h-post-dose levels in stable kidney transplant recipients with the fat mass above median [18]. On the other hand, the frequently observed overhydration in kidney transplant candidates could make the calculation of proper initial tacrolimus dose based on body weight even more difficult. Taking all above evidence together, we hypothesized that some parameters of recipient's body composition, describing the body water compartments and the proportion of fat and lean mass, would be of value for the optimization of initial tacrolimus dosing. Notably, we did not find such an analysis in the current literature.

Thus, the aim of our prospective study was to investigate the relationship between several recipient's body composition parameters acquired by bioimpedance analysis (BIA) and the tacrolimus metabolism rate in the first days after kidney transplantation.

2. Materials and Methods

2.1. Study Group

We prospectively analyzed all 153 consecutive patients who received their kidney transplant in our center between August 2019 and June 2021. After the exclusion of patients using tacrolimus prior to the most recent transplantation, those with insufficient data and those who withdraw consent to participate, 122 subjects were included in the final analysis (Figure 1). The study was conducted in concordance with the protocol of Helsinki. The Institutional Review Board accepted the study protocol (No KNW/0022/KB1/81/18) and all participants gave their written informed consent.

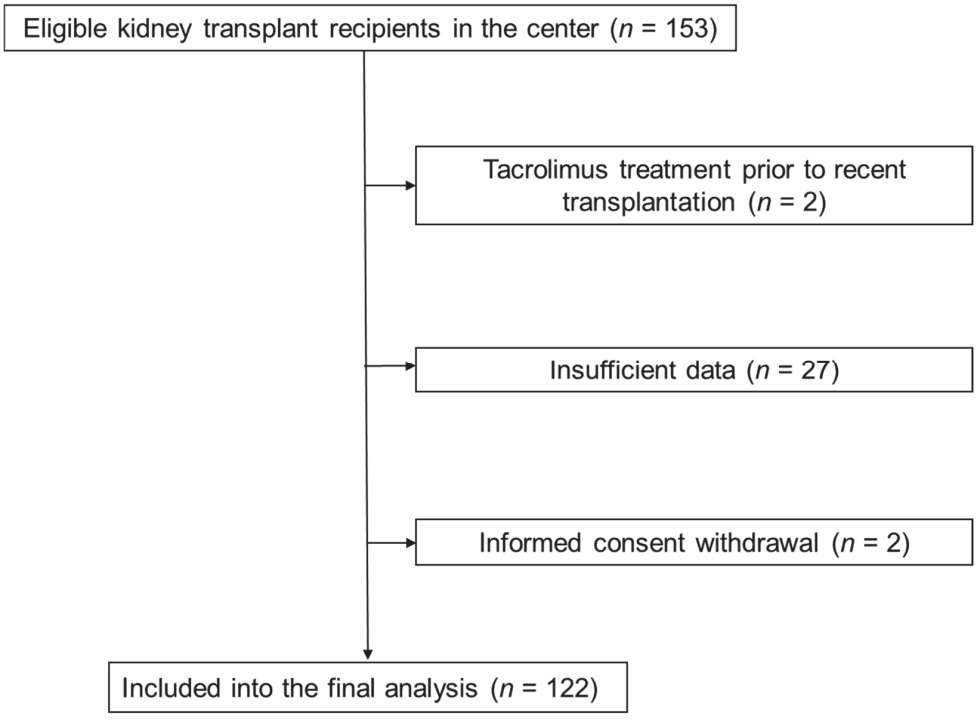


Figure 1. Study flow chart.

In each patient, the bioimpedance analysis of body composition was performed immediately prior to kidney transplantation procedure. The first tacrolimus blood trough level was determined within first day's post-transplantation.

2.2. Pre-Transplant BIA

The body composition analysis was performed within few hours before transplantation procedure using BIA device (InBody 770, InBody Japan Inc., Tokyo, Japan) with a multifrequency analyzer (1, 5, 50, 500 and 1000 kHz). All measurements were performed according to the manufacturer's instructions. Patients refrained from eating for minimum 5 h. During examination, patients stepped barefoot on the footplate containing separate foot electrodes and additionally held the right- and left-hand electrodes. Based on the measurements and BIA software program equations, intracellular water (ICW), extracellular water (ECW), total body water (TBW), ECW/TBW ratio, phase angle, visceral fat area (expressed in cm^2), lean body mass (LBM) and lean body mass index (LBMI, expressed in kg/m^2) were calculated. Additionally, the proportion of LBM as a percentage of total body mass was also calculated and analyzed. We also performed the segmental lean analysis in 5 different body sectors: both arms, trunk and both legs.

2.3. Immunosuppression Protocol and Tacrolimus C/D Ratio Calculation

All patients received routine immunosuppression regimen, consisted of twice daily tacrolimus (Prograf®, Astellas Pharma, Inc., Tokyo, Japan), mycophenolate mofetil and steroids, with an induction therapy using basiliximab or rabbit antithymocyte globulin (rATG). First doses of tacrolimus and mycophenolate were given pre-operatively. Mycophenolate mofetil was started from 750 mg BID. Steroids were given intravenously during operation (500 mg), then 125 mg i.v. the next day and subsequently 20 mg of oral prednisolone daily. Patient receiving rATG was given 125 mg of methylprednisolone instead of prednisolone before each dose.

According to the center protocol, the tacrolimus dose was prescribed based on patients' body weight. In order to avoid tacrolimus levels exceeding 15 ng/mL, the initial tacrolimus dose was decreased (by 32.9 % in an analyzed group) in patients older than 55 years, with BMI >25 kg/m^2 and those with the occurrence of anti-HCV antibodies. As patients treated with rATG induction routinely receive antifungal prophylaxis with 100 mg of fluconazole, those subjects were also prescribed a lower tacrolimus dose (usually 3 mg/day—for subjects with body weight below 60 kg—or 4 mg/day for other patients). Based on the first tacrolimus blood trough level measurement, the tacrolimus C/D ratio was calculated.

Delayed graft function (DGF) was defined as a need for dialysis therapy in the first post-transplant week. Acute rejection (AR) was diagnosed based on the results of protocol biopsy performed at a median 8th post-transplant day.

2.4. Statistical Analysis

Statistical analyses were performed using Statistica 13.3 PL for Windows (Tibco Inc., Palo Alto, CA, USA) and MedCalc v19.2.1 (MedCalc Software, Mariakerke, Belgium). Values are presented as means with 95% confidence interval, medians with interquartile ranges or frequencies. The main study comparison was performed between groups of patients allocated based on the initially calculated tacrolimus C/D ratio, using the Student t-test (for quantitative variables) or the χ^2 test (for qualitative variables). Variables with skewed distribution were compared using the Mann-Whitney U test. The second analysis compared patients with LBMI equal and above or lower than median value, using similar tests. Stepwise multiple regression analysis was performed for the tacrolimus C/D ratio as a dependent variable and age, BMI, the amount of residual diuresis, and LBMI as potential independent variables. For all analyses, a p value below 0.05 was considered statistically significant.

3. Results

3.1. Study Group Characteristics

There were 122 kidney transplant recipients recruited into this study. Based on the initial tacrolimus C/D ratio, study participants were divided into two groups, equal and above or below a median value. The baseline characteristics of study groups is given in

Table 1. Patients with slower tacrolimus metabolism (C/D ratio ≥ 1.48) were significantly older, more frequently treated with rATG induction as compared with fast metabolizers. Consistently, there was a significant correlation between age and tacrolimus C/D ratio ($r = 0.278$; $p < 0.01$). There was a tendency to more frequent re-transplants and lower HLA class II mismatch in the fast metabolizers group (Table 1).

Table 1. Baseline characteristics of study subgroups based on the median value of initial tacrolimus C/D ratio.

Parameter	Tacrolimus C/D Ratio		*p*
	Slow Metabolizers ≥1.48 *n* = 61	**Fast Metabolizers <1.48 *n* = 61**	
Patient			
Age (years)	51.6 (48.5–54.7)	44.7 (41.6–47.9)	<0.01
Sex (M/F)	40/21	36/25	0.46
BMI (kg/m^2)	25.6 (24.5–26.8)	25.7 (24.6–26.8)	0.95
Dialysis vintage (months) *	34 (25–55)	31 (20–44)	0.29
Residual diuresis (mL) *	300 (0–1000)	500 (100–1500)	0.35
Transplant procedure			
Retransplant (*n*, %)	4 (6.6)	10 (16.4)	0.09
HLA class I mismatch *	2 (2–3)	2 (2–3)	0.48
HLA class II mismatch *	1 (0–1)	1 (0–1)	0.09
CIT (h)	18.7 (16.9–20.4)	17.8 (16.2–19.4)	0.46
Induction therapy IL-2RB (*n*, %) ATG (*n*, %)	 29 (47.5) 32 (52.3)	 46 (75.4) 15 (24.6)	<0.01
DGF (*n*, %)	19 (31.1)	9 (14.8)	<0.05
Early acute rejection (*n*, %)	5 (8.2)	5 (8.2)	1.0
Tacrolimus dosing and metabolism			
Tacrolimus dose (mg/d) *	7.0 (4.0–12.0)	11.0 (8.0–13.0)	<0.001
Tacrolimus dose per kg (mg/kg) *	0.11 (0.06–0.14)	0.14 (0.12–0.16)	<0.001
Initial tacrolimus level (ng/mL) *	15.5 (9.0–21.6)	9.7 (6.4–12.0)	<0.001
Initial tacrolimus level > 15 ng/mL (%)	50.8	13.1	<0.001
Tacrolimus C/D ratio *	2.00 (1.71–2.50)	0.99 (0.74–1.24)	<0.001

Data presented as means with 95% confidence interval, * medians with Q1–Q3 values or frequencies, as appropriate. C/D, concentration-to-dose; BMI, body mass index; HLA, human leukocyte antigen; CIT, cold ischemia time; IL-2RB, interleukin-2 receptor blocker; ATG, antithymocyte globulin; DGF, delayed graft function.

There was no difference between the groups in the time from transplantation to the day of the first tacrolimus blood trough level measurement (median 2 (2–3) vs. 2 (2–3) days; $p = 0.1$). As expected, despite the substantially lower initial tacrolimus dosing, slow metabolizers presented significantly higher tacrolimus level and almost 4-fold more subjects reached the supratherapeutic drug level (Table 1). Nevertheless, after the subsequent dose adjustments, the tacrolimus trough levels in both study groups were similar thereafter until the discharge from the hospital (2nd post-transplant week: 9.5 (6.9–10.6) vs. 8.2 (6.8–9.6) ng/mL; $p = 0.19$, 3rd week: 8.4 (6.6–11.4) vs. 9.1 (7.1–10.8) ng/mL; $p = 0.88$, at discharge: 9.4 (7.2–10.9) vs. 8.7 (7.6–9.7) ng/mL; $p = 0.33$, respectively).

DGF was more frequently observed in the slow metabolizers group resulting in a tendency to longer median post-transplant hospital stay (17 (13–25) vs. 14 (13–19) in fast metabolizers, respectively; $p = 0.07$). However, the median serum creatinine concentrations during the hospital stay (3rd post-transplant day: 5.0 (2.8–8.1) vs. 3.5 (2.2–7.9) mg/dL; $p = 0.29$, 7th day: 2.3 (1.3–5.8) vs. 1.9 (1.2–5.5) mg/dL; $p = 0.33$, respectively) and at discharge (1.5 (1.1–2.1) vs. 1.5 (1.1–1.8) mg/dL; $p = 0.68$) were similar. Of note, the frequency of early AR episodes was also similar in both groups (Table 1).

3.2. Body Composition Parameters in Slow and Fast Tacrolimus Metabolizers

The BIA parameters describing the pre-transplant hydration status were also comparable between study groups, including the detailed lean mass analysis in 5 individual segments of the body. In line, the median post-transplant weight loss during the hospital stay was similar in both groups (3.1 (2.2–4.1) vs. 3.2 (2.3–4.1) kg in slow and fast metabolism groups, respectively; $p = 0.90$), with comparable values of systolic and diastolic blood pressure measured at 7th post-operative day 9SBP: 135 (130–150) vs. 140 (130–160); $p = 0.18$, DBP: 80 (80–90) vs. 85 (80–90); $p = 0.55$) and at discharge from the hospital (SBP: 130 (120–140) vs. 130 (125–140); $p = 0.38$, DBP: 80 (75–90) vs. 80 (78–85); $p = 0.49$).

Interestingly, we found a significant positive association between the post-transplant weight loss and the amount of pre-transplant residual diuresis ($r = 0.304$; $p = 0.01$). On the other hand, we also noted a weak reverse association between the residual diuresis and tacrolimus C/D ratio ($r = -0.186$; $p < 0.05$).

There were no significant differences between both analyzed groups in BIA parameters which reflect the proportion of fat and lean body mass (phase angle, visceral fat area, LBM, LBMI and the proportion of LBM as a percentage of total body mass) (Table 2). In the whole study group, BMI correlated the most with visceral fat area ($r = 0.772$; $p < 0.001$) and LBMI ($r = 0.562$; $p < 001$), whereas the correlations with LBM ($r = 0.389$; $p < 0.001$) and phase angle ($r = 0.249$; $p < 0.01$) were less pronounced. Of note, we found a weak reverse correlation between LBMI and tacrolimus C/D ratio ($r = 0.181$; $p < 0.05$). None of the remaining above analyzed parameters correlated significantly with tacrolimus C/D ratio.

Table 2. The comparison of the main results of bioimpedance analysis between both study subgroups.

Parameter	Tacrolimus C/D Ratio		*p*
	Slow Metabolizers ≥1.48 *n* = 61	**Fast Metabolizers <1.48 *n* = 61**	
Baseline body composition analysis			
Weight (kg)	74.9 (70.5–79.2)	76.1 (72.4–79.7)	0.67
ICW (L)	24.5 (23.1–25.9)	25.5 (24.1–26.9)	0.31
ECW (L)	15.7 (14.8–16.5)	16.2 (15.3–17.0)	0.42
TBW (L)	40.2 (37.9–42.5)	41.7 (39.4–44.0)	0.35
ECW/TBW	0.390 (0.387–0.393)	0.388 (0.385–0.390)	0.27
Phase angle (°)	5.0 (4.8–5.3)	5.2 (5.0–5.4)	0.32
Visceral fat area (cm^2) *	93.6 (59.1–126.3)	88.4 (51.5–129.9)	0.60
LBM (kg)	51.5 (48.6–54.4)	53.5 (50.5–56.4)	0.34
LBM (%)	69.2 (67.0–71.4)	70.5 (67.7–73.3)	0.45
LBMI (kg/m^2)	18.6 (17.9–19.3)	18.8 (18.2–19.5)	0.62

Data presented as means with 95% confidence interval, * medians with Q1-Q3 values, as appropriate. C/D, concentration-to-dose; ICW, intracellular water; ECW, extracellular water; TBW, total body water; LBM, lean body mass; LBMI, lean body mass index.

3.3. Body Composition Parameters in Groups Depending on the LBMI

In additional analysis, in which all study patients were assigned into two groups based on the value of BIA-derived LBMI equal and above or lower than median value, both groups were comparable in recipient age (49.0 (45.8–52.2) vs. 47.4 (44.1–50.8) years, respectively; $p = 0.50$), but the subjects with LBMI $\geq$ 18.7 kg/m^2 had significantly greater BMI (27.4 (26.5–28.4) vs. 23.7 (22.6–24.8) kg/m^2; $p < 0.001$) and residual diuresis (median 600 (250–1500) vs. 175 (0–1000) mL; $p < 0.01$) than subjects with LBMI below median value. They also presented significantly greater phase angle (5.4 (4.8–5.9) vs. 4.7 (4.2–5.3); $p < 0.001$), greater percentage of pre-transplant hydration measured in 5 different body compartments and greater median body weight reduction during the first post-transplant hospitalization (5.3 (2.8–6.3) vs. 1.9 (0.5–3.3) kg; $p < 0.001$).

At baseline, there were no differences in dialysis vintage, CIT, ECW/TBW, and visceral fat area between those groups. However, despite similar initial tacrolimus dose per kg of body weight (median 0.13 (0.09–0.15) vs. 0.14 (0.07–0.15) mg/kg/day; $p = 0.94$), the group of patients with LBMI $\geq$ 18.7 kg/m^2 had a significantly lower tacrolimus C/D ratio (median 1.39 (0.94–1.79) vs. 1.67 (1.00–2.28), respectively; $p < 0.05$) in comparison with the other group. As a consequence, regardless of the significantly higher initial tacrolimus dosing in subjects with the LBMI $\geq$ 18.7 kg/m^2 (median 12.0 (7.0–13.0) vs. 8.0 (4.0–10.0) mg/day; $p < 0.001$), the first tacrolimus blood trough level was similar in both groups (median 11.8 (7.8–18.5) vs. 10.4 (7.4–15.8) ng/mL, respectively; $p = 0.32$). Multiple regression analysis revealed that age ($r_{partial} = 0.322$; $p < 0.001$) and LBMI ($r_{partial} = -0.254$; $p < 0.01$) independently influence the tacrolimus C/D ratio.

4. Discussion

To our best knowledge, this is the first study which analyze the association between various parameters of body composition and the early post-transplant tacrolimus metabolism in kidney transplant patients. Hereby, we showed the independent association between the lean body mass index, calculated based on the bioimpedance measurement and the first post-transplant C/D ratio. This finding is of potential importance as it may be useful for the more precise tacrolimus dose determination immediately prior to kidney transplantation.

It is worth to noticing that tacrolimus C/D ratio calculated at 3-month or 6-month post-transplant time-point was previously shown to be a risk factor for significantly worse patient survival, worse kidney graft function and survival, higher rejection rate and the development of calcineurin inhibitor nephrotoxicity and BK nephropathy [19–21]. Of note, in our study we used tacrolimus C/D ratio calculated in the first day's post-transplant as a surrogate marker for tacrolimus metabolism. Interestingly, some metabolic differences defined based on C/D ratio were noted between LCP-tacrolimus and immediate-release tacrolimus during the early post-transplant period [22], however in our study all subjects were treated with an immediate-release drug formulation.

Recently, an increasing interest is observed in the literature in the role of body composition-based pharmacokinetic analyses in an effort to reduce severe drug toxicity. Particularly sarcopenia and body composition in cancer is being studied extensively, resulting in the emergence of body composition-tailored drug administration schemes [23]. As it was shown that dose per kilogram of LBM of the carboplatin was a significant predictor of severe hematologic toxicity, taking body composition into account was proposed for dose individualization of chemotherapeutic agents [24]. In line, computed tomography-derived body composition parameters were correlated with toxicity, dose reduction and termination of the treatment in patients with diffuse lymphoma receiving immunochemotherapy [25]. Moreover, based on cancer research, body composition-based dosing regimens were also proposed for quinolones [26] and anti-tumor necrosis factor medications [27].

In our transplant center, based on clinical observations and previous reports, we started to reduce the initial tacrolimus dose from 0.2 to 0.1–0.15 mg/kg/day in older and overweight/obese patients since 2011. However, as we summarized our experience, we

found that such a policy did not decrease the percentage of subjects with supratherapeutic first tacrolimus level substantially [28]. The present study protocol was designed to find potential parameters, which may be obtained prior to transplantation procedure and may help in the optimization of the initial tacrolimus dose. Finally, we confirmed that the bioimpedance body composition analysis of the potential kidney transplant candidate might be useful in such a dose tailoring, as the LBMI was found to be associated with the first post-transplant tacrolimus level independently of age and BMI. In line, of all anthropometric variables tested, a stepwise multiple regression analysis revealed that LBM was the only determinant of antipyrine clearance in apparently healthy subjects [29]. Moreover, LBM was found to best describe the average reported relationship between drug clearance and TBW in literature meta-analysis [30]. Interestingly, in chronic dialysis patients treated with vancomycin, the drug volume of distribution correlated best with LBM [31]. It was also shown in peritoneal dialysis patients that bioimpedance analysis can be used to estimate TBW and LBM with a correlation coefficient of 0.87 and 0.93, when using Deurenberg's formula [32].

Among the study limitations it should be noticed that the bioimpedance measurements were performed around the clock, when the potential recipients of a kidney from deceased donors were arriving to the transplant center. However, they abstained from eating for a minimum of 5–6 h prior to the examination, as they were aware of the planned transplantation and the pre-transplant dialysis session was performed. Another limitation is the lack of CYP3A5 genotyping in our patients. However, Polish Caucasian population is rather homogenic, with the vast majority of subjects (approximately 90%) being slow calcineurin inhibitor metabolizers [33]. Moreover, to date, CYP3A5 genotyping is neither recommended nor routinely performed prior to transplantation; thus, it cannot be used for initial tacrolimus dose calculation in daily clinical practice. Furthermore, according to some reports, the optimization of initial tacrolimus dose using pharmacogenetics testing was poorly predictive of tacrolimus clearance and did not improve clinical outcomes [15,34,35].

5. Conclusions

To conclude, in our present study we found the independent influence of LBMI on the very early tacrolimus C/D ratio post kidney transplantation. Thus, one could expect that based on this immediately assessed, non-invasive body composition parameter it will be possible to more precisely adjust the initial tacrolimus dose in order to minimize its potential toxicity. However, the clinical utility of this novel covariate for the optimization of tacrolimus dosing is still to be proven and needs the prospective validation in the randomized study.

Author Contributions: Conceptualization, A.K.; methodology, A.K.; formal analysis, A.K.; investigation, A.K., P.P., N.S.-B., B.C., H.K., P.K., G.P.; writing—original draft preparation, A.K.; writing—review and editing, G.P., N.S.-B., P.K, A.W.; supervision, A.K. and A.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from Medical University of Silesia No PCN-1-021-N/0/K.

Institutional Review Board Statement: The Institutional Review Board accepted the study protocol (No KNW/0022/KB1/81/18).

Informed Consent Statement: All study participants gave their written informed consent.

Data Availability Statement: The datasets generated and/or analyzed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bentata, Y. Tacrolimus: 20 years of use in adult kidney transplantation. What we should know about its nephrotoxicity. *Artif. Organs.* **2020**, *44*, 140–152. [CrossRef] [PubMed]
2. Saint-Marcoux, F.; Woillard, J.B.; Jurado, C.; Marquet, P. Lessons from routine dose adjustment of tacrolimus in renal transplant patients based on global exposure. *Ther. Drug Monit.* **2013**, *35*, 322–327. [CrossRef] [PubMed]
3. Staatz, C.E.; Tett, S.E. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin. Pharmacokinet.* **2004**, *43*, 623–653. [CrossRef] [PubMed]
4. Stratta, P.; Quaglia, M.; Cena, T.; Antoniotti, R.; Fenoglio, R.; Menegotto, A.; Ferrante, D.; Genazzani, A.; Terrazzino, S.; Magnani, C. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. *Eur. J. Clin. Pharmacol.* **2012**, *68*, 671–680. [CrossRef] [PubMed]
5. Krzyżowska, K.; Kolonko, A.; Giza, P.; Chudek, J.; Więcek, A. Which Kidney Transplant Recipients Can Benefit from the Initial Tacrolimus Dose Reduction? *Biomed Res. Int.* **2018**, *30*, 4573452. [CrossRef]
6. Vavic, N.; Rancic, N.; Dragojevic-Simic, V.; Draskovic-Pavlovic, B.; Bokonjic, D.; Ignjatovic, L.; Mikov, M. The influence of comedication on tacrolimus blood concentration in patients subjected to kidney transplantation: A retrospective study. *Eur. J. Drug Metab. Pharmacokinet.* **2014**, *39*, 243–253. [CrossRef]
7. Ro, H.; Jeong, J.C.; Kong, J.M.; Min, J.W.; Park, S.K.; Lee, J.; Koo, T.Y.; Yang, J.; Kim, M.S.; Hwang, S.; et al. The tacrolimus metabolism affect post-transplant outcome mediating acute rejection and delayed graft function: Analysis from Korean Organ Transplantation Registry data. *Transpl. Int.* **2021**, *34*, 163–174. [CrossRef] [PubMed]
8. Rodrigo, E.; de Cos, M.A.; Fernández-Fresnedo, G.; Sánchez, B.; Ruiz, J.C.; Piñera, C.; Palomar, R.; Cotorruelo, J.G.; Gómez-Alamillo, C.; de Castro, S.S.; et al. Higher initial tacrolimus blood levels and concentration-dose ratios in kidney transplant recipients who develop diabetes mellitus. *Transpl. Proc.* **2005**, *37*, 3819–3820. [CrossRef]
9. Saito, M.; Satoh, S.; Kagaya, H.; Tsuruta, H.; Obara, T.; Kumazawa, T.; Inoue, T.; Inoue, K.; Miura, M.; Yuasa, T.; et al. Thrombotic microangiopathy developing in early stage after renal transplantation with a high trough level of tacrolimus. *Clin. Exp. Nephrol.* **2008**, *12*, 312–315. [CrossRef]
10. Schiff, J.; Cole, E.; Cantarovich, M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. *Clin. J. Am. Soc. Nephrol.* **2007**, *2*, 374–384. [CrossRef] [PubMed]
11. Wang, P.; Mao, Y.; Razo, J.; Zhou, X.; Wong, S.T.; Patel, S.; Elliott, E.; Shea, E.; Wu, A.H.; Gaber, A.O. Using genetic and clinical factors to predict tacrolimus dose in renal transplant recipients. *Pharmacogenomics* **2010**, *11*, 1389–1402. [CrossRef]
12. Passey, C.; Birnbaum, A.K.; Brundage, R.C.; Oetting, W.S.; Israni, A.K.; Jacobson, P.A. Dosing equation for tacrolimus using genetic variants and clinical factors. *Br. J. Clin. Pharmacol.* **2011**, *72*, 948–957. [CrossRef] [PubMed]
13. Åsberg, A.; Midtvedt, K.; van Guilder, M.; Størset, E.; Bremer, S.; Bergan, S.; Jelliffe, R.; Hartmann, A.; Neely, M.N. Inclusion of CYP3A5 genotyping in a nonparametric population model improves dosing of tacrolimus early after transplantation. *Transpl. Int.* **2013**, *26*, 1198–1207. [CrossRef]
14. Størset, E.; Åsberg, A.; Skauby, M.; Neely, M.; Bergan, S.; Bremer, S.; Midtvedt, K. Improved Tacrolimus Target Concentration Achievement Using Computerized Dosing in Renal Transplant Recipients—A Prospective, Randomized Study. *Transplantation* **2015**, *99*, 2158–2166. [CrossRef] [PubMed]
15. Boughton, O.; Borgulya, G.; Cecconi, M.; Fredericks, S.; Moreton-Clack, M.; MacPhee, I.A. A published pharmacogenetic algorithm was poorly predictive of tacrolimus clearance in an independent cohort of renal transplant recipients. *Br. J. Clin. Pharmacol.* **2013**, *76*, 425–431. [CrossRef] [PubMed]
16. Rodrigo, E.; de Cos, M.A.; Sánchez, B.; Ruiz, J.C.; Piñera, C.; Fernández-Fresnedo, G.; Palomar, R.; Pérez-Ceballos, M.A.; Cotorruelo, J.G.; Zubimendi, J.A.; et al. High initial blood levels of tacrolimus in overweight renal transplant recipients. *Transpl. Proc.* **2005**, *37*, 1453–1454. [CrossRef] [PubMed]
17. St-Onge, M.-P.; Gallagher, D. Body composition changes with aging: The cause or the result of alterations in metabolic rate and macronutrient oxidation? *Nutrition* **2010**, *26*, 152–155. [CrossRef] [PubMed]
18. Han, S.S.; Kim, D.H.; Lee, S.M.; Han, N.Y.; Oh, J.M.; Ha, J.; Kim, Y.S. Pharmacokinetics of tacrolimus according to body composition in recipients of kidney transplants. *Kidney Res. Clin. Pract.* **2012**, *31*, 157–162. [CrossRef] [PubMed]
19. Tholking, G.; Fortmann, C.; Koch, R.; Gerth, H.U.; Pabst, D.; Pavenstadt, H.; Kabar, I.; Husing, A.; Wolters, H.; Reuter, S.; et al. The tacrolimus metabolism rate influences renal function after kidney transplantation. *PLoS ONE* **2014**, *9*, e111128. [CrossRef] [PubMed]
20. Nowicka, M.; Górska, M.; Nowicka, Z.; Edyko, K.; Edyko, P.; Wiślicki, S.; Zawiasa-Bryszewska, A.; Strzelczyk, J.; Matych, J.; Kurnatowska, I. Tacrolimus: Influcnce of the posttransplant concentration/dose ratio on kidney graft function in a two-year follow-up. *Kidney Blood Press Res.* **2019**, *44*, 1075–1088. [CrossRef]
21. Schutte-Nutgen, K.; Tholking, G.; Steinke, J.; Pavenstadt, H.; Schmidt, R.; Suwelack, B.; Reuter, S. Fast Tac metabolizers at risk—It is time for a C/D ratio calculation. *J. Clin. Med.* **2019**, *8*, 587. [CrossRef] [PubMed]
22. Suwelack, B.; Bunnapradist, S.; Meier-Kriesche, U.; Stevens, D.R.; Procaccianti, C.; Morganti, R.; Budde, K. Effect of concentration/dose ratio in de novo kidney transplant recipients receiving LCP-tacrolimus or immediate-release tacrolimus: Post hoc analysis of a phase 3 clinical trial. *Ann. Transpl.* **2020**, *25*, e923278. [CrossRef] [PubMed]

23. Hilmi, M.; Jouinot, A.; Burns, R.; Pigneur, F.; Mounier, R.; Gondin, J.; Neuzillet, C.; Goldwasser, F. Body composition and sarcopenia: The next-generation of personalized oncology and pharmacology? *Pharmacol. Ther.* **2019**, *196*, 135–159. [CrossRef] [PubMed]
24. Sjøblom, B.; Benth, J.Š.; Grønberg, B.H.; Baracos, V.E.; Sawyer, M.B.; Fløtten, Ø.; Hjermstad, M.J.; Aass, N.; Jordhøy, M. Drug Dose Per Kilogram Lean Body Mass Predicts Hematologic Toxicity from Carboplatin-Doublet Chemotherapy in Advanced Non-Small-Cell Lung Cancer. *Clin. Lung Cancer.* **2017**, *18*, e129–e136. [CrossRef] [PubMed]
25. Guo, J.; Cai, P.; Li, P.; Cao, C.; Zhou, J.; Dong, L.; Yang, Y.; Xuan, Q.; Wang, J.; Zhang, Q. Body Composition as a Predictor of Toxicity and Prognosis in Patients with Diffuse Large B-Cell Lymphoma Receiving R-CHOP Immunochemotherapy. *Curr. Oncol.* **2021**, *23*, 1325–1337. [CrossRef] [PubMed]
26. Jiao, Y.; Kim, T.H.; Tao, X.; Kinzig, M.; Landersdorfer, C.B.; Drescher, S.K.; Sutaria, D.S.; Moya, B.; Holzgrabe, U.; Sörgel, F.; et al. First population pharmacokinetic analysis showing increased quinolone metabolite formation and clearance in patients with cystic fibrosis compared to healthy volunteers. *Eur. J. Pharm. Sci.* **2018**, *123*, 416–428. [CrossRef]
27. Ding, N.S.; Malietzis, G.; Lung, P.F.C.; Penez, L.; Yip, W.M.; Gabe, S.; Jenkins, J.T.; Hart, A. The body composition profile is associated with response to anti-TNF therapy in Crohn's disease and may offer an alternative dosing paradigm. *Aliment. Pharmacol. Ther.* **2017**, *46*, 883–891. [CrossRef] [PubMed]
28. Krzyżowska, K.; Kolonko, A.; Giza, P.; Chudek, J.; Więcek, A. No Significant Influence of Reduced Initial Tacrolimus Dose on Risk of Underdosing and Early Graft Function in Older and Overweight Kidney Transplant Recipients. *Transpl. Proc.* **2018**, *50*, 1755–1759. [CrossRef] [PubMed]
29. Nawaratne, S.; Brien, J.E.; Seeman, E.; Fabiny, R.; Zalcberg, J.; Cosolo, W.; Angus, P.; Morgan, D.J. Relationships among liver and kidney volumes, lean body mass and drug clearance. *Br. J. Clin. Pharmacol.* **1998**, *46*, 447–452. [CrossRef]
30. McLeay, S.C.; Morrish, G.A.; Kirkpatrick, C.M.; Green, B. The relationship between drug clearance and body size: Systematic review and meta-analysis of the literature published from 2000 to 2007. *Clin. Pharm.* **2012**, *51*, 319–330. [CrossRef]
31. Hartinger, J.M.; Šíma, M.; Hronová, K.; Halouzková, B.A.; Szonowská, B.; Polakovič, V.; Bednářová, V.; Hladinová, Z.; Tesař, V.; Slanař, O. Vancomycin pharmacokinetics in patients treated with intermittent haemodialysis based on therapeutic drug monitoring. *J. Chemother.* **2021**, *23*, 1–8. [CrossRef] [PubMed]
32. De Fijter, W.M.; de Fijter, C.W.; Oe, P.L.; Ter Wee, P.M.; Donker, A.J. Assessment of total body water and lean body mass from anthropometry, Watson formula, creatinine kinetics, and body electrical impedance compared with antipyrine kinetics in peritoneal dialysis patients. *Nephrol Dial. Transpl.* **1997**, *12*, 151–156. [CrossRef] [PubMed]
33. Grenda, R.; Prokurat, S.; Ciechanowicz, A.; Piatosa, B.; Kaliciński, P. Evaluation of the genetic background of standard-immunosuppressant-related toxicity in a cohort of 200 paediatric renal allograft recipients—A retrospective study. *Ann. Transpl.* **2009**, *14*, 18–24.
34. Pallet, N.; Etienne, I.; Buchler, M.; Bailly, E.; Hurault de Ligny, B.; Choukroun, G.; Colosio, C.; Thierry, A.; Vigneau, C.; Moulin, B.; et al. Long-Term Clinical Impact of Adaptation of Initial Tacrolimus Dosing to CYP3A5 Genotype. *Am. J. Transpl.* **2016**, *16*, 2670–2675. [CrossRef] [PubMed]
35. Niioka, T.; Kagaya, H.; Saito, M.; Inoue, T.; Numakura, K.; Habuchi, T.; Satoh, S.; Miura, M. Capability of utilizing CYP3A5 polymorphisms to predict therapeutic dosage of tacrolimus at early stage post-renal transplantation. *Int. J. Mol. Sci.* **2015**, *16*, 1840–1854. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

The Influence of Parathyroidectomy on Osteoporotic Fractures in Kidney Transplant Recipients: Results from a Retrospective Single-Center Trial

Ulrich Jehn [1,*,†], Anja Kortenhorn [1,†], Katharina Schütte-Nütgen [1], Gerold Thölking [1], Florian Westphal [1], Markus Strauss [2], Dirk-Oliver Wennmann [1], Hermann Pavenstädt [1], Barbara Suwelack [1], Dennis Görlich [3] and Stefan Reuter [1]

1 Department of Medicine D, Division of General Internal Medicine, Nephrology and Rheumatology, University Hospital of Muenster, 48149 Muenster, Germany; anja.kortenhorn@ukmuenster.de (A.K.); katharina.schuette-nuetgen@ukmuenster.de (K.S.-N.); gerold.thoelking@ukmuenster.de (G.T.); florian.westphal@ukmuenster.de (F.W.); dirkoliver.wennmann@ukmuenster.de (D.-O.W.); Hermann.Pavenstaedt@ukmuenster.de (H.P.); Barbara.Suwelack@ukmuenster.de (B.S.); Stefan.Reuter@ukmuenster.de (S.R.)
2 Department of Medicine C, Division of Cardiology and Angiology, University Hospital of Muenster, 48149 Muenster, Germany; Markus.Strauss@ukmuenster.de
3 Institute of Biostatistics and Clinical Research, University of Muenster, 48149 Muenster, Germany; Dennis.Goerlich@ukmuenster.de
* Correspondence: ulrich.jehn@ukmuenster.de; Tel.: +49-251-83-47540; Fax: +49-251-83-56973
† These authors contributed equally to this work.

Citation: Jehn, U.; Kortenhorn, A.; Schütte-Nütgen, K.; Thölking, G.; Westphal, F.; Strauss, M.; Wennmann, D.-O.; Pavenstädt, H.; Suwelack, B.; Görlich, D.; et al. The Influence of Parathyroidectomy on Osteoporotic Fractures in Kidney Transplant Recipients: Results from a Retrospective Single-Center Trial. *J. Clin. Med.* **2022**, *11*, 654. https://doi.org/10.3390/jcm11030654

Academic Editors: Wisit Cheungpasitporn, Charat Thongprayoon and Wisit Kaewput

Received: 31 December 2021
Accepted: 26 January 2022
Published: 27 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Kidney transplant (KTx) recipients are a high-risk population for osteoporotic fractures. We herein aim to identify the role of pre-transplant parathyroidectomy (PTX) and other modifiable factors associated with osteoporotic fractures in KTx recipients. We conducted a retrospective study involving 711 adult patients (4608 patient-years) who were transplanted at our center between January 2007 and June 2015. Clinical data were extracted from patients' electronic medical records. Different laboratory and clinical parameters for mineral bone disease (MBD) and osteoporosis, including medication, were evaluated. We chose fracture events unrelated to malignancies or adequate trauma as the primary endpoint. Osteoporotic fractures occurred in 47 (6.6%) patients (median 36.7 months, IQR 45.9) after KTx (fracture incidence of 10 per 1000 person-years). Prior to KTx, subtotal PTX was performed in 116 patients (16.3%, median time 4.2 years before KTx, IQR 5.0). Of the patients with fracture (n = 47), only one (2.2%) patient had previously undergone PTX. After adjusting for the known fracture risk factors MBD and osteoporosis, PTX remained a protective factor against fractures (HR 0.134, CI 0.018–0.991, p = 0.049). We observed a reduced risk for pathological fractures in KTx patients who underwent PTX, independent from elevated parathyroid hormone at the time of KTx or afterwards.

Keywords: kidney transplantation; chronic kidney disease; mineral bone disorder; parathyroidectomy; parathyroid hormone; osteoporosis; bone fractures

1. Introduction

Kidney transplant (KTx) recipients are a high-risk population for osteoporotic fractures, yet their environment combines a number of risk factors that lead to mineral bone disorder (MBD) and osteoporosis. Osteoporotic fractures increase morbidity and mortality in elderly patients; therefore, their prevention is an important issue [1].

Since the majority of patients after KTx show impaired renal function/chronic kidney disease (CKD) and present a long history of end-stage renal disease (ESRD), CKD-related MBD also plays an important role in KTx recipients. It refers to CKD-MBD after kidney transplantation. Both high FGF-23 levels and hyperparathyroidism are present

post-transplant, contributing to hypophosphatemia and hypercalcemia [2]. Secondary hyperparathyroidism (sHPT) and uremic toxin accumulation enhance osteoclast-mediated bone resorption with increased release of calcium and phosphate [2].

After KTx, further transplantation-related factors associated with MBD and osteoporosis occur. In addition to electrolyte imbalances (such as hypophosphatemia, hypomagnesemia and hypercalciuria), reduced physical activity, sarcopenia, and immunosuppressive medication, which usually includes corticosteroids and calcineurin-inhibitors (CNI), increase bone resorption [3–5]. Therefore, KTx recipients have a significantly lower bone mineral density (BMD) compared to the general population [5].

Treatment of hyperparathyroidism (HPT) and MBD is primarily based on the assessments of laboratory values of phosphate, calcium, PTH level, alkaline phosphatase, and vitamin D. The primary therapeutic approach is drug-based and aims primarily to identify and treat long-term trends. PTX is indicated for ESRD patients with HPT who do not respond adequately to medical or pharmacologic therapy [6].

New pharmacological approaches with calcimimetics have significantly reduced the frequency of PTXs in ESRD patients [7]. However, improvements in mortality rate and bone metabolism with the use of calcimimetics have not been consistently confirmed.

In patients with primary hyperparathyroidism, Yeh et al. demonstrated that PTX is associated with a reduced risk of fractures [8]. In chronic hemodialysis patients with sHPT, Rudser et al. observed a reduced fracture risk in patients after PTX.

For patients after KTx who have secondary or tertiary HPT, data regarding fracture risk is sparse, and it is unclear to date whether PTX affects the risk of osteoporotic fractures in this particular setting. Thus, we aimed to clarify the effect of PTX and other factors available for retrospective analysis associated with MBD on osteoporotic bone fractures in KTx recipients.

2. Materials and Methods

2.1. Study Design and Population

We conducted a retrospective study involving 711 adult patients with a follow-up of 4608 patient-years transplanted at our center between January 2007 and June 2015. The follow-up period ended on 25 June 2019. Transplanted patients under the age of 18 years were excluded. Patients were censored for loss of allograft function and death with a functioning allograft. Patient demographic and clinical characteristics were recorded at the time of KTx. Informed written consent was obtained from all patients to collect their data at the time of transplantation. Patient data were anonymized before analysis. Regarding patients with PTX, we only considered those patients who received PTX before KTx. Seven patients in our collective who had PTX surgery after KTx were excluded. This study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved by the local ethics committee (Ethik Kommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität, 2014-381-f-N). Data were extracted from the electronic patient records. Induction therapy was chosen according to immunologic risks. One gram mycophenolate mofetil was given twice a day; the dosage was reduced if adverse events occurred. Prednisolone was started with 500 mg intravenously (i.v.) before KTx, followed by 100 mg for three days; then the dose was reduced by 20 mg/day. A dosage of 20 mg/day was maintained until day 30 and then slowly reduced to 5 mg/day. Maintenance immunosuppressive therapy usually consisted of a calcineurin inhibitor (tacrolimus), mycophenolate sodium or mycophenolate mofetil, and prednisolone. m-TOR-inhibitor-based immunosuppression after KTx was chosen only in a minority of patients, usually when they were enrolled in clinical trials.

2.2. Analyzed Markers and Parameters

To characterize the bone metabolism of the study subjects, we evaluated different laboratory markers and parameters, including intact PTH (iPTH) (normal range

15.6–65 pg/mL), total serum calcium levels, ionized calcium levels, medication with native and active vitamin D preparations, vitamin D analogues, bisphosphonates, calcimimetics and IgG2-anti-RANKL-antibodies (denosumab).

Each of the laboratory parameters was evaluated three months and one year after KTx. PTH was also evaluated immediately pre KTx. Medication was evaluated one, two, and three years after KTx.

We chose fracture events unrelated to malignancies or adequate trauma as the primary endpoint.

The primary endpoint was ascertained by reviewing all patient files manually. To evaluate a potential association of tacrolimus metabolism and MBD, we calculated the concentration/dose (C/D) ratio three months after KTx. The C/D ratio allows the classification of patients into slow (C/D ratio $\geq$ 1.05) and fast tacrolimus metabolizers (C/D ratio < 1.05) [9].

2.3. Statistical Analysis

The data were analyzed with IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA). The results are expressed as a median with interquartile range (IQR) or mean with standard deviation (SD). Non-continuous parameters were analyzed by Fisher's exact tests and chi-square tests and the continuous parameters were analyzed by a Mann–Whitney U-test and Kruskal–Wallis test, respectively, where appropriate. A p-value ≤ 0.05 was considered statistically significant.

Repeated measures ANOVA was applied to compare courses of PTH (before KTx, at 3 months, at 12 months) between different groups.

We analyzed the probability for fracture-free survival after KTx by Kaplan–Meier analysis and a log-rank test. To further, estimate the effect of PTX on the fracture incidence, we considered death as a competing risk. Cumulative incidence functions were estimated for both competing outcomes (death and fracture) and PTX groups were compared using Gray's test.

Univariate Cox proportional hazard models were fitted to identify the potential predictors for fracture events. Death was considered censored in this analysis; i.e., the model corresponds to a cause-specific hazard model. Variables that were considered statistically noticeable by univariate analysis were used for multivariable Cox proportional hazard regression analysis to identify independent prediction factors for fracture events. Besides these, the two well-known influencing factors on bone mineral disease, female sex and dialysis vintage were added to the multivariable Cox proportional hazard regression analysis.

We analyzed the effect of the estimated glomerular filtration rate (eGFR) at different time points after KTx on fracture risk in Cox proportional hazard models using a landmark approach. We fitted five Cox proportional hazard models, starting at 12 months, 24, 36, 48, and 60 months. Within each analysis, only patients who lived without a fracture at the landmark time point were included with their eGFR values, respectively. Event times were recalculated starting from the landmark time point. Thus, each model evaluates the eGFR as a risk factor for new fractures after the respective time point. Results are presented as a forest plot.

For all Cox proportional hazard models, hazard ratios (HR) and 95% confidence intervals (CI) were reported.

3. Results

3.1. Baseline Characteristics

Baseline characteristics of the study populations are given in Table 1. Median age at transplantation was 53.0 years (range 17.8–78.4), 430 (60.5%) were male, and 204 (28.7%) received a living donor transplant.

Induction therapy was performed using basiliximab in 588 (83.6%) cases. A total of 37 (5.3%) of the patients received thymoglobuline (Table 1).

Table 1. Patients' demographic and clinical characteristics at transplantation.

Variable	All ** (*n* = 711)	Fracture (*n* = 47)	No Fracture (*n* = 664)	*p*-Value
Age at Tx * (years), median (IQR)	53.0 (21.7)	60.8 (11.7)	52.39 (21.9)	0.000 [a]
Sex male, *n* (%)	430 (60.5%)	22 (46.8%)	406 (61.5%)	0.063 [b]
mismatch-HLA-A, *n* (%)				
none	250 (35.3%)	16 (34.0%)	232 (35.3%)	1.000 [b]
1	338 (47.7%)	23 (48.9%)	313 (47.6%)	
2	120 (16.9%)	8 (17.0%)	112 (17.0%)	
mismatch-HLA-B, *n* (%)				
none	163 (23.0%)	12 (25.5%)	151 (23.0%)	0.152 [b]
1	339 (47.9%)	27 (57.4%)	309 (47.0%)	
2	206 (29.0%)	8 (17.0%)	197 (30.0%)	
mismatch-HLA-DR, *n* (%)				
none	178 (25.1%)	12 (25.5%)	164 (25.0%)	0.607 [b]
1	340 (48.0%)	20 (42.6%)	320 (48.7%)	
2	190 (26.8%)	15 (31.9%)	173 (26.3%)	
PRA > 85%, *n* (%)	12 (1.7%)	1 (2.1%)	11 (1.7%)	0.566 [b]
PRA > 5%, *n* (%)	84 (11.8%)	4 (8.5%)	80 (12.2%)	0.641 [b]
Living donor Tx *, *n* (%)	204 (28.7%)	10 (21.3%)	194 (29.4%)	0.317 [b]
ABO incompatible Tx *, *n* (%)	40 (5.6%)	0 (0.0%)	40 (6.1%)	0.101 [b]
Cold ischemia time (hours), median (IQR)	7.93 (9.18)	7.67 (7.03)	7.96 (9.21)	0.779 [a]
Warm ischemia time (minutes), mean ± SD	32.94 ± 8.33	34.20 ± 8.93	32.82 ± 8.29	0.130 [a]
Dialysis prior to Tx *, *n* (%)	662 (93.1%)	45 (95.7%)	612 (92.7%)	0.764 [b]
Dialysis vintage (months) median (IQR)	54.59 (65.23)	54.59 (51.44)	53.82 (66.35)	0.622 [a]
Previous Tx *, *n* (%)	93 (13.1%)	6 (12.8%)	87 (13.2%)	1.000 [b]
CMV mismatch D/R, *n* (%)				
D^-/R^-	122 (17.2%)	6 (13.0%)	116 (17.6%)	0.489 [b]
D^-/R^+	123 (17.3%)	5 (10.9%)	118 (17.9%)	
D^+/R^-	165 (23.2%)	12 (26.1%)	152 (23.0%)	
D^+/R^+	300 (42.2%)	23 (50.0%)	274 (41.5%)	
Initial immunosuppression, *n* (%)				
Initial steroid use	700 (98.5%)	47 (100%)	649 (98.3%)	1.000 [b]
Initial MMF * use	683 (96.1%)	44 (93.6%)	635 (96.2%)	0.423 [b]
Initial CyA * use	24 (3.4%)	2 (4.3%)	22 (3.3%)	0.670 [b]
Initial tacrolimus use	687 (96.6%)	45 (95.7%)	638 (96.7%)	0.670 [b]
Initial mTOR * inhibitor use	29 (4.1%)	3 (6.4%)	26 (3.9%)	0.433 [b]
Diagnosis of ESRD, *n* (%)				0.004 [c]
Hypertension	55 (7.7%)	1 (2.1%)	54 (8.2%)	
Diabetes	43 (6.0%)	3 (6.4%)	40 (6.1%)	
Polycystic kidney disease	104 (14.6%)	15 (31.9%)	88 (13.3%)	
Obstructive Nephropathy	35 (4.9%)	2 (4.3%)	33 (5.0%)	
Glomerulonephritis	228 (32.1%)	6 (12.8%)	221 (33.5%)	
FSGS *	32 (4.5%)	3 (6.4%)	29 (4.4%)	
Interstitial nephritis	36 (5.1%)	2 (4.3%)	33 (5.0%)	
Vasculitis	23 (3.2%)	4 (8.5%)	19 (2.9%)	
Other	102 (14.3%)	6 (12.8%)	96 (14.5%)	
Unknown	53 (7.5%)	5 (10.6%)	47 (7.1%)	

[a] Mann–Whitney U-test, [b] Fisher´s exact test, [c] Chi square test, * Abbreviations: Tx: transplantation; HLA: human leukocyte antigen; PRA: panel reactive antibodies; MMF: mycophenolate, mofetil; CyA cyclosporine A; FSGS: focal segmental glomerulosclerosis, ** missing values: if total numbers are below 711, single values were not available in electronic patient records.

3.2. Outcome Data

Patients' eGFR values and landmark univariable Cox proportional hazard regressions for fracture risk depending on eGFR are depicted in Figure 1.

Landmark time	N	Events	HR	95%CI	p-value	Fracture eGFR (ml/min)	No fracture eGFR (ml/min)
12 months	626	36	0.984	0.967–1.002	0.086	47.3 ± 17.4	53.2 ± 21.0
24 months	586	30	0.973	0.952–0.993	0.010	43.5 ± 16.5	54.6 ± 26.2
36 months	485	24	0.964	0.933–0.997	0.034	41.1 ± 18.6	53.5 ± 20.7
48 months	415	17	0.977	0.940–1.016	0.246	45.4 ± 12.2	52.5 ± 20.1
60 months	437	11	0.972	0.936–1.010	0.144	42.7 ± 20.2	51.5 ± 20.6

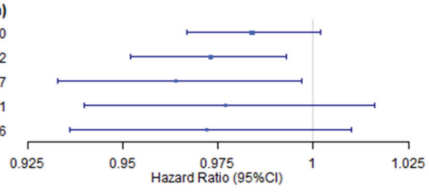


Figure 1. Landmark analysis of univariable Cox proportional hazard regressions for fracture risk depending on eGFR after 12, 24, 36, 48 and 60 months depicted as Forest plot. Mean eGFR values and standard deviations at each time point are given for patients with and without fractures.

Repeated measures ANOVA to compare the PTH courses is given in Figure 2.

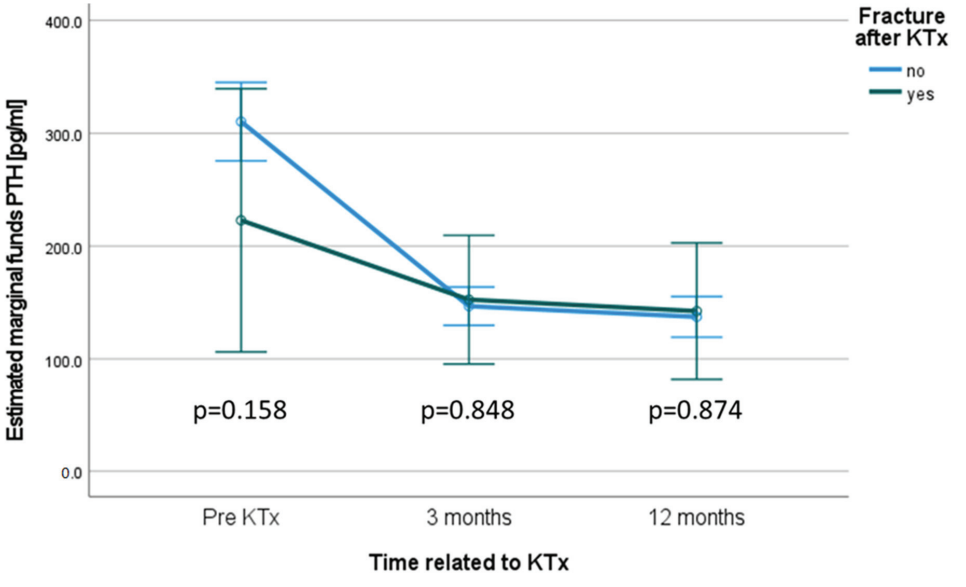


Figure 2. PTH courses in patients with and without fractures. Differences were tested for significance with repeated measures ANOVA. *p*-values are given within the figure.

Further outcome data are presented in Table 2.

Table 2. Characteristics of patients stratified by fracture events.

	All (n = 711)	Fracture (n = 47)	No Fracture (n = 664)	p-Value
Alkaline phosphatase at M3 (u/L) median (IQR)	83.50 (43)	84.50 (41)	83.00 (44)	0.696 [a]
Whole serum calcium at prior to KTX (mmol/L) median (IQR)	2.29 (0.29)	2.33 (0.3)	2.29 (0.29)	0.539 [a]
Ionized calcium at prior to KTX (mmol/L) median (IQR)	1.18 (0.12)	1.19 (0.09)	1.18 (0.12)	0.264 [a]
Whole serum calcium at M3 (mmol/L) median (IQR)	2.39 (0.24)	2.38 (0.29)	2.40 (0.24)	0.685 [a]

Table 2. *Cont.*

	All (n = 711)	Fracture (n = 47)	No Fracture (n = 664)	p-Value
Ionized calcium at M3 (mmol/L) median (IQR)	1.27 (0.14)	1.25 (0.14)	1.27 (0.13)	0.323 [a]
Serum phosphate at M3 (mg/dL) median (IQR)	2.70 (1.1)	2.70 (1.2)	2.70 (1.1)	0.502 [a]
Calcium phosphate product at M3 ((mg/dL)/(mmol/L))	6.55 (2.23)	6.48 (2.35)	6.55 (2.22)	0.579 [a]
Parathyroid hormone prior to KTX (pg/mL) median (IQR)	217.0 (329.5)	188.0 (209.8)	222.0 (333.6)	0.244 [a]
Parathyroid hormone at M 3 (pg/mL) median (IQR)	111.0 (130.5)	121.5 (174.1)	108.0 (126.6)	0.322 [a]
Parathyroid hormone at M 12 (pg/mL) median (IQR)	99.4 (104.0)	95.2 (114.4)	99.7 (102.6)	0.962 [a]
Use of calcimimetics within Y1-3, n (%)	47 (7.2%)	3 (7.0%)	44 (7.2%)	1.000 [b]
Use of bisphosphonates within Y1-3, n (%)	22 (3.3%)	7 (16.3%)	15 (2.5%)	<0.001 [b]
Use of nutritional Vitamin D within Y1-3, n (%)	355 (54.0%)	26 (60.5%)	327 (53.5%)	0.430 [b]
Use of VDR activators within Y1-3, n (%)	259 (39.4%)	15 (34.9%)	243 (39.8%)	0.629 [b]
Parathyroidectomy prior to KTX, n (%)	112 (15.8%)	1 (2.1%)	111 (16.7%)	0.003 [b]
Death censored allograft survival, n (%)	630 (88.6%)	37 (78.7%)	589 (89.2%)	0.053 [b]
Overall Graft Survival, n (%)	568 (79.9%)	30 (63.8)	535 (81.1)	0.008 [b]
NODAT, n (%)	116 (16.3%)	8 (17.0%)	107 (16.2%)	0.839 [b]
BK viremia, n (%)	165 (23.2%)	11 (23.4%)	154 (23.3%)	1.000 [b]
CMV viremia, n (%)	236 (33.2%)	13 (28.3%)	221 (33.5%)	0.520 [b]
Rejection yes, n (%)	280 (39.4%)	24 (51.1%)	255 (38.6%)	0.122 [b]
Tacrolimus use at M12, n (%)	331 (76.8%)	12 (42.9%)	317 (79.1%)	<0.001 [b]
Tacrolimus C/D ratio at M3, median (IQR)	1.30 (1.08)	1.43 (1.48)	1.30 (1.09)	0.187 [a]
Tacrolimus trough levels (ng/mL) at M3, mean ± SD	7.86 ± 2.64	7.91 ± 2.65	7.85 ± 2.63	0.881 [a]
Steroid use at M12	406 (57.1%)	27 (96.4%)	378 (94.0%)	1.000 [b]
Fracture Localisation				
Extremity		25 (53.2%)		
Femoral neck		6 (12.8%)		
Pelvis		5 (10.6%)		
Vertebrae		8 (17.0%)		
Thorax (rips, sternum, clavicle)		3 (6.4%)		

[a] Mann–Whitney U-test, [b] Fisher's exact test, Abbreviations: VDR: vitamin D receptor; CI: confidence interval; NODAT: New-onset diabetes after transplantation; crea: creatinine; CMV: cytomegalovirus; BK: BK-Polyomavirus; Tac: Tacrolimus; C/D ratio: concentration/dose ratio.

3.3. Fracture Events after KTx

Forty-seven patients (6.6%) from our study cohort suffered from at least one fracture event that was not related to malignancy or adequate trauma. The median time of occurrence of fracture events was 36.7 months (IQR 13.8–59.7 months) after KTx. Interestingly, a fracture occurred in only one PTX patient (Figure 3A). In contrast, the vast majority of the 46 (97.8%) patients with fracture events did not undergo PTX. Mortality was not different between patients with and without PTX (Figure 3B,C).

Levels of PTH were comparable between both groups at day 0 (p = 0.158), at 3 months (p = 0.848), and at 1 year (p = 0.874) (Figure 2).

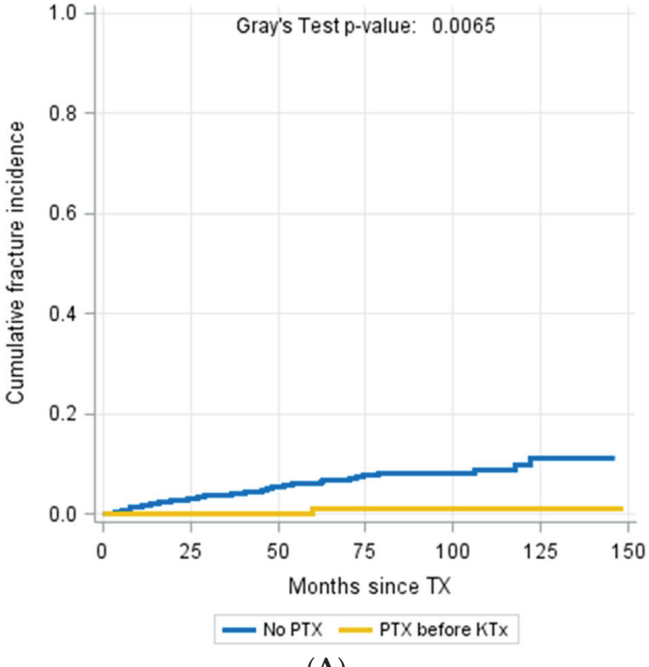
Patients with fracture events during the follow-up period were significantly older than those without fractures (59.47 ± 11.32 vs. 51.47 ± 14.06, $p < 0.001$). There was no statistical difference between the sexes.

To evaluate the influence of renal function displayed by eGFR on pathological fractures, we compared patients without fractures and those patients who developed fractures for their eGFR courses. Renal function in a course of five years was not consistently associated with fracture incidence; however, it was significantly associated with elevated fracture risk after 24 ($p = 0.01$, HR = 0.973) and 36 months ($p = 0.034$, HR = 0.964), but not after 12 ($p = 0.086$, HR = 0.984), 48 ($p = 0.246$, HR = 0.977) and 60 months ($p = 0.144$, HR = 0.972) (Figure 1). The mean eGFR tended to be lower in the fracture group, especially after years two and three (Figure 1).

PTX courses were not significantly different between these groups pre KTx ($p = 0.158$), after three ($p = 0.848$), and after twelve months ($p = 0.874$). However, PTH tended to be higher in the group without fractures at the timepoint prior to KTx (Figure 2).

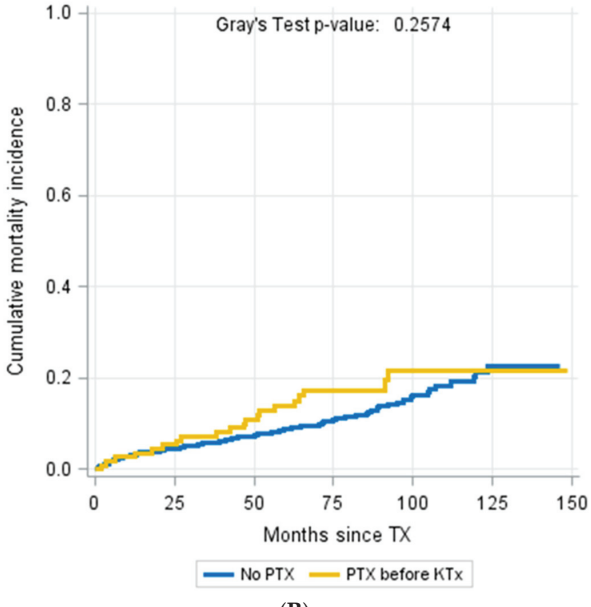
A total of 36.6% of patients showed hypophosphatemia (<2.5 mg/dL) three months after KTx without a significant difference between patients with and without fractures (33.3% vs. 36.8%, $p = 0.502$).

There was no significant association between fracture incidence and the common post-transplant complications BKV-viremia (23.4% vs. 23.3%, $p = 1.000$), CMV-viremia (28.3% vs. 33.5%, $p = 0.522$), NODAT (17.0% vs. 16.2%, $p = 0.839$) and rejection episodes (51.1% vs. 38.6%, $p = 0.122$).

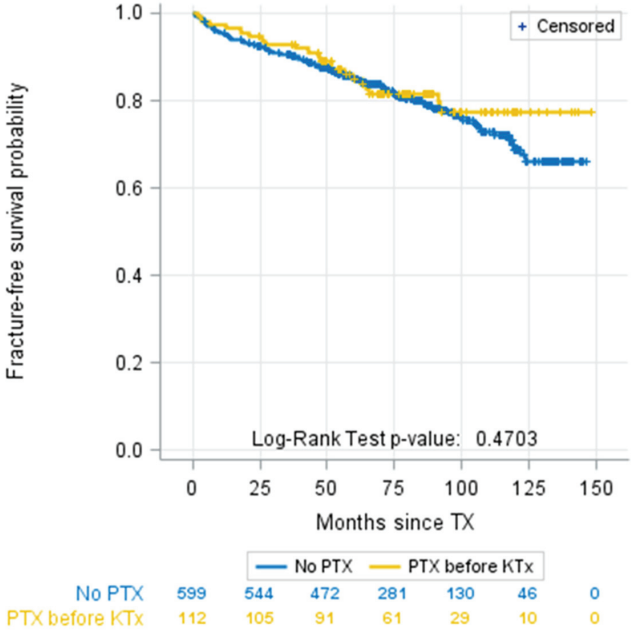


(**A**)

Figure 3. *Cont.*



(**B**)



(**C**)

Figure 3. Patients who underwent PTX showed a significantly reduced fracture incidence during the follow-up period (Gray´s test $p = 0.0065$) (**A**). Mortality incidence (Gray´s Test $p = 0.2574$, (**B**)) and fracture-free survival probability were not different between patients with and without fractures (Log-Rank $p = 0.4703$, (**C**)).

3.4. Parathyroidectomy

A total of 112 (15.8%) of the patients underwent subtotal PTX before KTx, whereas 599 patients (84.2%) did not receive PTX. None of the patients underwent PTX surgery after KTx.

PTX was performed at a median time of 4.2 years before KTx (IQR 5.0). Autotransplantation of partial parathyroid tissue was performed in 80 of the 112 patients (71.4%) undergoing PTX (Table 3). There are no significant differences in eGFR between patients with and without PTX at any timepoint.

Table 3. Characteristics of patients stratified by PTX.

	Patients with Parathyroidectomy before KTX (*n* = 112)	Patients without Parathyroidectomy before KTX (*n* = 599)	*p*-Value
PTX with partial autotransplantation, *n* (%)	80 (71.4 %)	-	-
Parathyroid hormone at KTx (pg/mL), median (IQR)	37.7 (169.5)	232.0 (353.5)	<0.001 [a]
Parathyroid hormone at M3 (pg/mL) median (IQR)	56.0 (111.1)	117.0 (132.8)	<0.001 [a]
Parathyroid hormone at Y1 (pg/mL) median (IQR)	57.6 (118.1)	104.0 (92.2)	<0.001 [a]
Alkaline phosphatase at M3 (u/L) median (IQR)	77.0 (41)	85.0 (45)	0.035 [a]
eGFR at Y1 (mL/min/1.73 m^2) (±SD)	53.6 (±20.0)	55.5 (±20.7)	0.763 [a]
eGFR at Y3 (mL/min/1.73 m^2) (±SD)	54.6 (±20.6)	55.2 (±20.3)	0.747 [a]
eGFR at Y5 (mL/min/1.73 m^2), mean (± SD)	50.9 (±22.0)	51.5 (±20.4)	0.868 [a]
Whole serum calcium at prior to KTX (mmol/L) median (IQR)	2.26 (0.38)	2.29 (0.28)	0.379 [a]
Ionized calcium at prior to KTX (mmol/L) median (IQR)	1.18 (0.11)	1.18 (0.11)	0.010 [a]
Whole serum calcium at M3 (mmol/L), median (IQR)	2.26 (0.32)	2.41 (0.22)	<0.001 [a]
Ionized calcium at M3 (mmol/L), median (IQR)	1.22 (0.26)	1.28 (0.13)	<0.001 [a]
Serum phosphate at M3 (mg/dL), median (IQR)	2.95 (1.5)	2.70 (1.0)	0.001 [a]
Calcium phosphate product ((mg/dL)/(mmol/L)) at M3, median (IQR)	6.62 (2.39)	6.54 (2.20)	0.237 [a]
Use of calcimimetics within Y1-3, *n* (%)	2 (1.7%)	45 (7.5%)	0.011 [b]
Use of bisphosphonates within Y1-3, *n* (%)	4 (3.6%)	17 (2.8%)	0.76 [b]
Use of nutritional Vitamin D within Y1-3, *n* (%)	58 (51.7%)	297 (49.6%)	0.749 [b]
Use of VDR activators within Y1-3, *n* (%)	62 (55.4%)	197 (32.9%)	<0.001 [b]

[a] Mann–Whitney U-test, [b] Fisher´s exact test, Abbreviations: PTX: parathyroidectomy; eGFR: estimated glomerular filtration rate, calculated using CKD-EPI formula; KTx: kidney transplantation; VDR: vitamin D receptor.

3.5. Medication with Calcimimetics

In total, 47 (6.6%) patients were treated with calcimimetics within the first three years after KTx. Two (4.3%) of these patients had previously undergone PTX. The calcimimetic use in patients with or without fractures (7.0% vs. 7.2%, $p = 1.000$) was comparable.

3.6. Levels of Parathyroid Hormone

Median levels of iPTH prior to KTx were 217.0 (329.5) pg/mL, 111.0 (130.5) pg/mL after three months, and 99.4 (104.0) pg/mL after one year. They were not significantly different between patients with and without fractures at any of the three times ($p = 0.208$ prior to KTx, $p = 0.366$ after 3 months, and $p = 0.906$ after one year).

Patients who underwent PTX showed lower PTH levels prior to KTx (37.7 (169.5) vs. 232.0 (353.5) pg/mL, $p < 0.001$), after 3 months (56.0 (111.1)) vs. 117.0 (132.8) pg/mL, $p < 0.001$, Figure 4), and also after one year (57.6 (118.1) vs. 104.0 (92.2) pg/mL, $p = 0.001$).

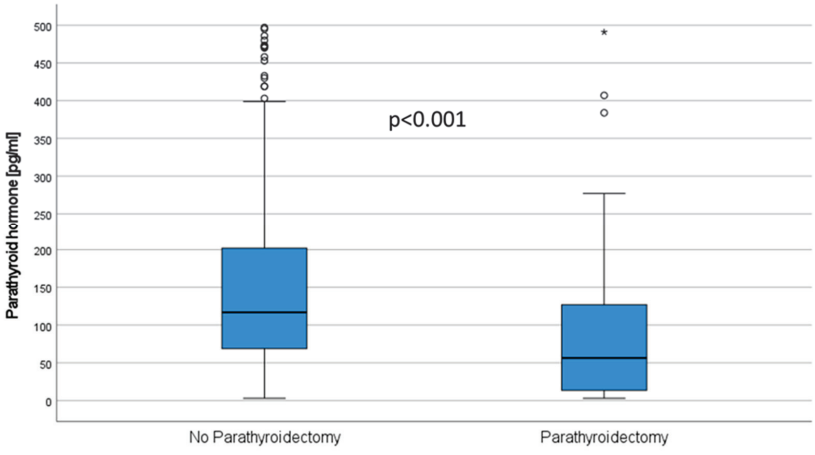


Figure 4. Levels of parathyroid hormone are significantly lower in patients after subtotal PTX compared to non-PTX patients three months after KTx.

Before KTx, 75.1% of recipients had iPTH levels within the range recommended by the KDIGO guidelines (2–9-fold above the normal range (15.6–65 pg/mL)), 14.1% of patients showed levels above the range, and 10.7% had levels below the range. For patients after KTx, the ideal PTH levels are not known. At one year after KTx, 23.8% of the patients showed iPTH within the assay range, 71.7% had values above, and 4.5% below [6].

Following a study by Perrin et al. who demonstrated a significant fracture difference between low and high intact PTH (cut-off 130 pg/mL) three months after KTx [10], we tested this cut-off after three months for our collective. However, we could not confirm a significant difference for fracture events between patients with PTH < 130 pg/mL and $\geq$ 130 pg/mL (see also Supplementary ROC Curve Figure S1). One patient who showed a fracture event within the first three months (1.4 months after KTx) was excluded from this analysis (Figure 5).

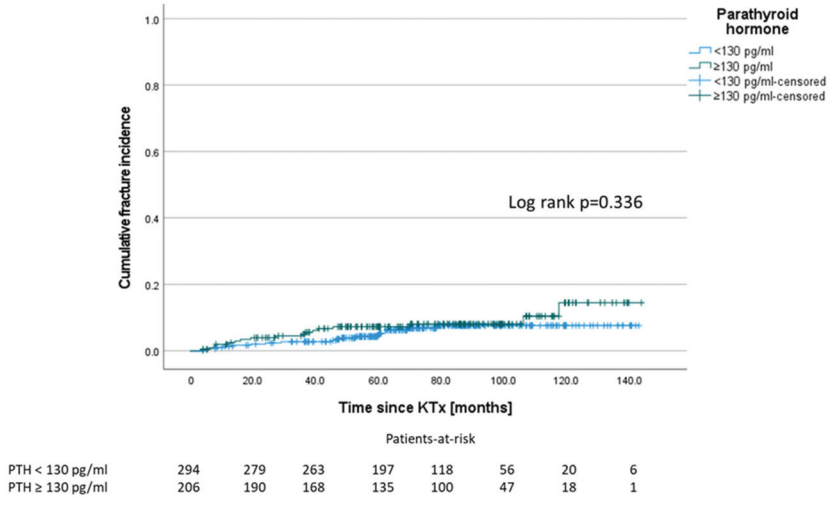


Figure 5. PTH levels ≥ 130 pg/mL after three months are not significantly associated with a higher fracture incidence. One patient, who showed a fracture event within the first three months, was excluded from this analysis.

3.7. Calcium Levels

For the analysis, whole serum calcium levels as well as levels of ionized calcium were evaluated. Whole serum calcium levels were reduced in PTX patients three months after KTx, $p < 0.001$). The same was true for levels of ionized calcium pre KTx ($p = 0.0109$) and three months after KTx ($p < 0.001$), Table 3.

Between patients with and without fractures, there were neither significant differences in calcium levels at day 0 ($p = 0.685$) nor three months after KTx ($p = 0.323$), Table 2.

3.8. Vitamin D Preparations and Bisphosphonates

Vitamin D receptor activators (VDRa) (calcitriol, paricalcitol)) were used more frequently in patients after PTX within the first three years after KTx (55.4% vs. 32.9%, $p < 0.001$). In contrast, we found no differences regarding the use of native vitamin D preparations (cholecalciferol, vitamin D3) between patients with or without PTX (51.7% vs. 49.6%, $p = 0.749$).

We addressed the question of whether the difference in the use of VDRa between patients with and without PTX could be the underlying reason for the reduced fracture rate of PTX patients (Table 3).

However, we could not find any relevant difference in the VDRa treatment of patients with and without fracture (34.9% vs. 39.8%, $p = 0.629$, Table 2).

During follow-up, seven (18.4%) patients with fractures received bisphosphonates. Of these, three patients were prescribed bisphosphonates as secondary fracture prophylaxis after a fracture event. In the remaining four patients, bisphosphonates were used due to diagnosed osteoporosis without a fracture event.

3.9. Immunosuppressive Medication

The vast majority of patients studied received a tacrolimus-based immunosuppressive regimen (96.6%). Mean trough levels were 7.86 ± 2.64 ng/mL. The median concentration/dose ratio was 1.3 (IQR 1.08). We did not find an association of steroid use ($p = 1.000$) or tacrolimus use ($p = 0.670$) or trough levels ($p = 0.881$), nor of tacrolimus metabolism (C/D ratio; $p = 0.187$), with fracture events after KTx. Moreover, the tacrolimus trough levels or C/D ratio and PTH levels were not associated.

3.10. Multivariate Cox Regression Analysis

To determine whether PTX is independently associated with a lower fracture risk after KTx, adjustment was made for the known risk factors age, sex and dialysis vintage and furthermore for the underlying renal disease, which was tested noticeable in the univariable analysis.

In this analysis, PTX remained a protective factor for fractures (HR 0.134 CI 0.018–0.991, $p = 0.049$). Age was confirmed as the most relevant risk factor (HR 1.051 CI 1.023–1.079, $p < 0.001$) (Table 4).

Table 4. Multivariable Cox regression for factors associated with fractures after KTx.

Variable	Hazard Ratio	95% CI	*p*-Value
Age at KTx	1.051	1.023–1.079	<0.001
Dialysis vintage	0.999	0.991–1.007	0.851
Female sex	1.692	0.919–3.115	0.091
Underlying renal disease	-	-	0.111
Parathyroidectomy	0.134	0.018–0.991	0.049

Abbreviations: CI: confidence interval; KTx: kidney transplantation.

4. Discussion

Bone fractures are a complication that greatly affects quality of life. In addition, immobility caused by fractures increases mortality [11]. KTx recipients have an increased risk of fractures. Recently, Evenepoel et al. observed a fracture incidence of 14.1 per 1000 person-years in 518 KTx recipients within a 5.2-year follow-up [12]. The authors found that low BMD was associated with fractures, independent of classic determinants, including history of fractures. In our larger study with a comparable length of follow-up, the incidence was 10 per 1000 person-years, but unlike the incidental fractures counted by Evenepoel et al., we excluded fractures related to adequate trauma or malignancy.

A recent Cochrane analysis addressed the question of the efficacy of different treatments for the prevention of MBD after KTx. The primary efficacy endpoint considered was bone fracture. It remained uncertain whether, apart from bisphosphonates, any other class of drugs reduced the fracture incidence. In the absence of trial data, the effect of PTX on fracture risk also could not be adequately assessed [13].

In chronic hemodialysis patients, Rudser et al. showed that patients who underwent PTX have a reduced fracture risk. Several mechanisms are discussed as potential mechanisms. First, PTH excess is avoided and therefore high bone turnover lesions are mitigated. Second, PTX-induced hungry bone syndrome, which increases bone mineral uptake, may inherit long-term protective effects on fractures [14]. A protective role of PTX against fracture events has already been shown for primary HPT [8], although the mechanisms in both settings are not comparable.

In 1994, Grotz et al. reported in 100 KTx patients that pretransplant PTX was associated with increased risk of post-transplant fractures [15]. However, this has not been confirmed in subsequent studies, and drugs, surgical techniques as well as indications for PTX have changed since then [16]. Nevertheless, adynamic bone disease, which could result from PTX beside malnutrition, uremic toxins, or CKD-related repressed WNT/β-catenin signaling [17], might have been responsible for the increased fracture risk of patients with PTX in the mentioned study.

In contrast, in the present era of calcimimetics, which probably cannot reduce the fracture risk in ESRD or KTx patients [13,18,19], we herein generate evidence that PTX may act as an independent protective factor against pathologic fractures in KTx recipients with HPT. This association persists when adjusted for the well-known risk factors of age, sex and dialysis vintage, as well as for underlying renal disease, which was associated with fracture events in univariate analysis. Since eGFR at different time points was inconsistently

identified as significant risk factor for fractures in univariate Cox proportional hazard regression analysis (after 2 and 3 years, but not after 1, 4, and 5 years, (Figure 1) we did not include this parameter in further analysis. Moreover, the addition of eGFR as reflection of renal function besides age to multivariable analysis seems problematic, because age is already considered in the eGFR estimation formula (CKD-EPI formula) (Levey et al., 2009). Further, age is known to be strongly associated with graft function and outcome in KTx recipients (Legendre et al., 2014). Reasons for this include the fact that living donations, which have a better graft outcome, are more common in younger recipients (Hart et al., 2019), and organs provided under the European senior program (ESP) have lower organ quality because they meet the expanded donor criteria (ECD) (Pascual et al., 2008).

Isaksson et al. matched a cohort of 590 PTX patients on dialysis or with functioning allograft for assessment of fracture risk [20]. It was observed that PTX reduced the hip fracture risk, but only in female patients compared with non-PTX patients. For this study, the authors distinguished only between KTx and non-KTx patients at the time of PTX. Whether patients received KTx after PTX was not considered. Nevertheless, these results point in the same direction as ours do.

Another study analyzed hypercalcemia control with subtotal PTX compared with cinacalcet use in KTx recipients. Cruzado et al. showed a superior effect of PTX for calcium and PTH normalization and an increase in BMD; but, due to the relatively short follow-up period of twelve months and small patient numbers, they were not able to sufficiently comment on fracture events [21].

Based on data suggesting that hypoparathyroidism after PTX in KTx recipients correlates with a significant decrease of renal function, PTX should be indicated with caution, and the initial treatment approach should be pharmacological with administration of calcimimetics [6,22,23]. This approach was largely followed in our center. Interestingly, Mathur et al. did not observe an association between treatment of sHPT and posttransplant delayed graft function, graft failure, or death, but the proportion of PTX-treated patients in their cohort of 5094 KTx recipients was small (4.5%) [24]. However, our data do not show a significant difference in eGFR courses between patients with and without PTX (Table 3).

In the group of patients in whom a drug approach was followed to lower PTH, it seems trivial that the PTH value remains elevated (Figure 3) [25]. This is paralleled by higher calcium levels and lower phosphate levels (Table 3). Nevertheless, there is also evidence to support a preference for PTX over the use of cinacalcet. In patients with tertiary hyperparathyroidism after KTx, cinacalcet can normalize the serum calcium levels, but unlike PTX, it cannot normalize the PTH levels [26,27]. However, there is no consensus on which PTH value clearly defines post-transplant HPT [28], but PTH levels were found to be an important negative independent predictor of MBD, intriguingly more deleterious than the cumulative dose of corticosteroids or inflammation [5]. In our cohort, one year after KTx the PTH levels decreased 2.2-fold compared to pre KTx. However, one year after KTx, only 23.8% of the patients showed PTH levels within the by KIDGO recommended range for patients with CKD, 71.7% had values above, and 4.5% below (see Results Section 3.6) [6]. This phenomenon has been observed in many studies [28]. Looking at both groups (PTX vs. non-PTX), there was no difference in PTH levels, pointing to the fact that many patients had undergone subtotal PTX and controlled HPT in the other group. Considering that the group without fractures included 17.8% PTX patients (vs. 2.2% in the fracture group), it becomes clear that the average PTH level in the group without fractures was higher in the non-PTX patients and therefore is probably not the decisive influencing factor (Table 3). Nevertheless, there is evidence that persistent HPT could be a risk factor for fractures after KTx. Therefore, it could be important that parathyroid recovery from CKD-induced HPT was incomplete even 1 year after KTx in a large subset of patients (Figure 1). While some studies link PTH-related stimulation of bone turnover to fractures [10], other sources describe (circulating) PTH levels as poorly predictive of underlying bone turnover [29]. In contrast to Perrin et al., who described a PTH above a cut-off of 130 pg/mL measured three months after KTx as a significant risk factor for fracture events in a cohort of 143 KTx

recipients with a total of 22 fracture events [10], we could not confirm this observation in our data (Figure 4). Moreover, according to our data, we could not support a clear cut-off for PTH being associated with an increased fracture risk (Supplementary Figure S1). In agreement with Perrin et al., we did not observe elevated alkaline phosphatase as a marker for bone turnover in the patients with fracture events. However, in contrast to Perrin et.al, we found alkaline phosphatase elevated in those patients without PTX. Nevertheless, it seems possible that bone abnormalities induced by HPT can be explained by the elimination of skeletal resistance to PTH occurring during CKD after renal transplantation.

VDRa (1.25(OH)2D3 (Calcitriol)) are more frequently applied in patients after PTX than in patients without PTX (Table 3). VDRa provide a well-studied protective effect on bone metabolism, whereas the efficacy of nutritional vitamin D preparations, frequently used in non-PTX KTx patients, has not been clearly established yet [30]. However, VDRa use did not differ in patients with and without fractures (Table 2).

In KTx recipients, immunosuppressive therapy is a specific contributing factor to MBD and osteoporosis. In addition to the osteoporotic effects of long-term steroid use [31], CNI medication may also affect bone metabolism. Luo et al. described increased bone resorption in patients with tacrolimus trough levels above 6 ng/mL [32]. Increased bone loss from CNI use has also been demonstrated in a rat model [33]. In contrast, we did not find an association between higher tacrolimus levels or tacrolimus metabolism, represented by the Tac C/D ratio [34], and pathologic fracture events in our cohort. Nevertheless, tacrolimus may influence bone resorption to an extent that is clinically not apparent and is overshadowed by more relevant factors. Recently, the effect of steroids on BMD was prospectively analyzed in de novo KTx patients using steroid minimization protocols. The authors showed that a cumulative methylprednisolone dose of 2.5 ± 0.8 g (after 1 year) and 5.8 ± 3.3 g (after 5 years) caused only limited BMD changes and was predominantly related to remodeling activity rather than corticosteroid exposure [35]. Since treatment per protocol in our center results in exposure of approximately 3.4 g prednisone after 1 year and 10.7 g after 5 years (in an average patient on steroids without rejection), we cannot exclude a relevant influence of steroids on BMD in our cohort. However, steroid use and rejection rates did not differ between the groups.

Interestingly, autosomal-dominant polycystic kidney disease (ADPKD) as an underlying disease for ESRD was associated with the incidence of fractures after KTx in univariate analysis. This fits with the observations of Gitomer et al. who describe a bone metabolism defect in ADPKD patients with CKD stages 1 and 2, although ADPKD was not associated with an increased risk of fractures in ESRD patients [36]. Nevertheless, we could not confirm this association in the multivariate analysis.

This study has several noteworthy limitations. Due to its retrospective nature, it is only hypothesis generating. Since we did not assess additional corticosteroid therapies, which were temporarily applied for the treatment of rejection episodes, nor the VDRa or vitamin D doses or levels, we cannot comment on that and cannot exclude the related effects. Moreover, additional information on FGF23 and BMD would have provided further valuable information on the bone homeostasis and morphology of patients after PTX compared to those without PTX. Nevertheless, these parameters were not routinely assessed in our patients. To at least partially address this limitation, we included known risk factors for lower BMD and fracture risk after KTx into our multivariable model, specifically, age, female sex, dialysis vintage, and cause of ESRD [37], to indirectly account for BMD.

5. Conclusions

In conclusion, our study points towards an association between PTX and fracture events after KTx, which is independent from the elevated PTH levels at the time of KTx or afterwards. Therefore, keeping in mind that PTX surgeries were performed considerably prior to KTx, the fracture events might be the result of long-lasting sHPT in the foregoing history of patients without PTX, to a significant extent. As pre-transplant PTX does not

influence eGFR after KTx and offers protective effects on fracture risk in our study, our data supports a more generous indication for PTX in ESRD patients with HPT waiting for KTx.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11030654/s1, Figure S1: ROC-analysis for PTH levels three months after KTx.

Author Contributions: U.J. and A.K. did research and wrote the paper; K.S.-N., G.T., F.W., M.S. and D.-O.W. did research and revised the paper; B.S. and H.P. revised the paper; D.G. performed statistical analyses and revised the paper; S.R. designed the research and wrote the paper; U.J. and A.K. contributed equally. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a grant from Innovative Medizinische Forschung, Medical Faculty, University of Münster, to U.J. The APC was funded by the Open Access Publication Fund of University of Münster.

Institutional Review Board Statement: This study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved by the local ethics committee (Ethik Kommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität, 2014-381-f-N).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors have declared that no competing interest exist.

References

1. Center, J.R.; Nguyen, T.V.; Schneider, D.; Sambrook, P.N.; Eisman, J.A. Mortality after all major types of osteoporotic fracture in men and women: An observational study. *Lancet* **1999**, *353*, 878–882. [CrossRef]
2. Hou, Y.-C.; Lu, C.-L.; Lu, K.-C. Mineral bone disorders in chronic kidney disease. *Nephrology* **2018**, *23* (Suppl. 4), 88–94. [CrossRef] [PubMed]
3. Bozkaya, G.; Nart, A.; Uslu, A.; Onman, T.; Aykas, A.; Doğan, M.; Karaca, B. Impact of calcineurin inhibitors on bone metabolism in primary kidney transplant patients. *Transplant. Proc.* **2008**, *40*, 151–155. [CrossRef] [PubMed]
4. Nitta, K.; Yajima, A.; Tsuchiya, K. Management of Osteoporosis in Chronic Kidney Disease. *Intern. Med.* **2017**, *56*, 3271–3276. [CrossRef] [PubMed]
5. Matei, A.; Bilha, S.C.; Constantinescu, D.; Pavel-Tanasa, M.; Cianga, P.; Covic, A.; Branisteanu, D.D. Body composition, adipokines, FGF23-Klotho and bone in kidney transplantation: Is there a link? *J. Nephrol.* **2021**. Ahead of print. [CrossRef]
6. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int. Suppl.* **2017**, *7*, 1–59. [CrossRef]
7. Tominaga, Y.; Matsuoka, S.; Uno, N.; Sato, T. Parathyroidectomy for secondary hyperparathyroidism in the era of calcimimetics. *Ther. Apher. Dial.* **2008**, *12* (Suppl. 1), S21–S26. [CrossRef]
8. Yeh, M.W.; Zhou, H.; Adams, A.L.; Ituarte, P.H.G.; Li, N.; Liu, I.-L.A.; Haigh, P.I. The Relationship of Parathyroidectomy and Bisphosphonates with Fracture Risk in Primary Hyperparathyroidism: An Observational Study. *Ann. Intern. Med.* **2016**, *164*, 715–723. [CrossRef]
9. Schütte-Nütgen, K.; Thölking, G.; Steinke, J.; Pavenstädt, H.; Schmidt, R.; Suwelack, B.; Reuter, S. Fast Tac Metabolizers at Risk—It is Time for a C/D Ratio Calculation. *J. Clin. Med.* **2019**, *8*, 587. [CrossRef]
10. Perrin, P.; Caillard, S.; Javier, R.M.; Braun, L.; Heibel, F.; Borni-Duval, C.; Muller, C.; Olagne, J.; Moulin, B. Persistent hyperparathyroidism is a major risk factor for fractures in the five years after kidney transplantation. *Am. J. Transplant.* **2013**, *13*, 2653–2663. [CrossRef]
11. Bliuc, D.; Nguyen, N.D.; Milch, V.E.; Nguyen, T.V.; Eisman, J.A.; Center, J.R. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. *JAMA* **2009**, *301*, 513–521. [CrossRef]
12. Evenepoel, P.; Claes, K.; Meijers, B.; Laurent, M.R.; Bammens, B.; Naesens, M.; Sprangers, B.; Pottel, H.; Cavalier, E.; Kuypers, D. Bone mineral density, bone turnover markers, and incident fractures in de novo kidney transplant recipients. *Kidney Int.* **2019**, *95*, 1461–1470. [CrossRef] [PubMed]
13. Palmer, S.C.; Chung, E.Y.; McGregor, D.O.; Bachmann, F.; Strippoli, G.F. Interventions for preventing bone disease in kidney transplant recipients. *Cochrane Database Syst. Rev.* **2019**, *10*, CD005015. [CrossRef] [PubMed]
14. Rudser, K.D.; de Boer, I.H.; Dooley, A.; Young, B.; Kestenbaum, B. Fracture risk after parathyroidectomy among chronic hemodialysis patients. *J. Am. Soc. Nephrol.* **2007**, *18*, 2401–2407. [CrossRef] [PubMed]

15. Grotz, W.H.; Mundinger, F.A.; Gugel, B.; Exner, V.; Kirste, G.; Schollmeyer, P.J. Bone fracture and osteodensitometry with dual energy X-ray absorptiometry in kidney transplant recipients. *Transplantation* **1994**, *58*, 912–915. [CrossRef] [PubMed]
16. O'Shaughnessy, E.A.; Dahl, D.C.; Smith, C.L.; Kasiske, B.L. Risk factors for fractures in kidney transplantation. *Tranplantation* **2002**, *74*, 362–366. [CrossRef]
17. Haarhaus, M.; Evenepoel, P. Differentiating the causes of adynamic bone in advanced chronic kidney disease informs osteoporosis treatment. *Kidney Int.* **2021**, *100*, 546–558. [CrossRef]
18. Zu, Y.; Lu, X.; Song, J.; Yu, L.; Li, H.; Wang, S. Cinacalcet Treatment Significantly Improves All-Cause and Cardiovascular Survival in Dialysis Patients: Results from a Meta-Analysis. *Kidney Blood Press. Res.* **2019**, *44*, 1327–1338. [CrossRef]
19. Moreno, P.; Coloma, A.; Torregrosa, J.V.; Montero, N.; Francos, J.; Codina, S.; Manonelles, A.; Bestard, O.; García-Barrasa, A.; Melilli, E.; et al. Long-term results of a randomized study comparing parathyroidectomy with cinacalcet for treating tertiary hyperparathyroidism. *Clin. Transplant.* **2020**, *34*, e13988. [CrossRef]
20. Isaksson, E.; Ivarsson, K.; Akaberi, S.; Muth, A.; Sterner, G.; Karl-Göran, P.; Clyne, N.; Almquist, M. The Effect of Parathyroidectomy on Risk of Hip Fracture in Secondary Hyperparathyroidism. *World J. Surg.* **2017**, *41*, 2304–2311. [CrossRef]
21. Cruzado, J.M.; Moreno, P.; Torregrosa, J.V.; Taco, O.; Mast, R.; Gómez-Vaquero, C.; Polo, C.; Revuelta, I.; Francos, J.; Torras, J.; et al. A Randomized Study Comparing Parathyroidectomy with Cinacalcet for Treating Hypercalcemia in Kidney Allograft Recipients with Hyperparathyroidism. *J. Am. Soc. Nephrol.* **2016**, *27*, 2487–2494. [CrossRef] [PubMed]
22. Evenepoel, P.; Claes, K.; Kuypers, D.; Maes, B.; Vanrenterghem, Y. Impact of parathyroidectomy on renal graft function, blood pressure and serum lipids in kidney transplant recipients: A single centre study. *Nephrol. Dial. Transplant.* **2005**, *20*, 1714–1720. [CrossRef] [PubMed]
23. Lau, W.L.; Obi, Y.; Kalantar-Zadeh, K. Parathyroidectomy in the Management of Secondary Hyperparathyroidism. *Clin. J. Am. Soc. Nephrol.* **2018**, *13*, 952–961. [CrossRef] [PubMed]
24. Mathur, A.; Sutton, W.; Ahn, J.B.; Prescott, J.D.; Zeiger, M.A.; Segev, D.L.; McAdams-DeMarco, M. Association Between Treatment of Secondary Hyperparathyroidism and Posttransplant Outcomes. *Transplantation* **2021**, *105*, e366–e374. [CrossRef]
25. Koh, E.Y.; van der Plas, W.Y.; Dulfer, R.R.; Pol, R.A.; Kruijff, S.; Rotmans, J.I.; Appelman-Dijkstra, N.; Schepers, A.; de Borst, M.H.; Hoorn, E.J.; et al. Outcomes of parathyroidectomy versus calcimimetics for secondary hyperparathyroidism and kidney transplantation: A propensity-matched analysis. *Langenbecks Arch. Surg.* **2020**, *405*, 851–859. [CrossRef]
26. Dulfer, R.R.; Koh, E.Y.; van der Plas, W.Y.; Engelsman, A.F.; van Dijkum, E.J.M.N.; Pol, R.A.; Vogt, L.; de Borst, M.H.; Kruijff, S.; Schepers, A.; et al. Parathyroidectomy versus cinacalcet for tertiary hyperparathyroidism; a retrospective analysis. *Langenbecks Arch. Surg.* **2019**, *404*, 71–79. [CrossRef]
27. Finnerty, B.M.; Chan, T.W.; Jones, G.; Khader, T.; Moore, M.; Gray, K.D.; Beninato, T.; Watkins, A.C.; Zarnegar, R.; Fahey, T.J. Parathyroidectomy versus Cinacalcet in the Management of Tertiary Hyperparathyroidism: Surgery Improves Renal Transplant Allograft Survival. *Surgery* **2019**, *165*, 129–134. [CrossRef]
28. Delos Santos, R.; Rossi, A.; Coyne, D.; Maw, T.T. Management of Post-transplant Hyperparathyroidism and Bone Disease. *Drugs* **2019**, *79*, 501–513. [CrossRef]
29. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int. Suppl.* **2009**, *76*, S1–S2. [CrossRef]
30. Kramer, H.; Berns, J.S.; Choi, M.J.; Martin, K.; Rocco, M.V. 25-Hydroxyvitamin D testing and supplementation in CKD: An NKF-KDOQI controversies report. *Am. J. Kidney Dis.* **2014**, *64*, 499–509. [CrossRef]
31. Buckley, L.; Humphrey, M.B. Glucocorticoid-Induced Osteoporosis. *N. Engl. J. Med.* **2018**, *379*, 2547–2556. [CrossRef] [PubMed]
32. Luo, L.; Shi, Y.; Bai, Y.; Zou, Y.; Cai, B.; Tao, Y.; Lin, T.; Wang, L. Impact of tacrolimus on bone metabolism after kidney transplantation. *Int. Immunopharmacol.* **2012**, *13*, 69–72. [CrossRef] [PubMed]
33. Kanda, J.; Izumo, N.; Furukawa, M.; Shimakura, T.; Yamamoto, N.; Takahashi, H.E.; Asakura, T.; Wakabayashi, H. Effects of the calcineurin inhibitors cyclosporine and tacrolimus on bone metabolism in rats. *Biomed. Res.* **2018**, *39*, 131–139. [CrossRef] [PubMed]
34. Schutte-Nutgen, K.; Tholking, G.; Suwelack, B.; Reuter, S. Tacrolimus—Pharmacokinetic Considerations for Clinicians. *Curr. Drug Metab.* **2018**, *19*, 342–350. [CrossRef]
35. Evenepoel, P.; Claes, K.; Meijers, B.; Laurent, M.R.; Bammens, B.; Naesens, M.; Sprangers, B.; Cavalier, E.; Kuypers, D. Natural history of mineral metabolism, bone turnover and bone mineral density in de novo renal transplant recipients treated with a steroid minimization immunosuppressive protocol. *Nephrol. Dial. Transplant.* **2020**, *35*, 697–705. [CrossRef]
36. Gitomer, B.; Pereira, R.; Salusky, I.B.; Stoneback, J.W.; Isakova, T.; Cai, X.; Dalrymple, L.S.; Ofsthun, N.; You, Z.; Malluche, H.H.; et al. Mineral bone disease in autosomal dominant polycystic kidney disease. *Kidney Int.* **2021**, *99*, 977–985. [CrossRef]
37. Iseri, K.; Carrero, J.J.; Evans, M.; Felländer-Tsai, L.; Berg, H.E.; Runesson, B.; Stenvinkel, P.; Lindholm, B.; Qureshi, A.R. Fractures after kidney transplantation: Incidence, predictors, and association with mortality. *Bone* **2020**, *140*, 115554. [CrossRef]

Journal of
Clinical Medicine

MDPI

Article

Kidney Perfusion in Contrast-Enhanced Ultrasound (CEUS) Correlates with Renal Function in Living Kidney Donors

Nasrin El-Bandar [1,†], Markus H. Lerchbaumer [2,†], Robert Peters [1], Andreas Maxeiner [1], Katja Kotsch [3], Arne Sattler [3], Kurt Miller [1], Thorsten Schlomm [1], Bernd Hamm [2], Klemens Budde [4], Lutz Liefeldt [4], Thomas Fischer [2] and Frank Friedersdorff [1,5,*]

[1] Department of Urology, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; nasrin.el-bandar@charite.de (N.E.-B.); robert.peters@charite.de (R.P.); andreas.maxeiner@charite.de (A.M.); kurt.miller@charite.de (K.M.); thorsten.schlomm@charite.de (T.S.)
[2] Department of Radiology, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; markus.lerchbaumer@charite.de (M.H.L.); bernd.hamm@charite.de (B.H.); thom.fischer@charite.de (T.F.)
[3] Department of General, Visceral and Vascular Surgery, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; katja.kotsch@charite.de (K.K.); arne.sattler@charite.de (A.S.)
[4] Department of Nephrology and Internal Intensive Care Medicine, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany; klemens.budde@charite.de (K.B.); lutz.liefeldt@charite.de (L.L.)
[5] Department of Urology, Evangelisches Krankenhaus Königin Elisabeth Herzberge, 10365 Berlin, Germany
* Correspondence: frank.friedersdorff@charite.de
† These authors contributed equally to this work.

Citation: El-Bandar, N.; Lerchbaumer, M.H.; Peters, R.; Maxeiner, A.; Kotsch, K.; Sattler, A.; Miller, K.; Schlomm, T.; Hamm, B.; Budde, K.; et al. Kidney Perfusion in Contrast-Enhanced Ultrasound (CEUS) Correlates with Renal Function in Living Kidney Donors. *J. Clin. Med.* **2022**, *11*, 791. https://doi.org/10.3390/jcm11030791

Academic Editors: Wisit Cheungpasitporn, Charat Thongprayoon and Wisit Kaewput

Received: 26 December 2021
Accepted: 30 January 2022
Published: 1 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Contrast-enhanced ultrasound (CEUS) is a widely used diagnostic tool for analyzing perfusion and characterizing lesions in several organs. However, to date, it has not been sufficiently investigated whether there is an association between CEUS findings and kidney function. This study aimed at identifying the potential relationship between kidney function and the renal perfusion status determined by CEUS in living kidney donors. A total of 30 living kidney donors examined between April 2018 and March 2020 were included in the study. All patients underwent various diagnostic procedures for evaluation of renal function. CEUS was performed in all 30 donors one day before nephrectomy. Kidney perfusion was quantified using a postprocessing tool (VueBox, Bracco Imaging). Various perfusion parameters were subsequently analyzed and compared with the results of the other methods used to evaluate kidney function. Of all parameters, mean signal intensity (MeanLin) had the strongest correlation, showing significant correlations with eGFR (CG) ($r = -0.345$; $p = 0.007$) and total kidney volume ($r = -0.409$; $p = 0.001$). While there was no significant correlation between any perfusion parameter and diethylenetriaminepentaacetic acid (DTPA), we detected a significant correlation between MeanLin and DTPA ($r = -0.502$; $p = 0.005$) in the subgroup of normal-weight donors. The results indicate that signal intensity in CEUS is associated with kidney function in normal-weight individuals. Body mass index (BMI) may be a potential confounder of signal intensity in CEUS. Thus, more research is needed to confirm these results in larger study populations.

Keywords: Contrast-enhanced ultrasound; kidney perfusion; kidney function; kidney transplantation; kidney donation

1. Introduction

Evaluation of kidney function is crucial in living donor candidates, who should not be exposed to avoidable health impairments. The Amsterdam Guidelines recommend a glomerular filtration rate (GFR) ≥80 as an essential prerequisite for living kidney donation [1]. Accurate assessment and verification of adequate kidney function is essential

during evaluation of a potential kidney donor [1]. Several methods are currently in clinical use for assessment of kidney function with differences in accuracy, complexity and duration [2]. However, accurate assessment of kidney function is not only needed in the context of living kidney donation. More importantly, it is needed whenever impairment of kidney function is suspected.

In routine clinical practice, the most frequently used method is measurement of serum creatinine. Determination of serum creatinine for assessment of renal function is limited by the fact that an increase only becomes evident after 50 % of kidney function is lost [2]. Estimated glomerular filtration rate (eGFR) is also widely used and provides a more accurate assessment of kidney function using one of the established formulas, such as "modification of diet in renal disease" (MDRD), "Cockcroft–Gault" (CG) or the "chronic kidney disease epidemiology collaboration equation" (CKD-EPI) [3–6]. Especially in the context of living kidney donation, use of an estimation formula for assessing kidney function is widely considered as imprecise and not appropriate [1,7–10]. Radioisotopic techniques provide more accurate information on kidney function and are widely used to measure split and total renal function [10,11]. Radiotracers that are used as exogenous filtration markers to measure GFR include ^{51}Cr-ethylenediaminetetraacetic acid (EDTA), ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA), ^{99m}Tc-mercaptoacetyltriglycine (MAG3) and ^{125}I-iothalamate [2,10].

Besides the widely used and established methods for the evaluation of kidney function, various imaging techniques have been reported to significantly correlate with kidney function—including measurement of cortical volume or total kidney volume on computed tomography (CT), measurement of kidney length in ultrasound (US) and assessment of kidney function in dynamic contrast-enhanced magnetic resonance imaging (MRI) [10,12–14]. However, it has not been sufficiently investigated if there is a relationship between kidney perfusion in contrast-enhanced US (CEUS) and kidney function. Since hyperperfusion can be considered an early sign of glomerular injury, kidney perfusion in CEUS could possibly provide information for detection of early kidney injury—especially when kidney function appears to be normal [15–17]. Thus, a proportion of the patients may be spared an invasive procedure for histological confirmation of kidney injury.

To date, CEUS has been primarily performed to characterize focal renal lesions and assess perfusion patterns based on microvascularization following administration of a strictly intravascular contrast agent [18,19]. Since CEUS has not yet been sufficiently investigated in the context of whole-organ perfusion and kidney function, this pilot study was conducted to analyze the potential relationship between kidney function and CEUS in living kidney donors.

2. Material and Methods

2.1. Study Design and Patient Population

The study was designed as a feasibility study to investigate a potential relationship between kidney function and kidney perfusion in CEUS. For this reason, 30 living kidney donors underwent CEUS one day prior to donor nephrectomy. Overall, 60 kidneys were examined between April 2018 and March 2020. The study was approved by the local institutional review board (Ethical Committee of Charité Universitätsmedizin Berlin) (EA1/406/16) and conformed to the amended Declaration of Helsinki. All donors were informed about the procedure and possible risks 24 h before the examination and provided written informed consent. The results of this study were not used to guide clinical management.

All living kidney donor candidates invariably undergo evaluation of total and split renal function using radioisotopic techniques prior to nephrectomy in our hospital. DTPA clearance was determined for assessment of total kidney function and MAG3-scintigraphy was used for evaluation of split renal function. Additional examination of kidney perfusion by CEUS enabled a direct comparison between CEUS-derived perfusion parameters and various established methods for evaluation of kidney function in individuals with healthy kidneys.

2.2. CEUS Examination Protocol

All examinations were performed by three examiners with many years of experience in CEUS, together with two assistants who administered the ultrasound contrast agent (UCA). SonoVue® (Bracco Imaging, Milan, Italy) was used as a second-generation UCA for all examinations and was administered via a 3-way stopcock in the antecubital vein, followed by a saline flush. The UCA was prepared according to the manufacturer's instructions.

All CEUS examinations were performed using a high-end ultrasound system (Aplio i900, Canon, Otawara, Japan) with a contrast-specific mode and the same multifrequency (i8CX1). CEUS was performed of the right kidney, followed by the left kidney. After positioning the transducer for renal imaging in the longitudinal plane in deep inspiration, a 1.6 mL UCA bolus was injected, followed by a rapid 10 mL saline flush. Approximately 10 to 20 s (s) after UCA administration, the first microbubbles appeared in the interlobar arteries, followed by rapid filling of the renal cortex and prolonged medullar enhancement (Figure 1). After the first contrast signal was displayed, microflow kinetics were recorded as a 30-s loop during a single breath hold. Since pulmonary elimination of the applied UCA takes approximately 5 to 10 min, CEUS of the left kidney was performed after a 10-min waiting time and when no CEUS signal was apparent in the left kidney.

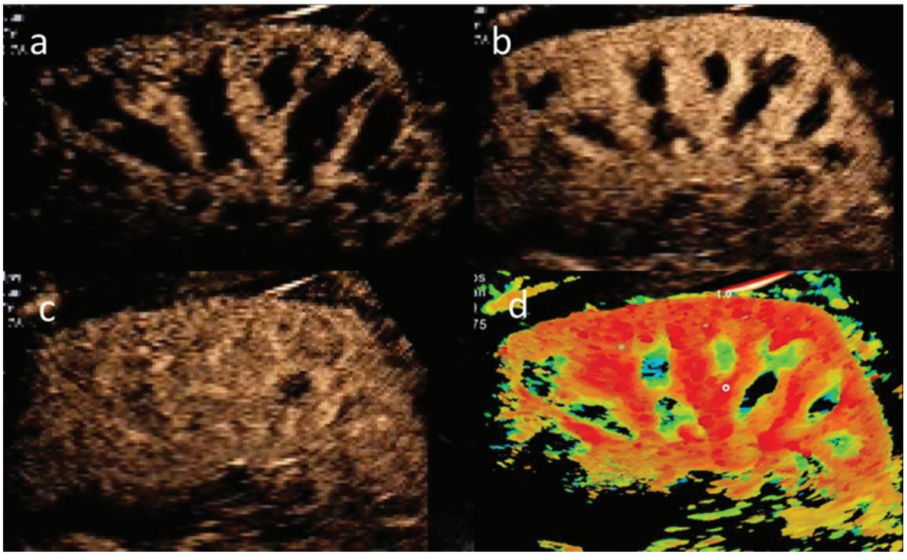


Figure 1. Example illustrating kidney perfusion in CEUS approximately 1 s (**a**), 4 s (**b**) and 13 s (**c**) after detection of the first signal (approximately 10–15 s after injection of the ultrasound contrast agent). In (**a**) the contrast agent enhances the interlobar arteries and part of the renal cortex. Full cortical enhancement is seen in (**b**), and perfusion of the whole kidney including the renal pyramids is shown in (**c**). Figure (**d**) shows the time course of successive enhancement in different colors in a single image. Red indicates earliest enhancement, followed by yellow, green and blue regions.

2.3. Ultrasound Settings

The UCA consists of microbubbles [20]. To avoid microbubble destruction, the UCA was administered in a straight direction. Moreover, scanning was performed with a low mechanical index (MI; 0.07–0.09) [21]. Beside the MI, there are other settings that may have influenced the received signal intensity. In this study population, Gain (G) ranged between 76 and 89 and the dynamic range (DR) was either 75 or 60.

2.4. Quantitative Perfusion Analysis

CEUS cine loops were stored as DICOM raw data and transferred to a software package for further analysis. Quantitative analysis of kidney perfusion with time-intensity curve measurements (TIC) was performed using the VueBox® postprocessing software package (Bracco Imaging, Milan, Italy). Motion compensation—which is offered by the software as an option for optimization of quality of fit (QOF)—was applied to all videos analyzed. Except for three video clips analyzed with QOF of 88, 78 and 74, QOF was over 90.

After motion compensation, a freehand region of interest (ROI) was manually placed in the renal cortex by the same person excluding artifacts. To ensure optimal comparability, the positions of the drawn ROIs had to be consistent. Therefore, all ROIs were drawn in a central position (middle third of the kidney) with adequate image quality. Based on the ROIs, the software generated time-intensity curves and computed different perfusion parameters.

2.5. Perfusion Parameters

Figure 2 shows the perfusion parameters automatically determined by VueBox® (Bracco Imaging, Milan, Italy). [22]. They can be classified into two categories: signal intensity parameters and time-related parameters (Table 1). Signal intensity parameters, such as peak enhancement (PE) and area under the curve (AUC), are determined to describe relative blood volume and mean transit time (mTTl) as a time-related parameter describes the mean blood flow velocity [21]. For simplicity's sake, and since signal intensities were very high, signal intensity parameters were divided by 1000.

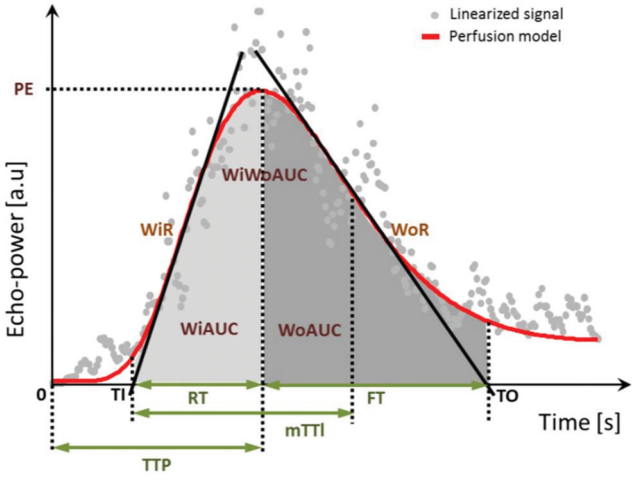


Figure 2. CEUS perfusion parameters in a model. TI defines the point in time when the tangent of the maximum increase (WiR) intersects the x-axis. TO is the point in time when the tangent of the maximum decrease intersects the x-axis. Parameters shown are described in Tables 1 and 2. WiWoAUC: Wash-in and wash-out Area Under the Curve; WoR: Wash-out Rate; WiR: Wash-in Rate; WoAUC: Wash-out Area Under the Curve; WiAUC: Wash-in Area Under the Curve; PE: Peak Enhancement; RT: Rise time; FT: Fall time; mTT1: Mean transit time local (mTT-Tl); TTP: Time to peak; S: seconds; a.u: arbitrary units; Figure source: VueBox—instruction for use [19]. CEUS: Contrast-enhanced ultrasound.

Table 1. CEUS parameters.

	CEUS Parameter	Label	Description
Time-related parameters †	RT	Rise time	-
	mTTl	Mean transit time local (mTT-Tl)	-
	TTP	Time to peak	-
	FT	Fall time	-
Signal intensity parameters *	MeanLin	-	Mean signal intensity
	PE	Peak enhancement	Maximum signal enhancement
	WiAUC	Wash-in area under the curve (AUC (TI:TTP))	AUC (area under the curve) during wash-in of UCA (between TI and TTP)
	WiR	Wash-in rate	Maximum increase
	WiPI	Wash-in perfusion index	WiAUC/RT
	WoAUC	Wash-out AUC (AUC (TTP:TO))	AUC (area under the curve) during wash-out of UCA (between TTP und TO)
	WiWoAUC	Wash-in and wash-out AUC	WiAUC + WiWoAUC
	WoR	Wash-out rate	Maximum decrease

* Signal intensity parameters in arbitrary units (a.u.). † Time-related parameters in seconds (s).

Table 2. Results for total and split kidney function.

			Mean	Standard Deviation	Total
Total kidney function prior to nephrectomy	DTPA clearance (mL/min/1.73 m^2)		97	14	30
	Serum creatinine (mg/dL)		0.83	0.13	30
	eGFR (CG) (mL/min/1.73 m^2)		95	25	30
	eGFR (CKD-EPI) (mL/min/1.73 m^2)		87	14	30
	eGFR (MDRD) (mL/min/1.73 m^2)		84	14	30
	Total kidney volume (cm^3)		323	74	30
Split kidney function prior to nephrectomy	Proportion in MAG3 (%)	right	47.6	3.3	30
		left	52.4	3.3	30
	Split DTPA clearance (mL/min/1.73 m^2)		49	8	60
	Split eGFR (CG) (mL/min/1.73 m^2)		47	13	60
	Split eGFR (CKD-EPI) (mL/min/1.73 m^2)		44	8	60
	Split eGFR (MDRD) (mL/min/1.73 m^2)		42	8	60
	Split kidney volume (cm^3)		162	38	60
Total kidney function after nephrectomy	eGFR (CG) (mL/min/1.73 m^2)		59	17	30
	eGFR (CKD-EPI) (mL/min/1.73 m^2)		50	11	30
	eGFR (MDRD) (mL/min/1.73 m^2)		49	9	30

Calculation of split DTPA clearance and split eGFR by multiplication with the proportion of split kidney function in MAG3-scintigraphy; no depth correction was applied for calculation of split kidney function.

2.6. Patient Data and Methods for Assessment of Kidney Function

Different methods for evaluation of kidney function were applied and the results documented for comparison with CEUS-derived perfusion parameters. MAG3-scintigraphy, DTPA clearance and serum creatinine were collected from the hospital's general documentation system. eGFR was calculated according to the CG, MDRD and CKD-EPI formulas [3–5]. Split kidney volume was determined by an automatic calculation after manually framing the kidneys in CT in all representative slices. Total kidney volume was calculated by adding both right and left split kidney volume. BMI was used to categorize donors into normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obesity ($\geq$30 kg/m^2). A total of 15 patients belonged to the normal-weight group.

DTPA clearance was used as the reference standard for measurement of kidney function. Donors were instructed to drink at least one liter prior to DTPA clearance testing.

DTPA clearance was determined using the Fleming formula [23]. MAG3 scintigraphy was performed to determine the proportion of split renal function for each kidney based on tubular extraction rate (TER) according to Bubeck [24]. Split DTPA clearance was calculated by multiplying the proportion of split kidney function derived from MAG3-scintigraphy with absolute DTPA clearance. Split eGFR was also calculated by multiplication with the result of MAG3-scintigraphy.

2.7. Statistical Analysis

First, established methods for evaluation of total kidney function prior to nephrectomy were compared with each other as a basis for comparing the results of these methods with CEUS-derived parameters and determining the degree of correlation. We analyzed whether CEUS parameters were related to (1) total kidney function prior to nephrectomy, (2) split kidney function prior to nephrectomy and (3) total kidney function after nephrectomy. For analysis of a potential relationship between CEUS parameters and postoperative total kidney function, CEUS parameters derived from the retained kidney before donor nephrectomy were used. Finally, confounders that could have an influence on CEUS parameters were analyzed. To investigate potential relationships between kidney function and renal perfusion in CEUS, Pearson correlations were carried out. Descriptive results are presented as mean +/− standard deviation.

A two-sided significance level of $\alpha = 0.05$ was defined to indicate statistical significance. All statistical analyses were performed using the SPSS software package (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, USA: IBM Corp.).

3. Results

3.1. Epidemiology and Descriptive Presentation of Kidney Function Data

Donors had a mean age of 54 +/− 9 years with a range of 37–75. The majority were female (76.7%), and mean BMI in the study population was 26 kg/m^2 (+/−4 kg/m^2).

Table 2 provides an overview of the kidney function tests performed both before and after nephrectomy. Prior to nephrectomy, mean DTPA clearance was 97 mL/min/1.73 m^2 (+/−14). Estimation of GFR yielded lower values than DTPA clearance. Among all estimation formulas, the CG formula yielded the mean value closest to that obtained by determination of DTPA clearance; however, scatter was also greatest with a standard deviation of 25 mL/min/1.73 m^2.

3.2. CEUS Parameters

Table 3 compiles the CEUS-derived values assessing signal intensity and time. Overall, there was large scatter of signal intensity parameters. All signal intensity parameters correlated strongly and significantly with MeanLin as a representative signal intensity parameter ($r = 0.930$ to $r = 0.984$; $p < 0.001$). In addition, CEUS-derived, time-related parameters also showed strong and significant correlation with each other. For correlation of time-related parameters, rise time (RT)—as a marker of arterial inflow—was chosen as a representative parameter.

Table 3. Computed CEUS values.

Parameter *	Mean	Interquartile Range	Correlation with MeanLin	Total
MeanLin †	12.9	17.1 (3.7–20.8)	-	60
PE †	27.6	38.1 (8–46.1)	r = 0.984; $p < 0.001$	59
WiAUC †	80	102.1 (22.4–124.5)	r = 0.987; $p < 0.001$	59
WiR †	8.8	13.4 (2.3–15.7)	r = 0.937; $p < 0.001$	59
WiPI †	17.5	23.8 (5.6–29.4)	r = 0.985; $p < 0.001$	59
WoAUC †	150.8	192.3 (41.3–233.6)	r = 0.983; $p < 0.001$	58
WiWoAUC †	230.6	292 (64–356)	r = 0.983; $p < 0.001$	58
WoR †	3.7	5.3 (0.9–6.2)	r = 0.930; $p < 0.001$	58
	Mean	**Interquartile Range**	**Correlation with RT**	**Total**
RT ‡	4.7	1.2 (3.8–5)	-	59
mTTI ‡	25.8	17.1 (14–31.1)	r = 0.387; $p = 0.002$	59
TTP ‡	7.4	2.1 (6.2–8.3)	r = 0.860; $p < 0.001$	59
FT ‡	9.5	2.2 (7.5–9.7)	r = 0.898; $p < 0.001$	58

† values in (a.u.); ‡ values in (s). Since the actual values were very large, they were divided by 1000. * All abbreviations are explained in Table 1.

3.3. Analysis of Total Kidney Function

Established methods for evaluation of total kidney function prior to nephrectomy were compared with each other. Table 4 shows correlations of various kidney function tests with DTPA clearance as the reference method for evaluation of total kidney function. DTPA clearance showed the strongest and most significant correlation with eGFR (CG) (r = 0.531; $p = 0.003$). In comparison, just a weak correlation was observed for serum creatinine and DTPA clearance (r = −0.228; $p = 0.225$). Because eGFR (MDRD) and serum creatinine showed only weak correlations, these parameters were not considered for further analysis.

Table 4. Parameters used for evaluation of kidney function prior to donor nephrectomy.

	Correlation with DTPA Clearance	*p*-Value
eGFR (CG)	r = 0.531	$p = 0.003$
Total kidney volume	r = 0.472	$p = 0.008$
eGFR (CKD-EPI)	r = 0.470	$p = 0.009$
eGFR (MDRD)	r = 0.377	$p = 0.040$
Serum creatinine	r = −0.228	$p = 0.225$

3.4. Comparison of CEUS and Kidney Function Parameters

Table 5 compiles correlations of CEUS signal intensity parameters with the results of different kidney function tests. All signal intensity parameters showed weak and nonsignificant correlations with DTPA and eGFR (CKD-EPI). Conversely, all signal intensity parameters correlated significantly with eGFR (CG) and total kidney volume. MeanLin (r = −0.345; $p = 0.007$ and r = −0.409; $p = 0.001$) and WiWoAUC (r = −0.346; $p = 0.008$ and r = −0.401; $p = 0.002$) showed the strongest correlations. In contrast to correlations between established methods for evaluation of total kidney function, correlations with CEUS signal intensity parameters were invariably negative. Higher signal intensities were associated with lower kidney function. These correlations were comparable with the correlation between DTPA clearance and eGFR (MDRD) and even stronger than the correlation between serum creatinine and DTPA clearance. Overall, correlations with total kidney volume were stronger than correlations with eGFR (CG). Time-related CEUS parameters did not show any significant correlation with kidney function test results (Table 5). Thus, these CEUS parameters were not taken into consideration for further analysis.

Table 5. Correlation of CEUS parameters with different methods for evaluation of preoperative total kidney function.

	Correlation with DTPA	Correlation with eGFR (CKD-EPI)	Correlation with eGFR (CG)	Correlation with Total Kidney Volume
MeanLin	r = −0.170; p = 0.194	r = −0.179; p = 0.172	**r = −0.345**; ***p* = 0.007**	**r = −0.409**; ***p* = 0.001**
PE	r = −0.162; p = 0.221	r = −0.182; p = 0.169	r = −0.322; p = 0.013	r = −0.392; p = 0.002
WiAUC	r = −0.189; p = 0.152	r = −0.214; p = 0.104	r = −0.339; p = 0.009	r = −0.402; p = 0.002
WiR	r = −0.112; p = 0.399	r = −0.111; p = 0.401	r = −0.274; p = 0.036	r = −0.357; p = 0.005
WiPI	r = −0.160; p = 0.225	r = −0.177; p = 0.179	r = −0.319; p = 0.014	r = −0.391; p = 0.014
WoAUC	r = −0.174; p = 0.192	r = −0.197; p = 0.138	r = −0.321; p = 0.014	r = −0.403; p = 0.002
WiWoAUC	r = −0.160; p = 0.229	r = −0.214; p = 0.106	**r = −0.346**; ***p* = 0.008**	**r = −0.401**; ***p* = 0.002**
WoR	r = −0.123; p = 0.356	r = −0.155; p = 0.245	r = −0.306; p = 0.019	r = −0.355; p = 0.006
RT	r = 0.037; p = 0.782	r = −0.118; p = 0.374	r = −0.084; p = 0.528	r = −0.018; p = 0.893
mTTl	r = −0.160; p = 0.226	r = 0.024; p = 0.860	r = 0.045; p = 0.733	r = −0.015; p = 0.913
TTP	r = −0.007; p = 0.959	r = −0.216; p = 0.100	r = −0.178; p = 0.178	r = −0.092; p = 0.489
FT	r = 0.113; p = 0.398	r = −0.105; p = 0.433	r = −0.047; p = 0.724	r = −0.013; p = 0.923

The strongest correlations are indicated in bold. MeanLin—analyzed as a representative parameter of signal intensity—shows the strongest correlations as a representative parameter of signal intensity.

In the assessment of preoperative split kidney function, split kidney volume and split eGFR (CG) also correlated with signal intensity parameters in CEUS (Table 6). Similarly, postoperative kidney function—to be precise eGFR (CG)—significantly correlated with CEUS intensity parameters of the remaining kidney (Table 6). For both preoperative split kidney function and postoperative kidney function correlation values were close to those for total kidney function.

Table 6. Correlation of CEUS-based signal intensity parameters with preoperative split kidney function and postoperative kidney function in donors.

	Correlation with Split DTPA	Correlation with Split eGFR (CKD-EPI)	Correlation with Split eGFR (CG)	Correlation with Split Kidney Volume
Preoperative split kidney function				
MeanLin	r = −0.150; p = 0.253	r = −0.176; p = 0.179	**r = −0.331**; ***p* = 0.010**	**r = −0.398**; ***p* = 0.002**
WiWoAUC	r = −0.151; p = 0.258	r = −0.216; p = 0.104	**r = −0.338**; ***p* = 0.009**	**r = −0.389**; ***p* = 0.003**
Postoperative kidney function				
MeanLin	n.A.	r = −0.133; p = 0.483	**r = −0.399; *p* = 0.029**	n.A.
WiWoAUC	n.A.	r = −0.148; p = 0.436	**r = −0.393; *p* = 0.032**	n.A.

The strongest correlations are indicated in bold. n.A.: not available.

3.5. Evaluation of Confounders

Although CEUS parameters showed significant correlations with eGFR (CG) and kidney volume, no correlations with the reference method DTPA clearance were detected. For identification of potential confounders, relationships of MeanLin and DTPA were depicted in a scatter diagram using three different colors to represent the three BMI subgroups investigated (Figure 3). In this diagram, higher values for MeanLin were associated with lower values for DTPA. On the other hand, smaller values for MeanLin were related to both high and small values for DTPA. However, the diagram also reveals that BMI may have had an impact on signal intensity. Donors with a higher BMI had low values for MeanLin, and no relation between MeanLin and DTPA could be identified. However, for normal-weight donors, the diagram shows a relation between MeanLin and DTPA (i.e., kidney function). The scatter diagram suggests that, in the normal-weight subgroup, higher values for MeanLin were associated with poorer kidney function, and lower values for MeanLin were associated with better kidney function.

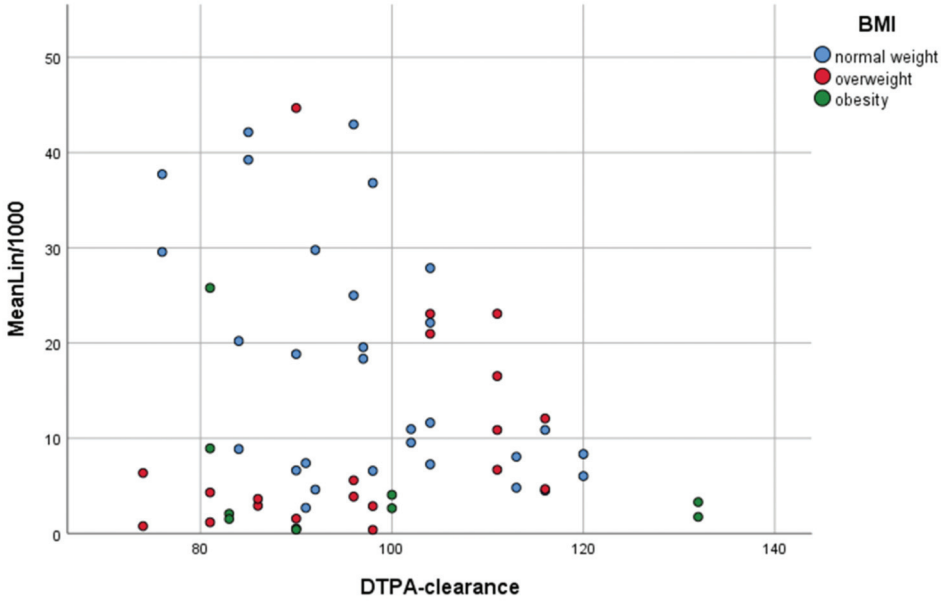


Figure 3. Scatter diagram of the association between MeanLin and DTPA clearance. Each dot represents one kidney. Data are presented in different colors for each of the three BMI-based subgroups. The results suggest that BMI could have had an impact on signal intensity and might thus be a confounder affecting the relationship between MeanLin and DTPA clearance.

Further analysis showed a significant negative correlation between BMI and MeanLin ($r = -0.366$; $p = 0.004$). A larger BMI was therefore associated with smaller MeanLin values.

Patients with normal weight (n = 15, defined as BMI of 18.5–24.9 kg/m^2) were selected to further analyze the relationship between DTPA and MeanLin. In this subgroup (30 kidney in total), MeanLin showed a significant and strong correlation with DTPA clearance ($r = -0.502$; $p = 0.005$).

4. Discussion

Although CEUS has been widely used in various clinical specialties, it has not been sufficiently investigated whether there is a relationship between CEUS-derived perfusion

and kidney function. To our knowledge, this is the first standardized and prospective study to analyze the potential relationship between kidney function and CEUS-based perfusion parameters in individuals with healthy kidneys. Our study revealed significant correlations between different methods for evaluating kidney function and CEUS signal intensity parameters. However, no correlations were identified with time-related CEUS parameters. MeanLin was analyzed as a representative signal intensity parameter and showed the strongest correlations, which were similar for preoperative total kidney function, preoperative split kidney function and postoperative kidney function.

Significant correlations were only identified for total kidney volume and eGFR (CG), but not for the reference method—DTPA clearance. Because of the importance of the correlation between CEUS and the reference method for evaluation of kidney function, we analyzed potential confounders for their effects on the relation between DTPA clearance and signal intensity in CEUS.

Since greater penetration depth can attenuate the signal in CEUS, BMI was analyzed as a potential confounder [21]. Indeed, there was a strong correlation between MeanLin and DTPA clearance ($r = -0.502$; $p = 0.005$) in the normal-weight subgroup.

While MeanLin did not correlate significantly with DTPA clearance in the total study population, it showed significant correlation with eGFR (CG). The estimation formula used for calculation of eGFR (CG) is the only formula that incorporates information on body weight [5]. This may explain why MeanLin, as a parameter that can be influenced by BMI and thus by body weight, was found to significantly correlate with eGFR (CG).

There was a large scatter for all signal intensity parameters. BMI and kidney depth may have contributed to the large scatter. However, the failure to use consistent US system settings may also have contributed to differences in signal intensities. Mechanical index (MI), frames per second (fps), gain (G) and dynamic range (DR) varied between the CEUS examinations performed in our study population. The fact that ultrasound system settings, in general, affect signal intensity, and may both attenuate and enhance it, hampers comparison of absolute signal intensities [21,25].

Participation of three different examiners may also have contributed to the observed variability in signal intensities. Both inter- and intra-observer variability have been described for CEUS before [26]. However, the fact that CEUS was performed by different examiners alone cannot explain the large scatter.

Another potential confounder of signal intensity is the amount of fluid intake prior to a CEUS examination. In our hospital, patients scheduled for radioisotopic measurement are instructed to drink one liter of water before the examination since the amount of drinking may influence results. In contrast, no recommendation was made regarding fluid intake prior to the CEUS examination in this pilot study. As a result, fluid intake may have influenced signal intensities in CEUS.

According to our results, kidney volume may also influence signal intensities in CEUS. A large kidney appears to attenuate the CEUS signal. The underlying mechanism should be addressed in future studies.

To our knowledge, two studies have been published that investigated possible associations between renal function and perfusion in CEUS [17,27]. Both studies were conducted in patients with diabetic kidney disease (DKD) in comparison to control groups. Similar to our study, patient groups were small, with 33 and 55 patients with diabetic nephropathy. One of these studies, conducted by Ma et al., showed a positive correlation between GFR and the area under the curve (AUC) determined as a signal intensity parameter in CEUS [27]. Wang et al. also reported a significantly increased area under the ascending curve for patients with early-stage DKD (eGFR (MDRD) $\geq$90 mL/min/1.73 m^2) compared to patients with moderate DKD (30–90 mL/min/1.73 m^2) [17]. In contrast, our results showed a negative correlation between kidney function and CEUS-derived signal intensity parameters. However, we only investigated kidney function in individuals without underlying kidney disease, while the two earlier studies analyzed kidney function and CEUS-based perfusion in patients with DKD.

Future studies should consider comparison of absolute values with scintigraphic results as presented by Krumm et al. for contrast-enhanced MRI [27]. In general, ultrasound contrast agents are associated with very low adverse event rates and do not interact with renal function or lead to contrast-agent nephropathy [28]. Moreover, microbubble-based contrast agents do not interact with thyroid function as they do not contain iodine. Ultrasound contrast agents are proven as strictly intravascular, allowing the assessment of organ perfusion on microcirculation level. [21] This advantage, combined with a dynamic examination, settles a further argument for CEUS compared to CT and MRI, since iodinated and gadolinium-based contrast media are known to be not strictly intravascular. Nevertheless, CEUS and US are known to be operator-dependent, especially in image/cineloop acquisition for parametric measurements. Although CEUS and US, in general, possess advantages, such as the absence of radiation, lower costs and high availability (nearly every mid-range system contains CEUS specific software), both tomographic modalities (i.e., CT, MRI) are more standardized in the assessment of scans or sequences. Thus, a volume dataset and not a single plane alone (as in US) can be used for whole organ perfusion. Our results demonstrated the potential influence of BMI or body weight on the parametric assessment of renal perfusion in CEUS (driven by lower image quality at higher penetration depth). These confounders may not affect image quality and parametric evaluation of organ perfusion in tomographic imaging.

Limitations

The most important limitation of this study is the small number of subjects included in this pilot study. Moreover, the lack of use of consistent US system settings and the involvement of several examiners could have affected our results. Additionally, since only patients in the subgroup of normal-weight donors showed a strong and significant correlation between MeanLin and DTPA clearance, a broad usage of CEUS is not applicable. Future studies are needed to analyze confounders and correlations in a larger population to enable a broad and standardized usage of this method.

5. Conclusions

This pilot study, for the first time, demonstrated a significant relationship between kidney perfusion in CEUS and kidney function in living kidney donors. However, its significance is limited to patients of normal weight and BMI, and the US settings appear to affect renal perfusion quantified by TIC measurements on CEUS. This pilot study provides important new insights and supports the role of CEUS in assessing whole organ perfusion. Future research should analyze the relationships and potential confounders identified here in a larger population.

Author Contributions: Conceptualization, N.E.-B., F.F. and T.F.; methodology, N.E.-B. and M.H.L.; software, N.E.-B.; validation, F.F., T.F. and B.H.; formal analysis, N.E.-B.; investigation, N.E.-B., M.H.L. and T.F.; data curation, N.E.-B., L.L. and F.F.; writing—original draft preparation, N.E.-B.; writing—review and editing, all authors; visualization, R.P.; supervision, F.F., T.F., M.H.L., T.S., K.K., A.S., K.B., K.M. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: "The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee) of Charité Universitätsmedizin Berlin (protocol code EA1/406/16, 26.01.2017)".

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: The authors thank Bettina Herwig for language editing of the manuscript, Annette Aigner for statistical advice and Christian Greis (Bracco Imaging) for scientific advice.

Conflicts of Interest: The authors declare no conflict of interest. Markus H. Lerchbaumer reports having received consultancy honoraria from Siemens Healthineers. Thomas Fischer reports having received consultancy honoraria from Siemens Healthineers, Bracco Imaging and Canon Medical Imaging.

References

1. Delmonico, F. A Report of the Amsterdam Forum On the Care of the Live Kidney Donor: Data and Medical Guidelines. *Transplantation* **2005**, *79*, S53–S66. [PubMed]
2. Durand, E.; Müller-Suur, R. Zur Messung der Nierenfunktion durch Clearancebestimmungen: Methoden und Indikationen—Ein Update. *Der Nukl.* **2010**, *33*, 145–153. [CrossRef]
3. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.L.; Castro, A.F., III; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T.; et al. A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* **2009**, *150*, 604–612. [CrossRef] [PubMed]
4. Levey, A.S.; Bosch, J.P.; Lewis, J.B.; Greene, T.; Rogers, N.; Roth, D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann. Intern. Med.* **1999**, *130*, 461–470. [CrossRef]
5. Cockcroft, D.W.; Gault, M.H. Prediction of creatinine clearance from serum creatinine. *Nephron* **1976**, *16*, 31–41. [CrossRef]
6. Levey, A.S.; Inker, L.A.; Coresh, J. GFR estimation: From physiology to public health. *Am. J. Kidney Dis.* **2014**, *63*, 820–834. [CrossRef]
7. Lentine, K.L.; Kasiske, B.L.; Levey, A.S.; Adams, P.L.; Alberú, J.; Bakr, M.A.; Gallon, L.; Garvey, C.A.; Guleria, S.; Li, P.K.-T.; et al. Summary of Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation* **2017**, *101*, 1783–1792. [CrossRef]
8. Abramowicz, D.; Cochat, P.; Claas, F.H.; Heemann, U.; Pascual, J.; Dudley, C.; Harden, P.; Hourmant, M.; Maggiore, U.; Salvadori, M.; et al. European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care. *Nephrol. Dial. Transplant.* **2015**, *30*, 1790–1797. [CrossRef]
9. Andrews, P.A.; Burnapp, L.; Manas, D.; Bradley, J.A.; Dudley, C. Summary of the British Transplantation Society/Renal Association U.K. guidelines for living donor kidney transplantation. *Transplantation* **2012**, *93*, 666–673. [CrossRef]
10. Halleck, F.; Diederichs, G.; Koehlitz, T.; Slowinski, T.; Engelken, F.; Liefeldt, L.; Friedersdorff, F.; Fuller, T.F.; Magheli, A.; Neumayer, H.-H.; et al. Volume matters: CT-based renal cortex volume measurement in the evaluation of living kidney donors. *Transpl. Int.* **2013**, *26*, 1208–1216. [CrossRef]
11. Brar, A.; Jindal, R.M.; Abbott, K.C.; Hurst, F.P.; Salifu, M.O. Practice patterns in evaluation of living kidney donors in United Network for Organ Sharing-approved kidney transplant centers. *Am. J. Nephrol.* **2012**, *35*, 466–473. [CrossRef]
12. Poggio, E.D.; Hila, S.; Stephany, B.; Fatica, R.; Krishnamurthi, V.; Del Bosque, C.; Goldfarb, D.; Herts, B.; Dennis, V.W.; Heeger, P.S.; et al. Donor kidney volume and outcomes following live donor kidney transplantation. *Am. J. Transplant.* **2006**, *6*, 616–624. [CrossRef] [PubMed]
13. Paleologo, G.; Abdelkawy, H.; Barsotti, M.; Basha, A.; Bernabini, G.; Bianchi, A.; Caprio, F.; Emad, A.; Grassi, G.; Nerucci, B.; et al. Kidney dimensions at sonography are correlated with glomerular filtration rate in renal transplant recipients and in kidney donors. *Transplant. Proc.* **2007**, *39*, 1779–1781. [CrossRef]
14. Buckley, D.L.; Shurrab, A.E.; Cheung, C.M.; Jones, A.P.; Mamtora, H.; Kalra, P.A. Measurement of single kidney function using dynamic contrast-enhanced MRI: Comparison of two models in human subjects. *J. Magn. Reson. Imaging* **2006**, *24*, 1117–1123. [CrossRef]
15. Raptis, A.E.; Viberti, G. Pathogenesis of diabetic nephropathy. *Exp. Clin. Endocrinol. Diabetes* **2001**, *109* (Suppl. 2), S424–S437. [CrossRef] [PubMed]
16. Hughson, M.D.; Johnson, K.; Young, R.J.; Hoy, W.E.; Bertram, J.F. Glomerular size and glomerulosclerosis: Relationships to disease categories, glomerular solidification, and ischemic obsolescence. *Am. J. Kidney Dis.* **2002**, *39*, 679–688. [CrossRef] [PubMed]
17. Wang, L.; Wu, J.; Cheng, J.F.; Liu, X.-Y.; Ma, F.; Guo, L.-H.; Xu, J.-M.; Wu, T.; Mohan, C.; Peng, A.; et al. Diagnostic value of quantitative contrast-enhanced ultrasound (CEUS) for early detection of renal hyperperfusion in diabetic kidney disease. *J. Nephrol.* **2015**, *28*, 669–678. [CrossRef] [PubMed]
18. Sidhu, P.S.; Cantisani, V.; Dietrich, C.F.; Gilja, O.H.; Saftoiu, A.; Bartels, E.; Bertolotto, M.; Calliada, F.; Clevert, D.A.; Cosgrove, D.; et al. The EFSUMB Guidelines and Recommendations for the Clinical Practice of Contrast-Enhanced Ultrasound (CEUS) in Non-Hepatic Applications: Update 2017 (Short Version). *Ultraschall Med.* **2018**, *39*, 154–180. [CrossRef]
19. Spiesecke, P.; Fischer, T.; Maxeiner, A.; Hamm, B.; Lerchbaumer, M.H. Contrast-enhanced ultrasound (CEUS) reliably rules out neoplasm in developmental renal pseudotumor. *Acta Radiol.* **2020**, *62*, 821–829. [CrossRef]
20. Greis, C. Ultrasound contrast agents as markers of vascularity and microcirculation. *Clin. Hemorheol. Microcirc.* **2009**, *43*, 1–9. [CrossRef]
21. Greis, C. Quantitative evaluation of microvascular blood flow by contrast-enhanced ultrasound (CEUS). *Clin. Hemorheol. Microcirc.* **2011**, *49*, 137–149. [CrossRef] [PubMed]
22. Bracco. User Manual | CEUS—Contrast Enhanced Ultrasound: Bracco Suisse SA; Copyright© 2019 Bracco Suisse SA All Rights Reserved. Available online: https://www.contrastultrasound-modality.com/quantification-software/support/user-manual (accessed on 22 December 2020).
23. Fleming, J.S.; Persaud, L.; Zivanovic, M.A. A general equation for estimating glomerular filtration rate from a single plasma sample. *Nucl. Med. Commun.* **2005**, *26*, 743–748. [CrossRef] [PubMed]
24. Bubeck, B. Renal clearance determination with one blood sample: Improved accuracy and universal applicability by a new calculation principle. *Semin. Nucl. Med.* **1993**, *23*, 73–86. [CrossRef]

25. Dietrich, C.F.; Averkiou, M.; Nielsen, M.B.; Barr, R.G.; Burns, P.N.; Calliada, F.; Cantisani, V.; Choi, B.; Chammas, M.C.; Clevert, D.A.; et al. How to perform Contrast-Enhanced Ultrasound (CEUS). *Ultrasound Int. Open* **2018**, *4*, E2–E15. [CrossRef] [PubMed]
26. Kunz, P.; Kiesl, S.; Groß, S.; Kauczor, H.-U.; Schmidmaier, G.; Fischer, C. Intra-observer and Device-Dependent Inter-observer Reliability of Contrast-Enhanced Ultrasound for Muscle Perfusion Quantification. *Ultrasound Med. Biol.* **2020**, *46*, 275–285. [CrossRef]
27. Krumm, P.; Hupka, T.; Haußmann, F.; Dittmann, H.; Mühlbacher, T.; Nadalin, S.; Königsrainer, A.; Nikolaou, K.; Heyne, N.; Kramer, U.; et al. Contrast-enhanced MRI for simultaneous evaluation of renal morphology and split renal function in living kidney donor candidates. *Eur. J. Radiol.* **2021**, *142*, 109864. [CrossRef]
28. Tang, C.; Fang, K.; Guo, Y.; Li, R.; Fan, X.; Chen, P.; Chen, Z.; Liu, Q.; Zou, Y. Safety of Sulfur Hexafluoride Microbubbles in Sonography of Abdominal and Superficial Organs: Retrospective Analysis of 30,222 Cases. *J. Ultrasound Med.* **2017**, *36*, 531–538. [CrossRef]

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland
Tel. +41 61 683 77 34
Fax +41 61 302 89 18
www.mdpi.com

Journal of Clinical Medicine Editorial Office
E-mail: jcm@mdpi.com
www.mdpi.com/journal/jcm

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland

Tel: +41 61 683 77 34
Fax: +41 61 302 89 18

www.mdpi.com

ISBN 978-3-0365-4008-5