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TRANSPLANT TRIAL WATCH
published: 07 July 2022
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Transplant Trial Watch

John M. O'Callaghan[1,2]*

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Keywords: kidney transplantation, anti-inflammatory drugs, squamous cell carcinoma, topical sirolimus, randomised controlled trial

To keep the transplantation community informed about recently published level 1 evidence in organ transplantation ESOT and the Centre for Evidence in Transplantation have developed the Transplant Trial Watch. The Transplant Trial Watch is a monthly overview of 10 new randomised controlled trials (RCTs) and systematic reviews. This page of Transplant International offers commentaries on methodological issues and clinical implications on two articles of particular interest from the CET Transplant Trial Watch monthly selection. For all high quality evidence in solid organ transplantation, visit the Transplant Library: www.transplantlibrary.com.

RANDOMISED CONTROLLED TRIAL 1

Efficacy and Safety of Iguratimod Supplement to the Standard Immunosuppressive Regimen in Highly Mismatched Renal Transplant Recipients: A Pilot Study.

by Tao, J., et al. Frontiers in Immunology 2021; 12: 738392.

Aims

This study aimed to assess the effect and safety of Iguratimod (IGU) combined with standard immunosuppressive regimen in highly HLA-mismatched kidney transplant patients.

Interventions

Patients were randomised to either the IGU or non-IGU group.

Participants

60 highly HLA-mismatched renal transplant recipients.

Outcomes

The primary outcomes were biopsy-proven acute rejection and functional allograft survival. The secondary outcomes were the safety profile, donor-specific antibody (DSA) and other indicators.

Follow-Up

52 weeks.

CET Conclusion

This small pilot RCT investigated whether the addition of the disease-modifying anti-rheumatoid drug (DMARD) Iguratimod (IGU) can improve outcomes in poorly-mismatched renal transplant recipients. The study itself was unblinded, but nephrologists scoring protocol biopsies were blinded to treatment allocation. Both modified intent-to-treat and per-protocol analyses are reported. Patients receiving IGU had numerically lower

Transplant Trial Watch

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incidence of biopsy-proven acute rejection, although not achieving statistical significance due to the small sample size. The results presented do show some promise for the use of IGU following renal transplantation, but larger studies will be required to confirm any benefit. It should be noted that the baseline rate for biopsy-proven acute rejection was relatively high for a Tac/MMF/Pred based regimen (29.6%). Another potential limitation is that patients were only eligible at least 2 weeks post-transplant—anti-inflammatory drugs of this nature may be most effective if given from the day of transplant.

Jadad Score
3.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
No.

Trial Registration
ClinicalTrials.gov—NCT02839941.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Chemoprevention of Cutaneous Squamous Cell Carcinoma and Its Precursors in Solid Organ Transplant Recipients Using Topical Sirolimus: A Randomized, Double-Blind, Placebo-Controlled Pilot Trial.

by Chong, S., et al. Journal of the American Academy of Dermatology 2022 [Online ahead of print].

Aims
This study aimed to examine whether topical application of sirolimus could safely reduce the incidence of keratinocyte cancer (KC) in solid organ transplant recipients.

Interventions
Forearms of patients were randomised to either receive topical sirolimus or placebo.

Participants
29 adult solid organ transplant recipients with a history of basal cell carcinomas or squamous cell carcinomas (SCC) as well as keratotic lesions on the back of the forearms and hands.

Outcomes
Number of keratotic lesions, change in keratotic lesions, number of intraepidermal carcinoma, and number of SCC.

Follow-Up
24 months.

CET Conclusion
This small, blinded pilot study randomised transplant recipients with a history of basal and squamous cell carcinoma (BCC/SCC) to apply topical sirolimus to one forearm/hand and placebo to the other for 12 weeks. There was a significant reduction in the risk of keratotic lesions in the sirolimus group at 12 weeks, which resulted in a significant reduction in the risk of intraepithelial carcinomas at 24 months (4 in the sirolimus arm vs. 12 in the placebo arm). Whilst clearly small and underpowered for firm conclusions, this pilot study does provide some evidence of efficacy and feasibility in support of a larger efficacy study.

Trial Registration
ACTRN12618001961235.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

This is a short letter in the Journal of the American Academy of Dermatology, yet it shows some interesting and clear-cut results from a study with elegant design.

Transplant recipients who were high risk for skin lesions, having at least five Basal Cell Carcinoma (BCC) or Squamous Cell Carcinoma (SCC) in the last 5 years, and at least 5 current keratotic lesions on the back of the forearms or hands, were selected. By randomising one hand/forearm from each patient to treatment and the other hand/forearm to placebo, the study introduced a paired design that not only effectively doubled the recruitment number but also reduced differences between study "arms" (no pun intended). The blinding was achieved by daily application of two topical preparations that were physically indistinguishable, one with additional 1% sirolimus.

At 12 weeks, 18 patients completed the regimen. 11 patients stopped applying the study preparations but had an average of 5 weeks of application and only 1 stopped due to contact dermatitis. The number of keratotic lesions was significantly reduced in each patient on the treated hand/forearm (31%) but was increased in the control hand/forearm (6%). Over 24 months of follow up there were 3-times fewer intraepithelial carcinomas on the treated hand/forearm, using intention to treat analysis (There was no significant difference in the SCC numbers).

The preparation of topical sirolimus appears to be quite effective at preventing intraepithelial carcinoma on the upper limbs of high-risk transplant patients. It would be good now to see longer term follow up to see how the effect is maintained.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

CONFLICT OF INTEREST

The author declares that this clinical impact summary has been written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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POINT OF VIEW
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Decision Making in the Context of Paediatric Solid Organ Transplantation Medicine

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This manuscript aims to outline ethical, legal, and psychosocial key situations in the context of transplantation under special consideration of children. Besides being particularly vulnerable, children as minors by law are not meant to consent to whatever medical procedure is applied to them. Rather their next-of-kin and medical staff are to decide. In the context of transplantation thus it needs to be reflected under which circumstances a child can become an organ donor or receive an organ. This essay will not provide answers to current questions in transplantation medicine but provide an overview of present European practices and juxtapose divergent courses of action which are based on an assumed similar social-cultural background. Data are drawn from a systematic comparison of the various national organ transplantation laws and tissue acts. Ethical reflections are based on a thematically targeted literature search using PubMed Central and PhilPapers databases.

Keywords: ethics, transplant ethics, psychology, solid organ transplant, paediatric

THE CHILD AS AN ORGAN DONOR

In order to transplant an organ it needs an organ donor. Essentially, there are two options: living and deceased donor donation.

A living donor is a person who gives a kidney, or a part of liver, lung, or (in experimental cases) intestines to be donated to another person who is in need for such transplant. A healthy donor, informed consent, and a decision made voluntarily and without undue pressure are legal prerequisites for living donation and insisted on by the declaration of Helsinki [1]. To protect the potential donor, European transplantation laws or tissue acts require an independent assessment, e.g., by an ethics committee, to secure donor voluntariness and avoid organ trading. While some countries allow for non-directed, altruistic living donation, others ask for donor and recipient to be closely related either emotionally or by blood. With regards to the five principles of biomedical ethics, the principle of non-maleficence is challenged in the context of living donation: Each surgical procedure will not only cause pain but is a potentially life-threatening event to the donor. The medical risk varies considerably between organs: while a kidney-transplantation is considered a low risk procedure, the kidney does not regenerate resulting in a slightly elevated life-long increased risk to the kidney donor for developing arterial hypertension, proteinuria or even end stage renal failure [2]. In contrast, it is more dangerous to undergo liver donation, yet liver function will fully restore without long-term side-effects. Finally, liver transplantation is an ultimately live-saving procedure whereas dialysis offers a way to successfully bridge waiting time to transplantation. Yet, refusing a potential donor to donate may lead to emotional and psychological harm due to the stress which is immanent to living with a relative who is suffering from a chronic, life-limiting condition.

Point of View

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To date only five European countries allow for minors to act as living organ donors: In Belgium and the UK the individual's maturity and capacity to make an autonomous decision and give fully informed consent is decisive. In Luxemburg, Norway and Sweden the parental permission is obligatory in addition to the assent of the potential donor [3].

Decision-making in the context of living-related donation poses a special challenge as the parent-child- and siblings-relationships are loaded with strong emotions, attachment, and perceived duties. As found by Russell et al. [4] merely raising the issue of live organ donation triggers an emotional cascade in parents which appears to be beyond their conscious control but leaves little room to freely decide. Rather, parents are restricted in their options given the thread to their child's health and life, and their wish for optimal treatment. If now a minor is to donate to a sibling, the situation becomes even more complex as the moral obligation to protect the vulnerable needs to be recognised. The donor situation becomes more complicated as the potential donor might feel obliged to help the sibling in order to restore a normal family live. Simultaneously, parents may find themselves potentially sacrificing the health of one child for the other. This pressure arising from emotional involvement and perceived moral obligation may result in an implicit moral imperative which undermines true informed consent and freedom of choice. Apart from sufficient cognitive capacity to fully understand the situation, inner strength is needed to identify and possibly resist such potential coercion. The question re the child's best interest appears unanswerable, weighing physical integrity against emotional burden.

A deceased donor in contrast is a person who donates any organ after his/her own death. The first challenge is to define death. In Europe the concept of brain death, as total and irreversible loss of all brain function, is widely accepted. However, by means of intensive care the brain-dead person will still have a functioning cardiovascular system. While most European countries demand full brain-death before proceeding into organ explantation, Poland, the UK, and Israel allow for brain-stem-death. The brain-stem controls basic regulatory functions such as breathing, blood pressure or heartbeat. The rational is that cessation of autonomous breathing is incompatible with life and thus brain-stem-death will result in full brain death as soon as artificial respiration is stopped.

But even the concept of brain-death as it was defined in the 1970 is disputed until today. For instance, in their "statement on brain death and the decision for organ donation" from 24th Febraury 2015 7 of 18 members of the German Ethics Council voted against declaring a brain-dead person as dead; rather they recommended to regard them as a dying person. By law this means, that they still have full personal rights, which are denied after death. While brain-death is a prerequisite to organ donation in most European countries and explicitly mentioned in the transplantation laws and tissue acts, the legal definition of death varies between countries or, in the case of Germany, is non-existent.

In addition to brain-death, some European Countries also accept donation after cardiac death (NHBD) for donation. In this case circulatory death is considered sufficient. While in the past NHB-donors were only acceptable for tissues (i.e., cornea, skin, bone, heart valves etc.), recent advances in medicine made it possible to recover kidneys, livers or lungs from humans following circulatory arrest.

The modified Maastricht Classification of donor after circulatory death defines 4 settings which vary regarding the circumstances of circulatory arrest and the potential use of organs [5]:

European legislation varies considerably regarding NHBD: Whereas in the UK in 2018 donation after cardiac arrest accounted for 40% of all deceased donation, it is strictly forbidden in Germany. While Italy quires a no-touch period of at least 20 min before organs can be recovered, most other countries accept 5 min as sufficient [6].

There are manifold reasons why NHBD transplantation is disputed. This includes the administration of drugs which do not benefit the donor, the risk to end resuscitation too early in order to retrieve organs, the active withdrawal of life-support, and potential harm to the dying person who might experience pain given that the brain is still functioning. In the case of Maastricht category II, it is necessary to perform cannulation and perfusion of a conserving liquid in order to preserve organs. This is done in high urgency, most likely before the donor's next-of-kin can be asked for the assumed consent. Category III asks for an active withdrawal of treatment which will cause death. This can only be acceptable if the decisions regarding non-survivability of the health condition is correct and any further treatment will be futile. Decisions must be made in the best interest of the patient regardless the potential of organ retrieval. In any case, the definition of death based on the time of cardio-circulatory arrest appears arbitrary as the Institute of Medicine in 2000 [7] concluded: "existing empirical data cannot confirm or disprove a specific interval at which the cessation of cardiopulmonary function becomes irreversible." Additionally, continuation of cardio-pulmonary-resuscitation can potentially restore cardiocirculatory activity even after hours, unless brain death has occurred. Finally, the need for high-end intensive care to preserve organs for donation may violate a person's wish for an end-of-life-care without high-tech medicine, particularly if defined by a Do-Not-Resuscitate-order.

Independently of the type of deceased donor donation European legislations vary with regards to who is considered to be an organ donor. The crucial difference is the type of consent that is required. In the case of an opt-in system explicit consent is required. This means that the potential donor has declared his/her wish to donate during life-time. If the potential donor's wish is not documented, the next-of-kin is asked for informed consent assuming the potential donor's will. Nowadays most European countries operate on an opt-out system which is based on presumed consent. In this case anyone fulfilling the requirements for organ donation is considered a donor unless they have explicitly expressed their unwillingness to donate during life-time (dissent solution). The biggest challenge to the latter is a potential to undermine autonomy and to force a decision. While proponents of this approach claim that anyone is free of choice to opt out, opponents argue that the decision to (not) donate is not a dichotomous choice. Rather,

there is a need for a third option which leaves room for the potential donor to delegate the decision to family members or a trusted person.

In 2021, Eurotransplant accounted for 55 deceased donors younger than 16 years which represents 2.9% of all deceased donors in the Eurotransplant countries [8]. The UK, which allows for brain-stem-death as valid criterion in adults excludes children younger 2 months of age from donation as it is considered rarely possible or even impossible to confidently diagnose death as a result of cessation of brain-stem reflexes in this age [9]. While infant donation thus is not possible in the UK, the import of infant organs from other countries is legally and socially accepted.

In countries with an opt-in system paediatric donation is different from adult donation to the extent that the parent or legal guardian always has to authorise the donation, irrespective the deceased minor's opinion. However, the minimum age required to declare one's intention varies considerably and can be as low as 12 years (NHS Scotland). In countries with an opt-out system, there is uncertainty how this can be applied to children without incapacitating the parents. In most cases the regulations are thus suspended and do not apply to minors and adults who lack the capacity to understand the implications; again, the legal guardian's consent is required.

Although parental consent is legally requested in case of paediatric donation, it needs to be questioned how informed such consent can be under the given circumstances. Little is known about how organ donation might conflict with parental expectations. Particularly, if a child's death does not occur suddenly in the context of an accident but comes gradually due to a progressive life-limiting condition parents frequently wish for the child to stay at home or to be hold when death occurs. This conflicts with the need for high-end intensive care necessary for organ recovery. Finally, one needs to ask whether merely raising the question of donation may cause further harm to the bereaved ones if not placed appropriately. This might be particularly the case, when parents find themselves in the stress-field of weighing the own and their child's assumed needs against the societal needs and perceived moral obligations.

Data show that parents of a minor decide differently than relatives of potential adult donors: In 2018 the NHS Blood and Transplant reports a consent in 48% of the cases of minor donors as compared to the average consent rate of 66% across all ages.

In any case, parents are approached in the moment of utmost tragedy and possibly largest emotional defencelessness in order to make an undirected gift to help some unknown other. Bennett et al. [10] report that clinicians fail to refer patients to the relevant donation organisation in 23% of all withdrawal-of-therapy cases. Numbers were found to be lowest in children age 1 month and younger with a non-referral rate of 39%. While Hawkins et al. [11] identify medical reasons such as perceived medical unsuitability, it is also reported that medical staff feels unsure about if and how to approach the relevant families [12]. It is disputable whether such structural barriers to donation are acceptable, given that they do not only deny a family the chance to donate but also might deny organs to patients on the waiting list.

THE CHILD AS AN ORGAN RECIPIENT

Since the first solid organ transplantations to children in the 1960s [13–15] paediatric transplantation medicine has come a long way. The transplantation of kidney, liver, heart, and lung has become a routine procedure to save and prolong lives of children with terminal organ failure even in infancy.

Legally, there are no clear restrictions as to under which circumstance a child may or may not receive a vital organ. Technically, there are some constraints based on the anatomic conditions. Questions however arise frequently in terms of.

- the child's ability and necessity to at least assent to transplantation and the related therapeutic procedures,
- the justifiability of organ transplantation in children with severe mental disabilities or crippling conditions where transplantation may result in extended suffering,
- the necessity of a good enough social and/or familiar support.

The need to assent becomes relevant with age. While most policies require paediatric patients to come of age in order to express their free will to most medical procedures, organ transplantation is different to the extent that it asks maximum commitment of the transplant recipient. If a young person mentally rejects the organ, non-adherent behaviour and subsequent biological rejection of the organ become more likely. Forcing a child into transplantation without the ability to secure consequent maintenance treatment means to potentially withhold an organ from someone who might have been more ready to accept it.

However: when is a child old enough to encompass the consequences of transplantation or its refusal and what happens if a child's wish conflicts with a child's wellbeing? Claiming that a child's decision may not be in the child's best interest asks for who is to define the best interest. Not only that "best interest" is a vague construct, it is susceptible to the bias and prejudice of the person interpreting this construct.

One of the most prominent cases on child decision-making in recent history is the one of Hannah Jones who at the age of 13 years denied heart transplantation. Hannah had suffered leukaemia at 4 years of age and subsequently developed severe cardiomyopathy as a complication to chemotherapy. As a teenager she decided that she no longer had the strength to fight her health conditions and rather wanted to spend her limited life-time outside hospitals and aggressive treatment. While her parents accepted her wish the medical team did not. Consequently, the case was meant to be taken to High Court aiming to define best interest and the acceptability of Hannah's wish. Legal actions were however dropped, after a member of the local child protection team advised the primary care trust that Hanna was competent to make her decision.

The United Nations Convention on the Rights of the Child clearly state in article 12 that:

"States Parties shall assure to the child who is capable of forming his or her own views the right to express those views freely in all matters affecting the child, the views of the child being given due weight in accordance with the age and maturity of the child."

Thus the question is not whether a child is old enough but capable of forming an own view and understanding the consequences. Above all this requires a constant dialogue between health care providers, patient, and family in order to hear the wishes, understand the needs, answer questions, and judge on the relevant capacities.

The justifiability of transplantation in multiply disabled children is highly debated and there is no consent across European approaches. While general disability-based discrimination is forbidden across Europe, reasons to deny access to transplantation include a potentially reduced life-expectancy, a lack of improvement in terms of quality of life, or a lacking ability to comply with the complex treatment following transplantation [16, 17].

Research shows that allograft function and survival of children with severe developmental delay but no other conflicting health condition does not significantly differ from the outcome of other recipients. If at all, adherence appears to be better and immunosuppressant trough-levels more stable in paediatric organ recipients with developmental delay. This is attributed to the continuous and consequent care provided by the parents or relevant custodian as well as to the lack of pubertal opposition. Thus, a possible decision against donation in case of mental disability is not based on possible medical outcome but on assumed concerns with regards to psycho-social management [16, 18].

Clinical practice differs in case of comorbidities in addition to developmental difficulties, particularly when the comorbidity causes uncontrollable suffering or significantly shortens life-expectancy [19]. Overall transplant-results, policies, and medical approaches vary considerably. In this light, individualised assessments which respect the patients' and relatives' wishes, and include an external review of other experts in the field become indispensable. The aim needs to be to balance the benefits and burdens on a case to case base [20].

Social support is essential in paediatric organ donation. Allograft maintenance asks for frequent visits to specialist doctors, home assessments of bodily functions, and a strict, life-long daily medication regime. Additionally, transplantation may interfere with developmental experiences and alienate a child from relevant peers both in appearance as well as in behaviour and psychosocial development. In cases where social support is lacking and follow-up care cannot be secured the success of transplantation is at significant risk. Facing the overall lack of available donor organs it is disputable, whether an organ can be provided to a patient with a poor outlook. Concurrent obligations occur with the potential recipient on the one side, and other candidates on the waiting list on the other side.

As in the case of developmental delay or comorbidities, lacking social support is not a strict exclusion-criterion to transplantation but demands careful consideration. Given the complexity of the situation Dionne et al. [21] recommend accounting for contextual and societal factors when considering organ donation. While it is comprehensible to provide a scarce good such as an organ only under the provision of a good perspective, social support requirements may reinforce social injustice further disadvantaging children from complex social context. Thus one might argue that the provision of sufficient social support in such cases needs to be improved instead of excluding the already marginalised.

In summary organ donation and transplantation are no straight forward processes by the means of psychology, sociology or ethics. Some challenges may only be approachable on an individual base and ask for thorough frameworks that facilitate just decision making. Other challenges may be addressed by legal guidelines however - as indicated - jurisdiction can vary considerably even in what is thought a common European socio-cultural background. Broadening the discussion to other geographical, cultural, religious or societal contexts might add to the complexity of the topic by adding further ethical ideas and legal frameworks, e.g., the acceptance of organ trading, and drawing a widely heterogeneous picture.

Advances in medicine have the potential to raise chances but with increasing options also more challenges may occur. An interdisciplinary discourse is needed to tackle the issues addressed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JP is the sole author of this manuscript, researched the literature and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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MEETING REPORT
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Ethics of Early Clinical Trials of Bio-Artificial Organs

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Regenerative medicine is the new frontier in the field of organ transplantation. Research groups around the world are using regenerative medicine technologies to develop bio-artificial organs for transplantation into human patients. While most of this research is still at the preclinical stage, bio-artificial organ technologies are gearing up for first-in-human clinical trials in the not-too-distant future. What are the ethical conditions under which early-phase clinical research of bio-artificial organs can be conducted safely and responsibly? What lessons can be learned from prior experiences with early-phase clinical trials in adjacent fields of research? This is a Meeting Report of an online international workshop organised in the context of the Horizon 2020-funded VANGUARD project, which is developing a bio-artificial pancreas for the treatment of patients with type 1 diabetes.

Keywords: regenerative medicine, tissue engineering, research ethics, first-in-human clinical trials, bio-artificial organs, ethics

INTRODUCTION

Although the advancement of medicine calls for clinical research on innovative medical treatments and technologies, early-phase clinical trials are known to be risky and ethically challenging. First-in-human trials especially are associated with serious—predictable and unpredictable—risks for research participants. To justify exposure of volunteers to the risks and burdens of participation in early-phase clinical trials, the research and the resulting intervention must have clear scientific and societal value (1). Today, research groups around the world are developing new applications of regenerative medicine in pre-clinical research settings for the purposes of organ transplantation. Tissue engineering, 3D bio-printing, and organoid technologies are used to generate bio-artificial organs for transplantation into human recipients (2). These technologies might save or improve patients' lives, and become part of a solution to the problem of donor organ shortage. In the not-too-distant future, they are expected to be ready to be tested in human research participants. It will be challenging for researchers and research ethics committees (RECs) to determine when, and under what conditions, these applications will be ready to make the leap to early-phase clinical research, in a safe and responsible manner.

On 3rd February 2022 an online meeting was held to bring together ethicists, researchers, and clinicians to discuss the ethics of early-phase clinical trials in regenerative medicine in transplantation. The meeting was organised in the context of the VANGUARD project, a European research project which aims to generate a vascularized and immune-protected bio-artificial pancreas that can be transplanted into non-immunosuppressed type 1 diabetes patients.[1] This project is one of 14 projects funded by the European Commission Horizon 2020 programme "Regenerative medicine: from new

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[1]VANGUARD. New generation cell therapy: bioartificial pancreas to cure type 1 diabetes. https://vanguard-project.eu/ (Accessed 4 May 2022).

insights to new applications".[2] Representatives of other recipients of grants from this call were invited to attend the meeting. The meeting was announced on the website and in newsletters of the European Society for Organ Transplantation (ESOT), and open to the public. In total, 102 people registered, and 74 people attended the meeting. The meeting commenced with a keynote lecture by Jonathan Kimmelman, Professor of Biomedical Ethics and Social Sciences of Medicine at McGill University in Montreal, Canada, and was followed by three brief presentations on three bio-artificial organ technologies, and a panel discussion.

A THEORY OF—ETHICALLY RESPONSIBLE—CLINICAL TRANSLATION

Kimmelman laid out his theory of clinical translation in a lecture titled "How to think about the ethics (and the science) of first-in-human trials". Phase I clinical trials, he said, are among the most vexing challenges in medical research ethics. He brought to mind some of the numerous cases in which either fully or relatively healthy volunteers had died from participating in first-in-human clinical trials, including gene therapy trials in the late 1990s (3). Yet for the advancement of medicine, such trials must be launched.

Kimmelman's theory is as follows: all drugs, surgeries, vaccines, and devices are "born guilty"; they are poisons, toxins. In one of his papers, Kimmelman cites Paracelsus: "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous" (4). Only by learning to understand how these poisons can be used to target medical conditions in patients, safely and effectively, they can be transformed into technologies of clinical utility. It is by going through the process of clinical translation, that poisons are converted into putative therapeutic interventions.

The process of clinical translation takes time and effort. It consists of two steps. First, we must identify the configuration of materials, practices and beliefs—which Kimmelman calls the "intervention ensemble" (5)—that we must combine with a pharmacological agent or another type of medical technology, to unlock its clinical utility. This includes finding the optimal dosage, mode of delivery, timing and frequency of administration, but also, for instance, knowing what accompanying therapeutic regimen to administer (e.g., immune-suppression), what side effects to look out for, which patients with which comorbidities to exclude, or, in the case of bio-artificial organs, what materials to use, how to assemble or combine them, and who, how, where and how much of them to transplant. Early-phase clinical trials are focused mainly on building this intervention ensemble, on exploring and establishing the approximately optimal conditions in which the investigational treatment will have the desired effects without having the undesired side effects. Second, in later-phase clinical trials, the intervention ensemble must be evaluated rigorously, ideally within randomised controlled trials, in order to demonstrate sufficient efficacy and safety and obtain marketing approval by regulatory authorities. In our online meeting, the focus was on early-phase clinical research.

Kimmelman suggested that there are several moral premises that ought to underwrite clinical translation. First, those of us who are involved in clinical research should maximise "moral efficiency," that is, for every medical breakthrough, we should minimise welfare loss. Thus, we should minimise the number of patients that are exposed to the risks and harms of research participation. Second, we must ensure that we generate information that healthcare systems need to support the practice of efficient and cost-effective medicine. We need to know how to use and how not to use medical technologies. This also means that we must understand the relative or incremental value of a new technology as compared to other, existing therapeutic approaches. Third, we should acknowledge that clinical translation is not like a pipeline but more like a web (6), a dense network of collaboration among various stakeholders who must trust one another. For example, research participants should be able to trust researchers when "lending their bodies to research." Therefore, we must advance rules and practices that protect and maintain the stability of these networks. Kimmelman believes that we may not currently be meeting these moral requirements in full. He discussed three areas of concern: risk-benefit assessment, subject selection, and informed consent.

Risk-Benefit Assessment

When sponsors and researchers are considering to set up a clinical trial, and when research ethics committees (RECs) are evaluating a protocol for a clinical trial, an assessment must be made of the balance between risks and potential benefits associated with the trial. There are widespread but mistaken assumptions about risk-benefit assessment, according to Kimmelman. For instance, while it is generally assumed that sponsors would not initiate trials unless there were a good prospect of success, in reality, they may do so in the absence of such prospect. Also, it is assumed that regulatory authorities will make risk-benefit assessments before trials are launched. In practice, however, regulatory authorities defer to RECs for assuring that the "risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may be expected to result" from trials (7). Moreover, there is little guidance on how RECs should judge whether an intervention is promising enough to launch a clinical trial (8–10). At minimum, RECs require solid pre-clinical evidence, for instance, evidence that is confirmed in multiple relevant animal models. The International Society for Stem Cell Research (ISSCR) lists key design principles for pre-clinical studies for the generation of rigorous evidence to support decision-making about clinical trials (11).

Patient Selection

In selecting subjects for participating in early-phase clinical research, a balance must be sought between the dual aims of maximising moral efficiency and generating useful evidence. Patients with late-stage or refractory disease will have less to lose—but possibly also less to gain—than healthy volunteers or patients with more recent disease onset. Starting with patients who are more severely ill—and without satisfactory alternatives—helps to avoid dramatic outcomes, and therewith, crises of confidence, such as the crisis of confidence in the gene therapy field in the late 1990s (12). When designing early-phase clinical trials, researchers should consider the

[2]European Commission. Regenerative medicine: from new insights to new applications. https://cordis.europa.eu/programme/id/H2020_SC1-BHC-07-2019 (Accessed 4 May 2022).

effects of patient selection on the maintaining of trust and the stability of the collaborative networks needed for clinical translation.

Informed Consent

Most patients are taking part in early-phase clinical trials in the hope of gaining medical benefit, even though, as part of the informed consent process, they are informed that it is uncertain whether they will benefit. Kimmelman suggested that researchers should be more forthright to research participants, and explain to them that major benefits are "highly unlikely" (13), and that patients are "more likely to experience side effects than benefit medically." Patients should understand that by participating in early-phase clinical research, they contribute to welfare gain to society but are likely to experience welfare loss themselves.

BIOARTIFICIAL ORGAN TECHNOLOGIES: THREE EXAMPLES

Following the lecture, three examples were presented by junior researchers Ollala Iglesias García, Ary Marsee, and Dide de Jongh of bio-artificial organ technologies that are currently under pre-clinical development within the context of the aforementioned Horizon2020-call.

In the project BRAVE, led by researchers of the University of Navarra, regenerative medicine and 3D-bioprinting are combined with computational modelling to develop a biological ventricularassist device, which is meant to provide lifelong support to patients with ischemic heart disease. The researchers are aiming to bring the device "as close to the bedside in the shortest time possible".[3] The device consists of a 3D-printed microfibre scaffold seeded with human induced pluripotent stem cells, to be integrated in the patient's heart and restore cardiac function. Computational modelling is used to assess cardiac geometry and tissue mechanics, such that the design of the assist device can be tailored to the individual patient's heart.

Researchers of the project OrganTrans, which is coordinated by the Swiss Centre for Electronics and Microtechnology (CSEM), are building a platform for liver tissue engineering as a "disruptive alternative to donor organs" for treating patients with chronic end-stage liver diseases.[4] The platform uses stem cells that are derived from the patient's residual healthy liver tissue, which self-assemble and self-organize into liver organoids. Organoids are supported by 3D bio-printed scaffolding made up of synthetic hydrogel and vascular networks made using endothelial cell ink, to reconstruct functional liver tissue for transplantation into patients.

Finally, in VANGUARD, which is led by researchers at the University of Geneva, a bio-artificial pancreas is being developed for the treatment of type 1 diabetes.[5] The bioartificial organ is composed of islets of Langerhans from deceased donors, an extra-cellular matrix consisting of genome-edited human amniotic endothelial cells derived from donated placentas to protect islet cells against inflammatory and hypoxic damage and to accelerate engraftment, and patient-own blood outgrowth endothelial cells for vascularisation and immune-protection.

PANEL DISCUSSION

In the panel discussion, Kimmelman was joined by three senior representatives of the above projects, Manuel María Mazo Vega (BRAVE), Mariana Pacheco Blanco (OrganTrans) and Ekaterine Berishvili-Berney (VANGUARD), and Anne-Floor de Kanter of Utrecht University, who is pursuing a PhD in ethics of regenerative medicine. During the panel discussion, several ethical issues were raised in response to the presentations of the three new bio-artificial organ technologies currently under development. Four issues that are most relevant to early-phase clinical research on bio-artificial organs are briefly discussed below.

Are Bioartificial Organs Special?

The ethical issues arising in early-phase clinical research on bio-artificial organ technologies, it was generally agreed by the panel, are not entirely novel or unique. Lessons can be learned from prior experiences in other areas in medicine, including other applications of regenerative medicine and gene therapy (14). However, there are not only similarities, but also differences, between the transplantation of bioartificial organs and, for instance, the administration of pharmaceutical agents or cell and gene therapies, or the implantation of (non-biological) medical devices. Firstly, transplantation of bio-artificial organs requires surgery, that is, making skin incisions, entering the body, and making changes to the anatomy of the patient. Thus, it is invasive—more so than pharmaceutical agents, which may be taken orally, or cell and gene therapy, which may be injected or infused. Secondly, as the "product" is composed of biological materials, and "metabolically active" (15), it may integrate with the body of the recipient and develop within the body over time (16). Consequently, the treatment is likely to be irreversible (15)—more so than treatment using non-biological medical devices, which can be removed integrally. Thirdly, bio-artificial organs are complex: they may be composed of biological materials derived from various sources. To develop the bio-artificial pancreas, for instance, researchers need access to biological materials derived from deceased donors and new mothers—raising ethical issues known from the field of organ transplantation generally, including informed consent from donors and the crucial importance of maintaining—and deserving—public trust. Bio-artificial organs are complex also in the sense that—in contrast to cell and gene therapies—they are organised in three-dimensional space. There is little experience yet with exploring the intervention ensemble in terms of requirements for the three-dimensional organisation of tissues.

Finally, what makes the coupling of regenerative medicine with transplant medicine potentially revolutionary is its aspect of "personalisation": by using patients' own cells to generate organs

[3]BRAVE. A therapy for life to restore the patient's heart function. https://projectbrave.eu/(Accessed 4 May 2022).

[4]OrganTrans. Controlled organoids transplantation as enabler for regenerative medicine translation. https://organtrans.eu/ (Accessed 4 May 2022).

[5]VANGUARD. New generation cell therapy: bioartificial pancreas to cure type 1 diabetes. https://vanguard-project.eu/ (Accessed 4 May 2022).

for transplantation, the major hurdle of the need for patients to take lifelong immune-suppressive medications and the associated long-term complications, can be overcome (2). As each bio-artificial organ is personalised, however, each "product" is different. It cannot be "constructed in uniform batches according to well defined standards to the same extent as medical devices or medicinal products" (16). This renders the generation of evidence of the product's safety and efficacy more difficult. Personalised technologies may need to be evaluated—and regulated—not as medicines, but as health services (17). In a services-based regulatory model, it would not be the product, but rather the service that is evaluated and approved for use. In OrganTrans, for instance, it would not be the personalised liver organoids that are approved for use, but the platform for liver tissue engineering—not the bio-artificial organ itself, but the methods used for its creation.

It will be clear to the reader that none of these characteristics—invasiveness, integration and irreversibility, complexity, and personalisation—are unique to bio-artificial organs. In fact, most Advanced Therapy Medicinal Products (ATMPs), a category of "medicines" for the European Medicines Agency (EMA) that includes gene therapy, somatic-cell therapy, and tissue-engineered medicines, will have one or more of these characteristics.[6] What is new about bio-artificial organs for transplantation, is that these characteristics are combined in one technology, and that they accumulate and may interact, thus heightening the ethical sensitivity of their application.

Social Value

In the panel discussion, there was a strong focus on the social value of research and development of bio-artificial organ technologies. This is not surprising, as three of the panellists were involved in research projects funded by a Horizon 2020 programme that is meant to stimulate clinical translation of regenerative medicine technologies.[7] Panellists were intent not only on advancing science, but first and foremost on developing technologies that may benefit patients. Bio-artificial technologies should lead to improvements in health or well-being of future patients.

Whether new technologies succeed in contributing to health or well-being depends not only on the safety and efficacy of the technology itself, but also on the scientific and societal context in which the technology is being developed. The social value of a new technology is defined as its clinical benefit to future patients relative to alternatives that may already be approved for marketing (18), or that are being developed in parallel, and may become available in the near future. For instance, in recent years, due to significant advances in continuous glucose monitoring and continuous subcutaneous insulin infusion technologies, treatment of type 1 diabetes has become more effective—also in patients at higher risk of hypoglycaemia or with hypoglycaemia unawareness—and less burdensome (19). Also, there are new classes of immunotherapeutic medicines, such as anti-CD3 antibodies, which seems to delay progression to type 1 diabetes in high-risk research participants (20), and possibly to halt the disease in newly diagnosed patients, although the evidence is not convincing (21). While these technological advances will have improved the treatment of many type 1 diabetes patients, there may be subgroups of patients who cannot be adequately treated using pharmaceuticals or automated sensor-pump combinations, and who thus qualify for more invasive interventions, ranging from conventional islet or pancreas transplantation to, possibly, bio-artificial organ technologies. Adding social value thus entails identifying the groups of patients who will benefit the most.

Further, social value can only be realized when new technologies actually reach those groups of patients who will benefit the most. Bio-artificial organs might help to reduce the global disease burden associated with ischemic heart disease, end-stage liver disease, or type 1 diabetes, but only if they can be accessed by patients who need them, including patients in developing countries. Tissue engineering and 3D-bioprinting technologies, however, require highly specialised personnel, equipment, information technology, and laboratory facilities, which may not in place everywhere in the world. Researchers and developers in developed countries should think about how bio-artificial organs—or rather the technologies used to generate them—can be distributed to other geographical areas.

Accessibility implies not only availability, but also affordability. The prices of bio-artificial organs are likely to be high (22). This may not be due to material costs: the bio-artificial pancreas, for instance, is composed, among other things, of patient-derived material, which should be free, and placenta, which is medical waste, and can be procured at low cost. However, clinical development of bio-artificial organ technologies will require major financial investments that are beyond the reach of research groups themselves (23). Manufacturers need to recover the costs of development and reward their investors within the—often limited—timeframe of market exclusivity (24), thus driving up the prices of new medical technologies. Over time, however, as patents expire and monopolies are rescinded, prices may decrease.

Randomised Controlled Trials

While the focus of our meeting was on early-phase clinical research, it was felt that researchers should already be anticipating ethical issues that will arise in later-stage clinical research, in which rigorous evidence must be generated of the clinical utility of bio-artificial organ technologies. To optimize the scientific validity of later-stage research, researchers should ideally conduct randomised controlled trials.

However, in the field of surgery, innovation has traditionally occurred mainly through gradual improvements on modi operandi in operating theatres, and it has not been customary for surgeons to conduct randomised controlled trials (25). Also, there is much less of a paradigm for funding clinical trials in surgery than there is for funding drug trials. Moreover, the design requirements for late-stage trials of bio-artificial organs are not clear, notably, in relation to the choice of a comparator. To ensure double blinding, the comparator should ideally be sham (or placebo) surgery. Patients are known to respond strongly to placebo in clinical trials of (minimally invasive) surgery (26).

[6]European Medicines Agency (EMA). Advanced therapy medicinal products: overview. https://www.ema.europa.eu/en/human-regulatory/overview/advanced-therapy-medicinal-products-overview (Accessed 4 May 2022).

[7]European Commission. Regenerative medicine: from new insights to new applications. https://cordis.europa.eu/programme/id/H2020_SC1-BHC-07-2019 (Accessed 4 May 2022).

Sham surgery, however, inherently implies physical harm and risks (27), and is not commonly used (28). It will likely be ethically acceptable to expose research participants to sham surgery only in the context of clinical equipoise (29), when the harms and risks of sham surgery may be deemed justifiable (27). This will probably not be the case in transplantation trials of vital bio-artificial organs, such as livers or hearts, as withholding standard of care may lead to severe illness or even death.

Panellists indicated that RECs had an important role to play in assessing the risks and benefits of clinical trials of bio-artificial organs, helping guide selection of research participants (30), evaluating and improving upon study design, and overseeing the adequacy of informed consent models. Panellists expressed the concern that RECs are currently not fully equipped for their role in evaluating protocols for clinical trials of regenerative medicine applications in organ transplantation, and that RECs must be strengthened, for instance through attracting and including expertise in regenerative medicine and organ transplantation.

Public Dialogue

Finally, panellists believed that researchers should communicate carefully about bio-artificial organs with patients and lay audience, without fuelling hype or crushing hope. Over the years, scientific advances in regenerative medicine have been surrounded by much hype and great expectations (31). After news about regenerative medicine technologies is reported in the media, panellists report, patients tend to ask their clinicians if and when the new treatment will be available to them, even though it may still take years—or even decades—for the treatment to be implemented in the clinic. It is important for researchers to stress that bio-artificial organ technologies are still being investigated in pre-clinical research settings, and to do so in language that is comprehensible to the public. Public dialogue is seen as serving two aims: firstly, to build and maintain (or even restore) trust in bio-artificial organ technology. This is necessary, as earlier research on bio-artificial organs in transplantation has raised some negative attention and scientific and clinical controversy (24). Secondly, clinical research can only be conducted as long as patients are willing to participate in research and there is general support within societies for the scientific endeavour. Participants in trials of bio-artificial organs may need to be followed up over long periods of time to monitor long-term adverse effects or complications, which requires long-term commitment. Researchers must therefore enter into long-term trust relationships with research participants. Representatives of the three European projects report that they have included patient organizations in their advisory boards, to ensure that patient voices are heard and used to help guide research questions, research design, and knowledge utilisation.

CONCLUDING REMARKS

Early-phase clinical transplantation trials of bio-artificial organs raise ethical issues in relation to risk-benefit assessment, patient selection, and informed consent. Although these issues are not new, clinical translation of bio-artificial organ technologies does present a new constellation of ethical challenges not found in other areas of clinical research. There are several ethical challenges that must either be thought through or acted upon. Researchers should think carefully about trial design, patient selection, and informed consent. To ensure that patients provide truly informed consent for early-phase clinical trials, the potential benefits of research participation should not be overstated. Transparent communication about risks and benefits helps to restore and maintain the trust of patients and publics alike. Clinical translation of rapidly advancing regenerative medicine technologies to the field of organ transplantation may be challenging, high-risk, laborious, and of uncertain commercial value, but without the effort, patients in need of organ replacement therapy will not be able to reap the fruits of these advancements. Researchers and manufacturers may need to think about ways of making their products or their technologies accessible to patient populations around the world, which might require the involvement of multi-stakeholder networks. Researchers must engage patient communities and the general public in clinical research to ensure that new bio-artificial organ technologies are aligned with patients' needs and preferences, and that societal concerns are adequately addressed. Finally, research ethics committees must be strengthened by including specific expertise in regenerative medicine and organ transplantation, so that they can help ensure that early-phase clinical trials of bio-artificial organs are conducted in a safe and ethically responsible manner.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Propensity Score and Instrumental Variable Techniques in Observational Transplantation Studies: An Overview and Worked Example Relating to Pre-Transplant Cardiac Screening

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Inferring causality from observational studies is difficult due to inherent differences in patient characteristics between treated and untreated groups. The randomised controlled trial is the gold standard study design as the random allocation of individuals to treatment and control arms should result in an equal distribution of known and unknown prognostic factors at baseline. However, it is not always ethically or practically possible to perform such a study in the field of transplantation. Propensity score and instrumental variable techniques have theoretical advantages over conventional multivariable regression methods and are increasingly being used within observational studies to reduce the risk of confounding bias. An understanding of these techniques is required to critically appraise the literature. We provide an overview of propensity score and instrumental variable techniques for transplant clinicians, describing their principles, assumptions, strengths, and weaknesses. We discuss the different patient populations included in analyses and how to interpret results. We illustrate these points using data from the Access to Transplant and Transplant Outcome Measures study examining the association between pre-transplant cardiac screening in kidney transplant recipients and post-transplant cardiac events.

Keywords: observational studies, causal inference, confounding, propensity score, instrumental variable

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Abbreviations: ATE, average treatment effect; ATT, average treatment effect on the treated; ATTOM, Access to Transplant and Transplant Outcome Measures Study; CI, confidence interval; HR, Hazard ratio; IMD, index of multiple deprivation; IV, instrumental variable; IPW, inverse probability weighting (using propensity scores); IQR, interquartile range; LATE, local average treatment effect; MACE, major adverse cardiac event; PS, propensity score; RCT, randomised controlled trial; SD, standard deviation.

INTRODUCTION

Randomised controlled trials (RCTs) are the gold standard study design for determining causal associations between clinical interventions and outcomes (1, 2). In transplantation, RCTs have shaped immunosuppression practice (3, 4), informed the management of cardiovascular risk (5), and guided infection prophylaxis (6). By randomly assigning individuals to treatment or control groups, two populations with similar characteristics are created, meaning differences in outcome likely result from differences in treatment.

In some situations RCTs are inappropriate or impractical, for example if there are ethical concerns or excessive costs (7). In transplantation, the small numbers of recipients compared to general populations can make achieving required sample sizes for small treatment effects challenging. Further, standard practice (often used as the comparator in RCTs) varies between centres, the time between waitlisting and transplantation may necessitate long follow up, and the lack of control over transplant timing can put pressure on the informed consent process (8). If individuals recruited to trials are healthier or sicker than the overall population, results may also not be generalisable.

When RCTs are impractical, observational data can inform practice. However, as the exposure is not randomly assigned, differences in case-mix can occur between exposed and unexposed groups. This generates confounding bias: a situation where the treatment and outcome have a common cause, resulting in a lack of exchangeability between treated and untreated groups. This can result in the association between treatment and outcome differing from the true effect measure (9). Confounders are identified using causal diagrams that depict potential pathways between treatment and outcome (10, 11). However, only known confounders can be adjusted for in multivariable regression models and unmeasured confounding can persist. Further, multivariable models may be overfitted if the number of covariates is large relative to the number of outcome events. To minimise confounding and improve the validity of causal inference from observational studies, propensity score and instrumental variable analyses are increasingly being used (12). These techniques do not minimise other forms of bias that make emulating an RCT from observational data challenging (13, 14), so whilst they have advantages over traditional methods they don't solve all issues with observational studies.

In kidney transplantation, there is no contemporary RCT examining the utility of screening for asymptomatic coronary artery disease prior to transplant listing. Screening is frequently performed but there is variation in practice between centres, likely influenced by local opinion (15). An RCT to examine if screening before transplant listing reduces post-transplant cardiac events would be challenging (16). Individuals would need to be identified at the point of screening, far in advance of transplantation. The low cardiac event rate would necessitate a large study population and high recruitment rates (17) which may be difficult to achieve if there is anxiety around recruiting patients, especially higher-risk individuals, meaning a study may be underpowered or not have generalisable results.

Given these challenges, we use observational data from the Access to Transplant and Transplant Outcome Measures (ATTOM) study (18) on pre-transplant coronary artery disease screening to describe the principles and assumptions of propensity score matching, inverse probability weighting, and instrumental variable analyses. We illustrate how these techniques are performed and interpreted and compare their results.

THE PROPENSITY SCORE

The propensity score (PS) refers to the predicted probability of an individual receiving a treatment by collapsing measured confounders into a single value, ranging from 0: no probability to 1: absolute probability of them receiving the treatment of interest (19).

The PS is typically estimated using a logistic regression model specifying the exposure as the dependent variable and measured confounders as independent variables. Measured confounders are those known at baseline that are predictive of both treatment and outcome. Variables that are predictive of treatment but not outcome should not be included as this may increase the variance of the estimated exposure effect (20). Confounders should not be chosen based on a statistically significant association with the exposure but based on prior knowledge and clinical judgement as formalised and summarised in a directed acyclic graph (10, 11, 20).

Once the model has been created, each individual's PS is generated based on their measured confounders. The score reflects their propensity for receiving the treatment, not whether this actually happened. Two balanced groups with a similar distribution of PS can then be created using matching or weighting techniques. Key features of PS analyses are shown in **Table 1**, and a detailed description of PS assumptions is in **Supplementary Table S1**.

Propensity Score Matching

In propensity score matching, treated and untreated individuals are "paired" based on their PS (**Figure 1**). Depending on the prevalence of the treatment, individuals can be matched on a 1:1 or 1:many basis. Nearest-neighbour matching identifies pairs with the closest PS. In "matching without replacement," an individual can only be matched once before being removed from the matching pool. This means pairs generated later in the matching process may have larger differences in their PS (21). Matching with replacement allows control patients to be matched to more than one treated patient. An alternative to nearest-neighbour matching is optimal matching, which minimises the difference in PS between pairs across the whole population. In large populations, nearest-neighbour and optimal matching give similar results (22). Both techniques include a "caliper" to avoid the inclusion of poorly matched pairs. This specifies the maximum acceptable difference in PS for a pair to match, generally accepted as 0.2 times the standard deviation of the logit of the PS to provide the optimal balance of matching quantity and quality (23, 24). Individuals who are unmatched are excluded from further

TABLE 1 | Comparison of propensity score and instrumental variable techniques.

	Propensity score matching	Propensity score weighting	Instrumental variable
Assumptions	Positivity Exchangeability/ignorability Consistency	Positivity Exchangeability/ignorability Consistency	Relevance assumption Exclusion restriction Independence assumption Monotonicity or homogeneity
Unmeasured confounding	Not eliminated	Not eliminated	Eliminated/reduced
Study application	Smaller studies or low event rate	Smaller studies or low event rate	Large multi-centre studies
Analysis and interpretation	Patient-level	Patient-level	Instrument level e.g. centre, physician
Causal effect	Average treatment effect on the treated	Average treatment effect	Average treatment effect or local average treatment effect depending on assumptions
Advantages	Simple to analyse and interpret	Retains data from all patients	Does not require modelling on confounders, minimises unmeasured confounding
Disadvantages	Exclusion of unmatched patients means results may not be applicable to whole study population	Results can be unstable if extreme weights are present	Analysis assumptions difficult to test Challenging to find suitable instrument

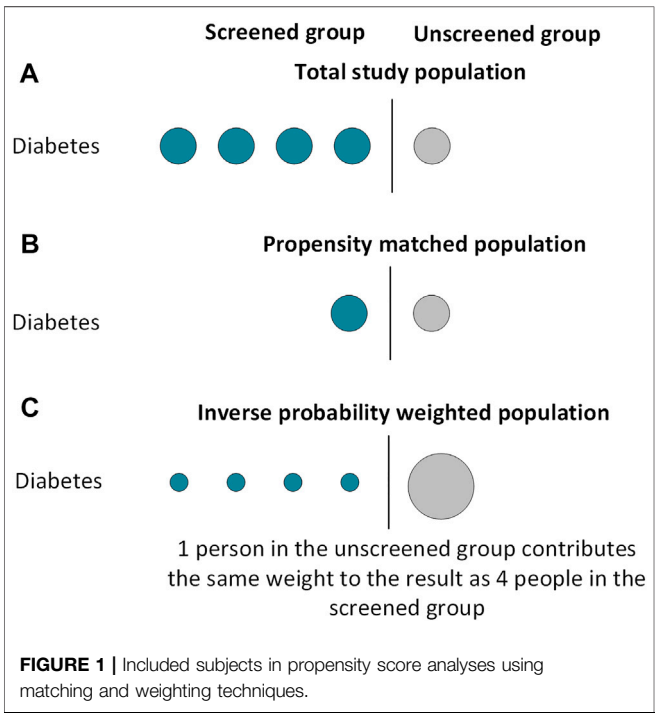


FIGURE 1 | Included subjects in propensity score analyses using matching and weighting techniques.

analyses. In practice, as it isn't always clear what the "ideal" statistical method is, performing analyses using a number of these techniques can help assess how sensitive results are to method specification.

The matching technique should create two groups with an equal distribution of measured covariates (**Figure 1**). The balance of covariates between groups can be examined using standardised differences, calculated by dividing the difference in proportion (for binary variables) or sample mean (for continuous variables) by the pooled standard deviation. There is no definite consensus on an acceptable standardised difference; a value below 0.1–0.2 is generally accepted (25). Visual diagnostic tools can also be used to examine covariate balance, as demonstrated in our worked example (26). Once the groups are balanced, they can be compared using standard regression analyses. These analyses can be univariable or multivariable, with the multivariable technique including the variables used to generate the PS. A multivariable model compensates for imperfect covariate balance and, if specified correctly, minimises the risk of a biased estimator (27). However multivariable models lose the advantage of having only 1 covariate in the final model, so could be overfitted if the number of covariates is large relative to the number of outcome events. Further, in the event of misspecification of the PS model, this method could increase bias (28).

Inverse Probability Weighting Using Propensity Scores

Inverse probability weighting (IPW, also known as propensity score weighting) creates a pseudo-population informed by all patients with a balanced distribution of measured covariates between groups (29). By doing so, IPW avoids excluding individuals from analyses and may result in better covariate balance than PS matching (30).

Each individual is assigned a "weight" depending on their measured covariates and the treatment they receive. For individuals who receive treatment, their weight is 1/PS, whilst individuals who do not receive treatment have a weight of 1/(1-PS). This means individuals receiving an "unexpected" treatment contribute larger weights to the analysis than individuals receiving their "expected" treatment (**Figure 1**). Each crude weight is greater than or equal to 1. If some patients have large weights, this can make results unstable. To minimise this risk, weights are frequently "stabilised" before further analysis. This is relevant if a multivariable regression model is being used; stabilisation does not affect univariable models which contain only the treatment indicator (31). Stabilisation involves multiplying the weight by the proportion of exposed patients for the treated group, and by the proportion of unexposed patients in the untreated group (32). Once stabilised, the mean

weight for the population should be approximately equal to 1. A regression analysis where each individual is weighted by their inverse probability of receiving treatment can then be performed. As with PS matched analyses, this regression can be univariable or multivariable. The same caveats of the multivariable model in PS matched methodology apply to IPW analyses.

Strengths of Propensity Score Analyses

PS techniques have several advantages over conventional multivariable regression models. First, conventional multivariable Cox models require around 10 events per covariate to produce a stable estimate, and combining covariates into a single PS is useful when the population is small, event rate is low, or number of covariates is large (33, 34, 35).

Second, in conventional regression models the treated and untreated groups can systematically differ. This means estimating the effect of treatment on a patient, who would never have been considered for treatment in real life, can be unreliable as the estimation is based on model extrapolations beyond the support of the data. PS matched analyses refer to only those patients who could feasibly exist in either the "treated" or "untreated" group. Whilst PS matched analyses can therefore provide improved real-world results, identifying the population to whom the results are applicable to can be challenging, especially where there is variation in treatment practice between centres.

Third, PS models highlight the limitations within which results should be interpreted. If a large proportion of individuals are unmatched in PS matched analyses, or there are patients with large PS weights in IPW analyses, this signifies poor overlap in covariate distributions between treated and untreated groups and means the likelihood of individuals being allocated to either treatment group is low. As traditional multivariable models extrapolate results to individuals in under-represented covariate strata, this could lead to bias in effect estimates. PS methods can alert researchers to these issues and highlight the limits within which comparisons of treatment options can be made.

Limitations of Propensity Score Analyses

PS assumptions (exchangeability, positivity, and consistency) are described in **Supplementary Table S1**, and it may be difficult to prove these assumptions hold. If the treatment is rare, there may be insufficient data to generate the PS. Further, the PS only encompasses measured confounders. Confounders that are unknown, poorly recorded, or not measurable cannot be controlled for and may not be balanced between groups, leading to unmeasured confounding bias.

In PS matching, unmatched individuals are "lost," reducing the study size. Individuals with the highest and lowest PS (the "always treated" and "never treated") are less likely to be matched and are under-represented in the regression models. Whilst there is no "required" proportion of patients that must be matched, the causal effect is only applicable to matched patients, not the whole study population.

In IPW, data from all participants is retained. However, if individuals contribute large weights to analyses, results may be unstable. There is no consensus on what a "large" weight is, and weight stabilisation is often used to minimise this risk. Some advocate truncating weights to a maximum of 10 for more precise estimates, (36) but this may re-introduce some of the confounding that the method aims to remove.

For interested readers, more detailed information on propensity scores can be found at the following references (9, 37, 38, 39).

INSTRUMENTAL VARIABLE ANALYSIS

Instrumental variable (IV) analyses were developed for economic studies and subsequently adopted in the medical setting. They aim to minimise confounding by indication by examining individuals based on an "instrumental variable": a variable that influences treatment and has no confounder with the outcome. This allows the IV to be capitalised on as a type of natural randomisation (40). Individuals are analysed according to the instrument rather than by the treatment they receive akin to an intention to treat analysis, whereby individuals in RCTs are analysed according to their randomisation group rather than by received treatment. Their advantage is they do not assume an absence of unmeasured confounders to the treatment-outcome relationship, allowing an independent treatment effect to be estimated as in an RCT. Key features are shown in **Table 1**.

To perform IV analyses, the IV is recommended to meet key assumptions (**Figure 2A**): (41).

(1) It must be strongly associated with the exposure (relevance assumption).
(2) It must only affect outcome through its association with the exposure (exclusion restriction).
(3) There must be no unmeasured confounders to the instrumental variable and the outcome (independence assumption).
(4) A fourth assumption is either that of effect homogeneity or effect monotonicity. Effect homogeneity states that the treatment should have a constant effect on the outcome across all individuals. In effect monotonicity, no patients should receive the opposite treatment to expected at all levels of the instrument i.e., at both the instrument to which they were assigned and instrument(s) to which they were not assigned (so called "defier" patients; **Supplementary Figure S1**) (9, 42). Identifying which "compliance type" a patient belongs to however is impossible. Further, when instruments are multi-categorical or preference-based, even defining compliance types (and thus effect monotonicity) is complex and can limit the clinical applicability of results.

A potential IV is initially identified using empirical evidence. The analysis then involves a two-stage regression model. As the technique originated in economics this was traditionally two sequential linear regressions using a two-stage least squares procedure (41). In medical studies the outcome cannot always be assessed using linear regression so here we simply refer to the technique as a two-stage instrumental regression method. In the

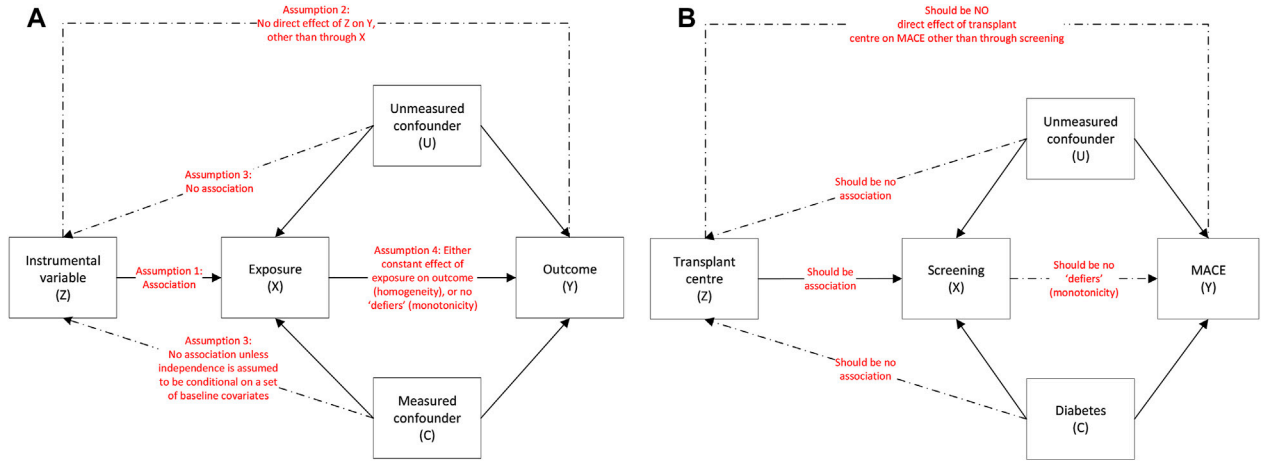


FIGURE 2 | (A): Instrumental variable assumptions and the associations between the instrumental variable (Z), exposure (X), outcome (Y), measured confounders (C) and unmeasured confounders (U) and **(B)**: using the example of screening on MACE.

first stage, the exposure (treatment) is regarded as the outcome and predicted from a regression model containing the instrument as an independent variable alongside other covariables. A linear regression is frequently used for the first stage even if the exposure is binary, though if the model contains additional covariates the predicted treatment value can lie outwith the range 0–1 (43). As such a linear model is only advised if few additional categorical covariates are added to the model (44).

In the second stage, a regression model examines the outcome of interest as the dependent variable, and the "predicted treatment" generated in the first stage is included as an independent variable instead of the received treatment ("predictor substitution" method). This regression can be univariable or multivariable. A multivariable model enables adjustment for potential confounding of the instrument-outcome relationship. Whilst instrument-outcome confounding represents a violation of the independence assumption, conditioning on pre-exposure covariates in the first and second stages of the IV model can reduce the impact of this and also increase the plausibility of the homogeneity assumption. (9) As such, multivariable models which include confounders of the instrument-outcome (in addition to treatment-outcome) relationship may be beneficial. Other methods of estimating the predicted treatment variable, how to include it in the second stage model, and type of second stage model exist. Broadly speaking, population effects can be interpreted using a range of first-stage regression techniques and a second-stage Cox model with the predictor substitution approach is a straight forward method for time-to-event analyses, though Cox models are not universally recommended in IV analyses unless the outcome is rare due to their potential to introduce bias (45-51).

As the analysis is performed, potential violations of IV assumptions should be assessed. Results must be interpreted in the context of how likely it is for the assumptions to be met.

(1) Relevance assumption: this is examined using the F statistic and partial R-squared values. An F statistic under 10 typically is used to identify a weak instrument (52). The greater the partial R-squared the greater the contribution of the instrument to treatment allocation, however this value varies with sample size and there is no consensus on what a satisfactory value is (53).
(2) Exclusion restriction: there is no statistical test to definitively confirm that the IV does not influence the outcome other than through treatment allocation. (54). Examining the association between the IV and the outcome can provide information on how likely a direct association is but requires careful conduct and interpretation.
(3) Independence assumption. This cannot be tested and is usually argued based on empirical evidence.
(4) Effect monotonicity or homogeneity. These assumptions may be implausible and are complex to define and assess. In effect monotonicity, identifying which compliance group (**Supplementary Figure S1**) a patient belongs to is impossible, and even defining compliance groups is challenging in the case of multi-categorical instruments (42).

Limitations

Finding a suitable IV can be challenging and large multicentre studies are often required. Ensuring assumptions of the IV are met may not be possible (55). Weak instruments may also amplify bias through violation of the exclusion restriction or independence assumption and result in more biased estimates than other analyses (9). Finally, whilst IV analyses can overcome unmeasured confounding, they are less precise as individuals are examined based on estimated not actual exposure (56).

INTERPRETING RESULTS FROM CAUSAL INFERENCE MODELS

Average Treatment Effects

When analysing causal inference studies, it is necessary to consider to whom the causal effect is applicable to. Terms used include the "average treatment effect" (ATE), "average

treatment effect on the treated" (ATT) and "local average treatment effect" (LATE).

ATE refers to the effect of treatment on the whole population. This is typically estimated by IPW techniques, which include all study participants. ATT refers to the effect of treatment on only those individuals potentially eligible to receive it and is typically estimated by PS matched analyses. In IV analyses, the causal effect depends on whether effect homogeneity or monotonicity hold. If homogeneity is assumed, the estimate refers to the ATE. If monotonicity is assumed, the estimate refers to the LATE. This reflects the effect of treatment on the subgroup of "complier" patients who receive the expected treatment given their instrument (**Supplementary Figure S1**). As complier patients cannot be identified from within the study population, the LATE has limitations in informing practice/policy decisions.

As the ATE, ATT and LATE refer to different groups of patients, their effect sizes can differ. Differences can aid the interpretation of study findings by providing insights into the effect of treatment on different groups of patients, and do not necessarily signify failure of a technique.

Conditional and Marginal Treatment Effects

In each of the above analyses, the final regression model that generates the causal effect can either be "marginal" or "conditional." Models which contain only the treatment (or predicted treatment in the IV analysis) and outcome generate marginal treatment effects. Although the characteristics of treated and untreated individuals should be similar through the PS matching, IPW or IV techniques, generating truly "exchangeable" groups of treated and untreated patients remains difficult. Models which condition on (and hence adjust for) confounders in the final regression may reduce such residual imbalances and generate conditional treatment effects.

The effect sizes from marginal and conditional regression models differ and cannot be directly compared (57, 58). If the model has been correctly specified, marginal models estimate the average effect of treatment on the population (i.e., the effect of moving the population from being untreated to treated), whilst conditional effects are more individualised and apply to groups of patients within covariate levels (i.e., the effect of moving an individual person from being untreated to treated). Marginal treatment effects are frequently used for health policy decisions, whilst conditional treatment effects are helpful at an individual patient level. Further, even if conditional models from PS matching, IPW and IV techniques contain the same variables, unavoidable differences between analyses mean results are still not directly comparable. For example, PS matching is conditional on the covariates and the PS, whereas the other analyses are just conditional on the covariates.

DOES SCREENING FOR CORONARY ARTERY DISEASE REDUCE POST-TRANSPLANT CARDIAC EVENTS?

To demonstrate the above techniques, a worked example is provided using data from the ATTOM study. ATTOM was designed to examine factors associated with transplantation in the UK, recruiting patients between 2011 and 2013 (59). Data on transplant assessment was collected for patients who were waitlisted or transplanted at study recruitment. In this analysis, individuals receiving a kidney transplant between 1st November 2011 and 31st December 2017 were included. This patient selection has implications on other forms of bias in the study, outlined in **Table 2**.

We wished to examine whether cardiac screening reduced post-transplant major adverse cardiac events (MACE). MACE was defined as unstable angina, myocardial infarction, coronary revascularisation, or cardiac death. Data on non-fatal cardiac events were obtained through linkage of the ATTOM dataset with routinely collected hospital data (60). Death data were obtained from the UK Renal Registry and NHS Blood and Transplant. Patients were followed up until 31st December 2017, with censoring for non-cardiac deaths.

Over the study period, 2572 individuals received a transplant. The mean age was 50 years (SD 13) and 61% were male. Ethnicity was White in 76%, Black in 14% and Asian in 9%. There was a history of diabetes in 13% and ischaemic heart disease in 7%. Overall, 51% underwent screening for asymptomatic coronary artery disease with a stress test (exercise tolerance test, stress echocardiogram, myocardial perfusion scan), CT coronary angiogram or invasive coronary angiogram before transplant listing. The proportion of individuals screened across the 18 transplant centres in England ranged from 5%–100% (**Figure 3**).

Median follow up was 5.0 years (IQR 3.8–5.5), over which time 211 individuals experienced MACE. Median time to MACE was 2.3 years (IQR 1.0–3.7; range 1 day–6.6 years). Over follow up, 227 patients died (8.9%); 40 had a cardiac death that was counted as MACE.

To examine whether screening has a causal effect on MACE at 90 days, 1 year or 5 years post-transplant, Cox regression models were performed using propensity score matching, inverse probability weighting, and instrumental variable analysis techniques.

Competing Risks and "Direct" and "Total" Treatment Effects

Non-cardiac death is a competing risk for post-transplant MACE, as patients dying of non-cardiac causes cannot subsequently develop MACE. The analyses presented in the following section determine the "direct" effect of screening on MACE as patients are censored at non-cardiac death, as opposed to the "total" effect of screening on MACE which would include causal pathways involving non-cardiac death (61).

Interpreting direct treatment effects is challenging as they assume an unrealistic situation where competing events do not occur. Further, direct treatment effects have additional causal assumptions such as no unaccounted confounding of the relationship between the competing event (non-cardiac death) and outcome of interest (MACE). If there is likely to be a confounding relationship between the censoring event and the outcome of interest, techniques such as inverse probability of censoring weighting may be required to derive valid estimates of the direct treatment effect—such analyses require sufficient data availability for the

TABLE 2 | Design of a potential randomised control trial to investigate the utility of cardiac screening prior to kidney transplant listing, and the design of the worked example, highlighting areas of residual bias.

Component	Ideal randomised control trial	Worked example and residual bias
Eligibility	Individuals with chronic kidney disease being worked up for kidney transplantation	Patients who were recruited to the ATTOM study and received a kidney transplant. Whilst these patients are representative of the UK kidney transplant population, information was not available on all patients who commenced transplant workup and it is not known if results are applicable to this whole population. Selection bias and survivor bias may be present
Treatment strategies	Receive a cardiac screening test (and any subsequent recommended cardiac intervention) vs. not receive a cardiac screening test prior to kidney transplant listing	Receiving a cardiac screening test (and any subsequent recommended cardiac intervention) as per local standard practice vs. not receiving a screening test prior to kidney transplant listing
Treatment assignment	Eligible individuals would be randomly assigned to one of the two treatment strategies and would be aware of the treatment which they were assigned to	Patients were selected for screening based on pre-determined local protocols or clinical judgement of the medical team. As treatment assignment was not randomised and there were not strict eligibility criteria, inferences are limited to those patients who might be considered for screening, rather than patients who would never or always be screened
Follow up	Follow up would start at the time of assignment to a treatment strategy (i.e. when randomised to receive cardiac screening or not) and would continue for a set period of time over which some patients would be activated on the waitlist and receive a transplant. This is likely to require long follow up, for example 3–5 years	Follow up started at the point of kidney transplantation and was for up to 5 years. This start point was chosen as the date transplant workup commenced was unknown, and data were not available on patients who commenced workup but were not waitlisted. This risks survival bias as all patients survived until the point of transplantation. Further, the misalignment of treatment assignment and follow up start means there could be fundamental differences between patients who are transplanted after screening vs. those transplanted without screening. As screening may not have a uniform effect on individuals unobserved in this study, there is a risk of selection bias
Primary end point	Post-transplant MACE. The exact time frame post-transplant that should be examined could be debated, but given screening aims to reduce short-term morbidity and mortality a time frame of around 1 year could be considered	Post-transplant MACE at 90 days, 1 year and 5 years post-transplant. Patients were censored for non-cardiac death, therefore estimates refer to the direct effect of screening on MACE and not the total effect of screening on MACE through all causal pathways, including through any effect on non-cardiac death
Secondary end point	Activation on transplant waitlist Time to waitlisting Time to transplantation Waitlist MACE Patient reported outcomes	Not captured
Causal contrast	Intention-to-treat effect—effect of being randomised to screening or no screening, even if off-protocol screening tests were performed Per protocol effect - effect of adhering to the treatment strategy over follow up	Per protocol effect—effect of adhering to the treatment strategies over follow up
Statistical analysis	Intention-to-treat; consideration would need to be made as to how to analyse patients not transplanted over follow up	Per protocol analysis

probability of censoring (i.e., non cardiac death) to be modelled accurately over time (61).

As the purpose of this paper is to demonstrate the application of different causal inference techniques, for pragmatic reasons the following analyses represent the direct effect of screening on MACE. Information on competing risk analyses, which can navigate this issue by generating total treatment effects, are found at the following references (62, 63, 64).

Propensity Score Matching and Inverse Probability Weighting

To generate the PS, variables deemed to potentially relate to screening and MACE were determined and included in a logistic regression model. These comprised: age, sex, ethnicity, socioeconomic status, smoking status and history of ischaemic heart disease, diabetes, cerebrovascular disease, and peripheral vascular disease. Transplant centre was not included as it should not independently associate with MACE, would prevent us capitalising on variation in practice to create groups screened and unscreened patients, and could result in violation of the positivity assumption (**Supplementary Table S1**).

As the proportion of screened and non-screened individuals was roughly equal, PS matching was performed on a 1:1 basis without replacement using a caliper of 0.2 times the standard deviation of the logit of the propensity score. Matching was possible in 1760 individuals. The distribution of the PS before and after matching is shown in **Supplementary Figure S2**. The standardised mean difference after matching showed appropriate covariate balance between groups (**Supplementary Table S2**). The characteristics of screened and unscreened patients in PS matched and unmatched groups are shown in **Figure 4**. The 812

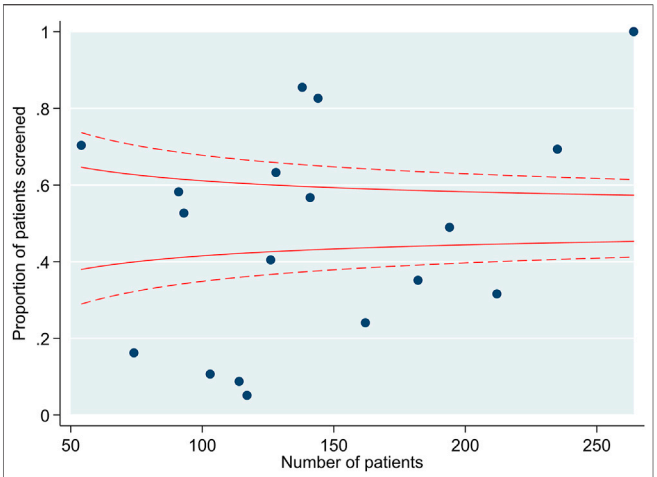


FIGURE 3 | Funnel plot demonstrating the number of individuals screened by transplant centre.

unmatched individuals were more likely to be male, of Asian ethnicity, and have a history of cardiovascular disease (**Supplementary Table S3**). In the PS matched population, screening did not reduce MACE at 90 days (conditional HR 0.80, 95% CI 0.31–2.05), 1 year (conditional HR 1.12, 95% CI 0.51–2.47) or 5 years (conditional HR 1.31, 95% CI 0.86–1.99) (**Table 3**). These results reflect the ATT: the causal effect of screening in screened patients eligible to receive either treatment (and thus "matched"), representing transplant recipients at low-medium cardiac risk.

For IPW, inverse probability of treatment weights were calculated. Weights were stabilised by multiplying them by the proportion of individuals who underwent screening in the exposed group, and proportion of individuals who did not undergo screening in the unexposed group (32). The mean of the stabilised weights was 1.00 (SD 0.47, range 0.53–8.45). Characteristics of the 57 patients with stabilised weights greater than or equal to 2 are in **Supplementary Tables S4, S5**. These patients were more frequently unscreened. Higher-weighted unscreened patients were older and more likely to have cardiovascular disease.

In total 2502 individuals were examined in the IPW analysis; 70 individuals were excluded due to missing data in variables used to generate the PS. Cox regression models were performed incorporating the IPW (**Table 3**). There was no evidence screening reduced MACE at 90 days (conditional HR 0.95, 95% CI 0.44–2.05) or 1 year (conditional HR 1.28, 95% CI 0.72–2.26). There was weak evidence that patients undergoing screening were at higher risk of MACE at 5 years (conditional HR 1.38, 95% CI 1.00–1.90), but this analysis did not meet the Cox proportionality assumption with a greater rise in MACE in screened patients over time. These results reflect the ATE: the causal effect of screening on the transplanted population. They do not provide information on the effect of screening on the total population who begin transplant workup.

It is important to note that these results represent a complete case analysis, as the 70 individuals with missing data were excluded. Complete case analyses assume data are missing completely at random, though other missing data mechanisms and their potential implications need to be considered (65).

Instrumental Variable Analysis

Transplant centre is determined by geographical location so is largely randomly allocated. We determined centre had the potential to be an IV as it (at least partly) met the following assumptions (**Figure 2B**):

(1) Relevance assumption: the likelihood of undergoing screening is associated with transplant centre (**Figure 4**), even after adjustment for patient-level characteristics (18). On an individual patient level, screening is associated with older age, male sex, and a history of vascular disease (**Supplementary Table S6**) but when examining patients based on whether they are registered at a centre with a low, medium, or high screening use, differences in these variables is reduced (**Table 4**).
(2) Exclusion restriction: this assumption cannot be guaranteed as there could be non-screening differences in centre-level practice that influence outcome, e.g., use of medical therapy, but this would not be expected given there is national guidance on cardiovascular risk management (66), and transplant outcomes are similar between centres (67).
(3) Independence assumption: this assumption cannot be proven, as acknowledged in IV literature. Whilst it may be assumed that if measured confounders are balanced across IV groups, unmeasured confounders will be too, this is purely speculative.
(4) Homogeneity or monotonicity. Screening may not have a uniform effect on individuals, for example it could benefit those with high cardiovascular risk but not low risk patients, thus violating homogeneity. Monotonicity (no patients receiving the opposite treatment to what would be expected at any level of the instrument) may be more likely to hold as patients receive screening based on defined protocols at their transplant centre. This assumption however cannot be proven and defining the four compliance types (**Supplementary Figure S1**) is complex.

In the first stage, a linear regression containing potential confounders of the treatment-outcome relationship (deemed to be those used to create the PS) and transplant centre was used to predict the likelihood of an individual undergoing screening. Linear regression was selected for this analysis as opposed to logistic regression as described in IV literature (43), which also prevented individuals from centres who screened all recipients (n = 264) being dropped given instrument was a "perfect" predictor of outcome. Whilst using centre as an instrument addresses unmeasured patient-level confounding (i.e., unmeasured confounding between X and Y via U in **Figure 2**), centre-level

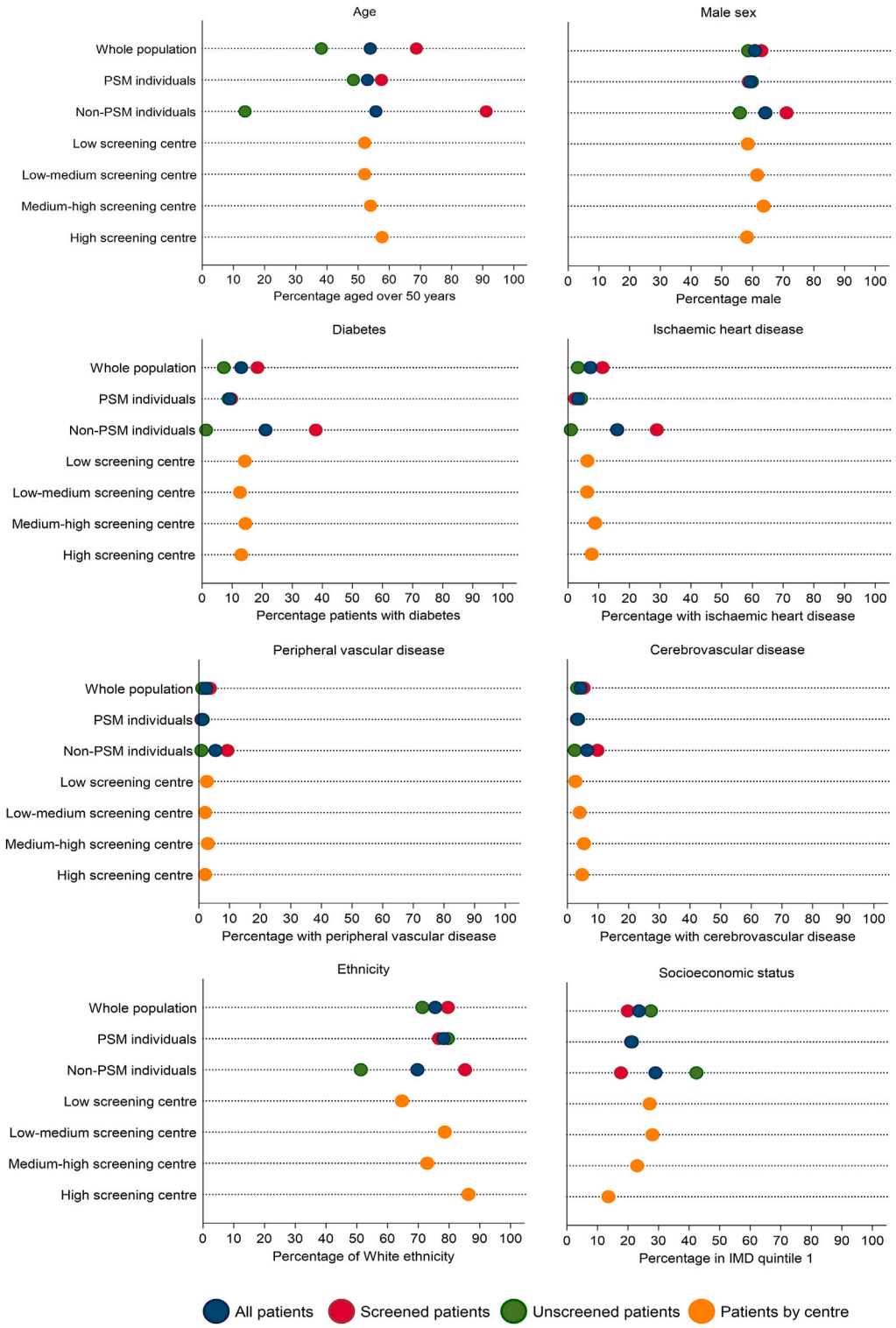
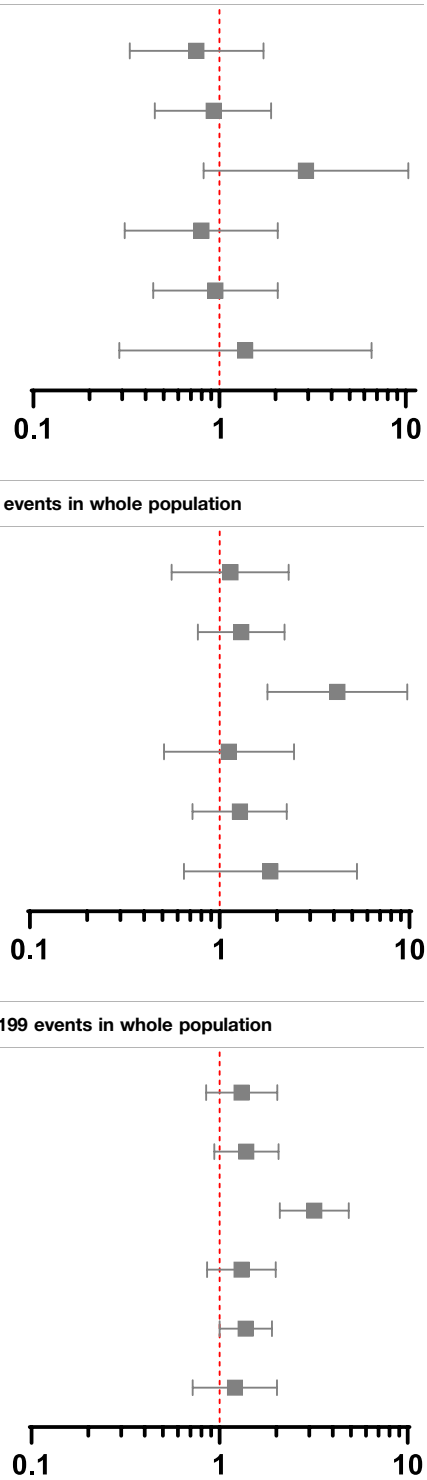


FIGURE 4 | Characteristics of screened and unscreened groups across the whole population and in propensity score matched and unmatched groups, followed by characteristics by centre screening use: low volume of screening (<25% of transplant patients screened; n = 570), low-medium volume of screening (25%–49% screened; n = 714), medium-high (50%–74% screened; n = 742) or high volume of screening (>74% screened; n = 546). Note that although there is variation in patient characteristics by those screened or unscreened, this variation reduces when patients are stratified by centre screening volume, suggesting centre could be a strong instrument.

TABLE 3 | Association between screening and post-transplant MACE at 90 days, 1 year and 5 years using propensity score matching, weighting and instrumental variable techniques.

Method and treatment effect	HR	95% CI	*p*-value	Hazard ratio with 95% confidence interval
Association between screening and MACE at 90 days post-transplant 14 events in PS matched group, 23 events in whole population				
PS match marginal	0.75	0.33–1.72	0.50	
IPW marginal	0.93	0.45–1.89	0.83	
IV marginal	2.91	0.82–10.33	0.10	
PS match conditional	0.80	0.31–2.05	0.64	
IPW conditional	0.95	0.44–2.05	0.90	
IV conditional	1.37	0.29–6.55	0.69	
Association between screening and MACE at 1 year post-transplant 32 events in PS matched group, 52 events in whole population				
PS match marginal	1.14	0.56–2.31	0.72	
IPW marginal	1.30	0.77–2.20	0.33	
IV marginal	4.18	1.79–9.76	0.001	
PS match conditional	1.12	0.51–2.47	0.77	
IPW conditional	1.28	0.72–2.26	0.40	
IV conditional	1.85	0.65–5.29	0.25	
Association between screening and MACE at 5 years post-transplant 117 events in PS matched group, 199 events in whole population				
PS match marginal	1.31	0.85–2.03	0.22	
IPW marginal	1.39	0.94–2.06	0.10	
IV marginal	3.19	2.09–4.87	<0.001	
PS match conditional	1.31	0.86–1.99	0.20	
IPW conditional	1.38	1.00–1.90	0.05	
IV conditional	1.21	0.72–2.02	0.48	



CI, confidence interval; HR hazard ratio; IV, instrumental variable; PS, propensity score; IPW, inverse probability weighting. Multivariable includes variables used to estimate the propensity score in the outcome regression model.

TABLE 4 | Patient characteristics based on the prevalence of screening pre-transplant by centre. The Kruskall-Wallis test was used to examine continuous variables and the Chi square test for categorical variables.

Percentage of individuals screened by centre	<25% 4 centres *n* = 570	25%–49% 5 centres *n* = 714	50–74% 6 centres *n* = 742	≥75% 3 centres *n* = 546	*p* value
Median age (years)	50 (40–60)	50 (41–59)	52 (40–60)	52 (42–62)	0.22
Male sex (%)	58.8	61.5	63.6	58.2	0.17
White ethnicity (%)	64.7	78.6	72.9	86.3	<0.001
IMD quintile 1 (%)	27.1	28.0	23.0	13.6	<0.001
Diabetic nephropathy (%)	23.2	22.0	23.9	23.8	0.29
Diabetes (%)	14.2	12.5	14.4	10.2	0.12
Ischaemic heart disease (%)	6.3	6.2	8,8	7.7	0.20
Peripheral vascular disease (%)	2.6	2.0	2.9	2.0	0.56
Cerebrovascular disease (%)	2.6	4.0	5.4	4.8	0.09
Pre-emptive transplant (%)	20.9	20.9	24.1	20.7	0.34

confounding remains possible due to other institutional differences in practice (i.e., confounding between Z and Y in **Figure 2** that may be distinct from U and/or C). (68) We considered including centre-specific variables which could influence outcome e.g. proportion of living donor or pre-emptive transplants, but these were not included in the final model due to collinearity with centre.

The first stage generated a predicted value, representing the likelihood of each individual being screened. The F statistic was 70 and the partial R-squared value was 0.33, indicating centre was a strong IV.

In the second stage, univariable and multivariable Cox regression models were performed using the predicted value from the first stage (predictor substitution method). This step can be considered as including the proportion of patients screened by centre as a patient characteristic, rather than whether each individual was screened. The multivariable model included the same confounders used to create the PS as these were deemed to potentially confound both the instrument-outcome and treatment-outcome relationship, and therefore including these confounders makes the independence assumption more likely to hold. Screening did not reduce MACE in the conditional model at 90 days (conditional HR 1.37, 95% CI 0.29–6.55), 1 year (conditional HR 1.85, 95% CI 0.65–5.29) or 5 years (conditional HR 1.21, 95% CI 0.72–2.02). These results reflect the LATE: the causal effect of screening on the 'complier' patients in the population.

Interpretation of Results

Results from PS matched, IPW and IV analyses are shown in **Table 3**. In the conditional models, screening did not reduce MACE in any analysis, which each had overlapping confidence intervals, but there was variation in estimates between methods. The hazard ratios using PS methods rose over time, crossing 1 between 90 days and 1 year, whilst in the IV analysis the hazard ratio was above 1 throughout. These differences can help result interpretation by considering which patients are included in each analysis.

In the PS matched analysis, the results are only applicable to 1760 transplant recipients with low-medium baseline risk of MACE, not the overall population. The 812 individuals excluded from the analysis were more likely to be male, of Asian ethnicity, have a history of cardiovascular disease and be of a lower socioeconomic status and thus have the greatest baseline cardiovascular risk. Whilst these results suggest no benefit to screening, this cannot be directly applied to these highest risk patients.

The IPW analysis includes all patients and represents the whole transplanted population. Similar findings were observed to the PS matched analysis at 90 days and 1 year. At 5 years, there was weak evidence that individuals who had undergone screening were more likely to experience MACE in the conditional model but it should be noted that this analysis did not meet the Cox proportionality assumption.

In the IV analysis, screening did not reduce MACE on conditional analyses with a hazard ratio above 1 throughout,

BOX 1 | Selected transplant studies using propensity score and instrumental variable techniques.

Propensity score techniques

- Comparison of outcomes in recipients receiving a living versus standard criteria deceased donor kidney transplant (74).
- Comparison of outcomes in donation after brainstem death and donation after cardiac death donors in liver transplantation (75).
- Association between immunosuppression regime (triple or quadruple therapy) in heart transplant recipients and death and rejection episodes (76).

Instrumental variable techniques

- Association between dialysis duration and patient outcome following kidney transplantation, using blood group as an instrumental variable (77).
- Examining whether delayed graft function is associated with long term outcomes after kidney transplantation using cold ischaemic time as an instrumental variable (78).
- Comparison of deceased and living organ donation rates in countries with an opt-in and opt-out policies using legal system and non-health based philanthropy as instrumental variables (79).

suggesting "complier" screened individuals had a higher risk of MACE than complier non-screened individuals, although confidence intervals were extremely wide. Given these results represent the LATE, it is not known whether the effect of screening on non-complier patients differs. Whilst the IV technique minimises unmeasured confounding, these results raise the possibility that unmeasured patient level characteristics associate with centre and outcome (i.e., clinicians screen their patients as they see their population as being inherently higher risk), or there are unmeasured differences in centre level practice, e.g., use of medical therapy that could bias results. Alternatively, it is possible that the PS matched and IPW analyses are prone to bias due to unmeasured confounding, and the IV analysis provides a result that is closer to the truth. Some studies suggest IV techniques provide less biased results than PS analyses, (69) but the challenges in identifying an appropriate instrument must be considered and results interpreted with caution until further studies examining both techniques are available (70).

The marginal hazard ratios presented in **Table 3** reflect the effect of screening on the study population as opposed to an individual patient. In the PS matched and IPW analyses, screening did not reduce MACE. The results of the IV analysis differed, with screened individuals having a greater risk of MACE at 1 year (HR 4.18, 95% CI 1.79–9.76) and 5 years post-transplant (HR 3.19, 95% CI 2.09–4.87). This may reflect deviation from the independence assumption of no confounders to the instrument and outcome, the impact of which is lessened by adjusting for confounders in the conditional model.

Limitations

Whilst the causal inference techniques applied to our worked example reduce confounding by indication, other forms of bias remain (**Table 2**). The worked example only examines patients who received a transplant. Data were not available for those who were screened and not listed due to an abnormal screening test, or listed but not transplanted due to MACE that occurred on the waitlist. Screening results are just one factor in a complex assessment of patients for transplantation, with the proportion of patients excluded due to cardiac screening abnormalities estimated at 1%–4% (71, 72, 73). In a target trial examining whether cardiac screening improves post-transplant outcomes these data would ideally be known, and neither PS or IV techniques specifically address this issue. Results therefore cannot be applied to the population who begin transplant workup nor determine the impact of screening on outcomes outwith post-transplant MACE.

SUMMARY

Propensity score and instrumental variable techniques reduce confounding in observational studies and are suited to areas where treatment decisions vary with clinician or facility preference. Whilst RCTs minimise confounding through the random allocation of treatment, results may not be generalisable if the individuals recruited to a trial are not representative of the population of interest, e.g., if individuals with less severe disease who are "lower risk" or with more severe disease who have "most to gain" are preferentially recruited. Population observational data allows all patients within clinical practice to be examined, but treatment effects from causal inference techniques still may not be applicable to the whole population due to limited overlap in confounder distributions between patient groups. Techniques deal with this issue in different ways. For example, in PS matching patients are excluded from analyses if a "suitable" match cannot be found. In IPW analyses, the presence of large weights can highlight instances where regression adjustment would result in the model being extrapolated to groups with little or no overlap in confounder distribution. Whilst large weights can make the ATE estimate unstable and results in wide confidence intervals, IPW techniques provide an "honest" reflection of the uncertainty in the estimate which might be underestimated in regression adjustment. Causal effects from each technique therefore permit inferences on different populations, which is important when interpreting study results.

Our case study demonstrates how causal inference techniques can estimate comparative effectiveness of interventions using observational data, but don't eliminate all forms of bias and may still not allow firm conclusions to be drawn. Differences in results may reflect the different populations the estimates are applicable to, the presence of unmeasured confounding, or imperfections in the instrument. It is difficult to know which analysis provides the closest result to the "true" estimate, and results should be interpreted in the context of the limitations of each method.

Despite these challenges, the unique issues in performing RCTs in transplantation, combined with the increase in size and granularity of routine healthcare datasets are likely to result in wider use of propensity score and instrumental variable techniques. Examples of transplantation studies using these techniques are shown in **Box 1**. There is potential to explore areas such as the optimal timing of pre-emptive transplantation, identifying which patients may benefit from transplantation, and how outcomes differ based on donor type. By identifying areas where there is variation in practice and clinical equipoise, these analyses can provide preliminary data to guide clinical trials. We welcome the possibility of this in the field of cardiac screening prior to kidney transplant listing.

AUTHOR CONTRIBUTIONS

AN performed the analyses, produced the figures and tables and wrote the manuscript under the supervision of RR, DT, and JF. JF contributed to study design, statistical analyses and manuscript preparation. NL contributed to statistical analyses and manuscript preparation. GO, RR, and DT contributed to study design and manuscript preparation.

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AUTHOR DISCLAIMER

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

CONFLICT OF INTEREST

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10105/full#supplementary-material

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GLOSSARY

Propensity score a value ranging between 0 and 1 that summaries the likelihood of an individual receiving a treatment based on their measured covariates

Propensity score matching process through which individuals in treated and untreated groups are matched to each other based on their propensity score. This can be done on a 1:1 (1 patient in the untreated group matched to 1 treated individual) or many-to-one (many patients in the untreated group matched to 1 treated individual) basis

Matching without replacement once an individual from the untreated group has been matched, they cannot be used as a comparator for any further treated individuals

Matching with replacement an individual in the untreated group can be used as a match for more than 1 treated individual. Useful if the number of untreated individuals is small.

Nearest neighbour matching matching process which pairs treated and untreated individuals based on them having the closest propensity scores, irrespective of whether the untreated individual is a better match for another treated individual.

Optimal matching matching process which aims to minimise the difference in propensity scores between pairs across the whole population. May be preferred over nearest neighbour matching if the proportion of untreated individuals in the population is small.

Inverse probability weighting technique which weights individuals based on their propensity score to create a pseudo-population with balanced measured covariates in treated and untreated groups

Instrumental variable a variable that is causally associated with the exposure, only affects outcome through its association with that exposure, and has no confounders with the outcome. Allows individuals to be examined based on the instrument to minimise the risk of unmeasured confounding.

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MINI REVIEW
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Artificial Intelligence: Present and Future Potential for Solid Organ Transplantation

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Artificial intelligence (AI) refers to computer algorithms used to complete tasks that usually require human intelligence. Typical examples include complex decision-making and-image or speech analysis. AI application in healthcare is rapidly evolving and it undoubtedly holds an enormous potential for the field of solid organ transplantation. In this review, we provide an overview of AI-based approaches in solid organ transplantation. Particularly, we identified four key areas of transplantation which could be facilitated by AI: organ allocation and donor-recipient pairing, transplant oncology, real-time immunosuppression regimes, and precision transplant pathology. The potential implementations are vast—from improved allocation algorithms, smart donor-recipient matching and dynamic adaptation of immunosuppression to automated analysis of transplant pathology. We are convinced that we are at the beginning of a new digital era in transplantation, and that AI has the potential to improve graft and patient survival. This manuscript provides a glimpse into how AI innovations could shape an exciting future for the transplantation community.

Keywords: organ transplantation, machine learning, artificial intelligence, deep learning, result prediction, healthcare 4.0, digital pathology

INTRODUCTION

Artificial intelligence (AI) refers to the use of algorithms (machine learning and deep learning) to perform tasks that are usually associated with human intelligence, "such as the ability to reason, discover meaning, generalize, or learn from past experience to achieve goals without being explicitly programmed for specific action" (1, 2). AI is already changing industry through new forms of interaction between man and machine. Driven by AI, this industrial revolution (known as I4.0) brought intelligent factories where humans and cyber-physical systems interact through deep-learning algorithms. These technologies are increasingly in demand in all industries which seek to ensure manufacturing competitiveness.

Powered by increasing availability of healthcare data and rapid development of analytical techniques, AI is also growing exponentially in all areas of medicine, including solid organ transplantation. The pre- and post-transplantation patient care requires complex decision-making. In this context, AI can drive a real paradigm shift as it enables analyzing and synthesizing of huge amounts of data, and transforming them into clinical recommendations. AI-based classifiers have been principally explored for the optimization of four key areas: organ allocation and donor-recipient pairing, transplant oncology, real-time immunosuppression regimes,

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TABLE 1 | AI Glossary table.

Term	Definition
Computer Algorithms	Computer algorithms are automated instructions
Machine Learning (ML)	Machine learning is a subfield of artificial intelligence intended as a sets of automated computer algorithms
Deep-Learning (DL)	Deep learning is a type of ML that imitates the way humans gain certain types of knowledge including statistics and predictive modeling
Neural Networks (NN)	Neural networks reflect the behavior of the human brain, allowing computer algorithms to recognize patterns and solve common problems in the fields of AI, ML and DL.
Cyber Physical System	Cyber Physical System is referred to computer-human networks, controlling physical processes, where physical processes affect computations and vice versa
Internet of Things	The Internet of Things represents a system of interralated computing devices, capable of operating without human-to-human or human-to-computer interaction

and precision transplant pathology. The aim of AI is to identify hidden trends and complex relationships within large datasets to obtain logical results while optimizing resources. AI is still in its infancy and, so far, we lack validated algorithms that could accurately drive organ selection, predict potential rejections or attenuate postoperative complications. Nevertheless, in the last few decades, AI applications have already contributed to lower incidence of rejection, and fine-tuning of the transplantation and organ preservation processes. In this review, we discuss emerging AI, machine learning and deep learning strategies applied to solid organ transplantation and their potential future applications (**Table 1**).

AI IN ORGAN ALLOCATION AND DONOR-RECIPIENT MATCHING MODELING

From an exclusively mathematical point of view, transplantation can be reduced to a list of problems in which the characteristics of the donor must be combined with the variables of the recipient in order to achieve one of the following three outcomes (2): the survival of the graft and the recipient, the loss of the graft or the loss of the graft and the recipient.

The allocation systems used by Eurotransplant in Europe, and the United Network for Organ Sharing (UNOS) in the US, are intended as objective and transparent procedures to make the best possible match (3, 4). The allocation systems, which are one of the cornerstones of transplantation, are based on two major principles: expected outcome and emergency. Additionally, the allocation (and donor-recipient matching) process depends on the timeframe during which the organ remains viable once harvested, which ranges from a few to 36 h, depending on the organ (5). Organ-matching characteristics may differ between organs, but they are crucial for the selection of the best possible allocation and donor-recipient matching. The Child-Pugh classification, the Model of End Stage Liver Disease (MELD), the Kidney Allocation System (KAS) and the Lung Allocation System (LAS) are the most important algorithms currently used (6). Whilst well integrated into clinical practice, these systems cannot prioritize recipients in real time and need constant modifications (7) and addition of exceptions. AI could significantly strengthen the decision-making, by automatically harmonizing principles of optimal use (utility) and equal access (equity) in a context of organ shortage and an ever-growing waiting list. In 2019, Bertsimas and co-workers proposed a machine learning-based model for alternative liver allocation (8). This model, named Optimal Prediction of Mortality (OPOM), predicts the probability of a patient's 3-month mortality or waitlist removal given their characteristics. Using the Standard Transplant Analysis and Research dataset (1618966 observations), OPOM provided more accurate and objective predictions than MELD. Additionally, the OPOM simulation reduced mortality on average by 417.96 deaths for 6139 liver transplantations by assigning different priority to liver transplant candidates. External validation still needs to be performed.

Organ allocation could also benefit from the Internet of Things (IoT). IoT refers to a network of interconnected smart devices such as smartphones, tablets, and laptops, but also wearables, cars, and data transmission devices (9). An IoT ecosystem of web-enabled connected devices using sensors, processors and communication hardware can be used to store, transmit and react appropriately to data from the surroundings. During the organ procurement and transplantation process, the distance between the donor and the recipient is a key factor influencing the time needed for organ transfer. Even if routinely preserved in ice-cold preservation fluids, organs are sensitive to cold ischemia time. IoT could be useful for real-time tracking of organs: during transport, the organ packaging can be equipped with a global positioning system (GPS) that can continuously track the organ's location and record shocks caused by rapid acceleration/deceleration or barometric pressure incidents (10). These data can be used to accurately approximate time of organ arrival in the recipient's transplant center, minimize downtime and optimize the workflow (**Figure 1**).

Organ allocation is strictly connected to donor-recipient matching. Although thoroughly analyzed and refined, the traditional donor-recipient matching models still leave room for improvement and could potentially benefit from AI. In 2013, Cruz-Ramirez et al. reported the use of AI artificial neural networks (AI-ANNs) to improve donor-recipient pairing. AI-ANNs analyzed data on 1,003 liver transplants including donor/recipient matching, graft retrieval and pre-transplant analysis (11). The following year, a large Spanish

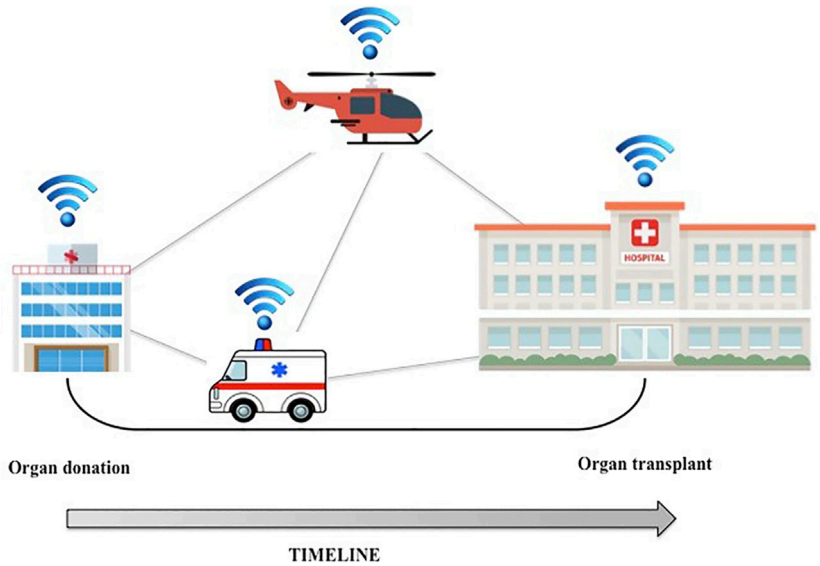

FIGURE 1 | GPS tracking technology could be applied to organ transport, thus minimizing downtime and optimizing the workflow.

multicenter study (Model for Allocation of Donor and Recipient in España [MADR-E]) documented the impressive advantages of using AI-ANNs rather than standard algorithms (12). In their work, Briceño et al. designed a 3-month graft mortality prediction model based on 64 donor and recipient characteristics and performed a binary analysis (graft survival/loss) for donor-recipient matching via AI-ANNs. AI-ANNs' new algorithms predicted graft survival (AUC, 0.81) and graft loss (AUC, 0.82) better than the isolated donor/recipient scores. Similarly, Rana and coworkers used the Organ Procurement and Transplantation Network (OPTN)/Scientific Registry of Transplant Recipients (SRTR) data to develop the Survival Outcome Following Liver Transplant (SOFT) score that integrates recipient and donor characteristics to predict liver transplant 3-month prognosis (13). The SOFT score demonstrated a predictive accuracy similar to those of other models (14, 15) with a C-statistic of 0.70.

The reports on impact of diabetes on the outcome of liver transplantation have been contradictory. Recently, Yasodhara et al. demonstrated the value of AI for successful liver donor-recipient matching, by including the metabolic status of the recipient (16). Based on the SRTR registry, the authors used machine learning to establish survival predictors in liver transplant recipients with preexisting and/or post-transplant diabetes. They tested survival models to predict general and cardiovascular mortality and evaluated the effects of preexisting and post-transplant diabetes on mortality. The model performance achieved C-statistics between 0.58 and 0.66. Additionally, the model was externally validated on a cohort of patients (University Health Network dataset from Toronto, Canada). While the study had some limitations (retrospective design, missing data on patients' comorbidities, unclear information regarding immunosuppression, unusually few patients with steatohepatitis), it is nevertheless one of the largest studies to address risk factors in liver transplant patients with diabetes. AI and machine learning enabled the authors to analyze the huge and heterogeneous dataset and conclude that diabetes is a superior predictor of outcome than obesity, which resulted in changes in practice in donor-recipient matching.

AI-ANNs algorithms have been also applied to donor-recipient matching for kidney transplants. In 2019, Bae et al. proposed an online tool (https://www.transplantmodels.com/kdpi-epts/) (17) to maximize benefits form marginal kidney donors. The authors estimated the 5-year patient survival using a random survival forest (RSF), with the combination of expected post-transplant survival (EPTS) score (variables: age, diabetes, time on dialysis and previous solid organ transplant) and Kidney Donor Profile Index (KDPI) (variables: age, race, height, weight, hypertension, diabetes, serum creatinine, hepatitis-C seropositivity and cause of death). The result of the evaluation yielded a C-statistic of 0.637 for the RSF algorithm, which is slightly higher than the Kidney Donor Risk Index (KDRI) model's 0.6. This prediction model could support personalized decision-making on kidney offers in clinical practice.

AI IN TRANSPLANT ONCOLOGY

Transplant oncology is defined as a combination of various fields of transplant medicine and oncology, aiming to extend the treatment limits of hepatobiliary cancer including hepatocellular carcinoma (HCC), cholangiocarcinoma or colorectal liver metastases (18, 19).

In the past this discipline relied on simple variables such as the number of tumor lesions and their size. In recent years, transplant

oncology was refined and a multitude of new variables identified as central, making AI a potentially important tool. Identification of key clinical and pathological variables is a crucial step in the use of AI for the prediction of tumor recurrence and graft survival after transplantation (20). AI has been used by different groups to predict oncological outcomes in patients undergoing liver transplantation for HCC. Halazun KJ et al. developed a model (MORAL - Model of Recurrence after Liver Transplant) which identified predicting factors of tumor recurrence pre- and post- liver transplantation (21). Specifically, neutrophil-lymphocyte ratio ≤5, alpha-fetoprotein (AFP) > 200 ng/ml and tumor size >3 cm have been classified as pre-transplantation predictive factors of decreased recurrence-free survival. Likewise, HCC grade 4, tumor size >3 cm, > 3 tumor lesions, and vascular invasion have been identified as post-transplantation negative predictive factors. Both scored (pre- and post-transplant) demonstrated predictive superiority (C-statistic of 0.82 and 0.86, respectively) when compared to Milan criteria for forecasting tumor recurrence (C-statistic of 0.63). When combined, the two scores achieved a C-statistic of 0.91.

The Metroticket 2.0 score proposed by Mazzaferro and coworkers (22) predicts survival after liver transplantation for HCC through competing-risk analysis. The authors enrolled 1018 patients from an internal cohort in Italy, while the score was validated by an external Chinese cohort of 341 patients. Preoperative characteristics such as AFP level, tumor volume and number of tumors were included. The validation set showed an accuracy of 0.721 (95% CI, 0.648%–0.793%) in predicting 5-year survival after liver transplant. This model was compared to Milan, Up-to-7 and UCSF criteria, demonstrating a superior predictive ability.

Recently, the group lead by Prof. Sapisochin described the use of AI for predicting the post-transplant recurrence of HCC based on preoperative patient and tumor characteristics (23). To do this, the group included HCC patients listed for liver transplantation between 2000 and 2016 (n = 739). This AI-based HCC-recurrence calculator (CoxNet-based) was then compared to alternative available recurrence risk scores (AFP, MORAL and HALT-HCC scores). The CoxNet-based algorithm outperformed AFP by 0.118, MORAL by 0.130 and HALT-HCC by 0.102. These findings confirm, pending an external validation, that an AI-based calculator can generate a comprehensive prediction of post-transplant HCC recurrence with higher accuracy than alternative scores.

AI AND REAL-TIME ADAPTATION OF IMMUNOSUPPRESSIVE THERAPY

The discovery of cyclosporine was a cornerstone of modern transplantation (24) and constant refinement of immunosuppressive regimens drastically improved outcomes for transplant patients (25). However, immunosuppressive regimens are burdened with adverse effects ranging from nephrotoxicity to malignancies, and significantly reduce the quality of life and life expectancy of transplant patients (26, 27). Furthermore, response to immunosuppressive therapy is highly individual. While some patients do not require any immunosuppression at all, others reject their organs on maximum immunosuppression (28–30). The individual optimization of immunosuppression is therefore of the utmost importance.

Many factors come into play when choosing the optimal immunosuppression regimen, and the decision-making is complex. One relatively simple example of machine learning use is to predict the stable dose of tacrolimus in kidney transplant patients. Three studies compared the logistic regression approach to machine learning algorithms (31–33). All studies showed a superior predictive ability of machine learning tools over the linear regression models, albeit with a relatively small difference. Using combination of genomic data and clinical factors was shown more important than the choice of algorithm. The improved prediction performance highlights the importance of integrating data from different sources (31).

Taking a more general approach, Nitski et al. analyzed large retrospective datasets with machine learning algorithms to predict mortality in liver transplant patients (34). The models were longitudinally updated with patients' data at every follow-up. Interestingly, the model provided meaningful predictions based on readily available data such as graft age, blood values, donor age, and postoperative complications, making a potential clinical implementation relatively straightforward. This dynamic model could be a valuable tool for clinicians to personalize immunosuppressive therapy based on the most likely complication, and therefore reduce graft-related mortality (35). Biomarkers surveillance plays an important role in predicting transplant rejection in patients on immunosuppression.

Suthanthiran *et al.* used the transcriptomes of urinary cells from 220 patients to predict acute rejection based on kidney biopsies (36). The authors obtained an AUC of 0.85 with a three-gene expression signature for the discrimination between acute rejection and no rejection in their own cohort, and an AUC of 0.74 upon external validation. However, the authors used a predefined gene set, while a genome-wide association study would have likely revealed better gene candidates (37). Deep learning tools could have been helpful in this big-data context to not only find these candidates but also to further improve the already working prediction model (38).

AI can integrate high-complexity information from many sources into the decision-making tree used in individualized immunosuppression. A wealth of information about donors and recipients is still underutilized. Data from pre-transplantation histology, recipient's genome, gene expression analysis, blood and urine analysis, and clinical observation can all deliver important clues on the state of a transplanted organ (39–41). AI can help us tap into this potential to fine-tune immunosuppression, and optimize graft and patient survival.

AI IN TRANSPLANT PATHOLOGY

AI has proven highly efficient in image processing. An image contains a high density of structured and unstructured

information that is often inaccessible to the untrained eye (42). A pathologist has the experience and the training to recognize subtle patterns and interpret them in the context of a particular patient and their disease. Unfortunately, trained pathologists are in short supply. This is where AI steps in to extract, process, analyze and even learn from the wealth of information contained in pathological slides (1), that can guide therapy or improve diagnostic accuracy. More than 2 decades ago, Furness et al. developed a machine learning algorithm that diagnosed the acute kidney allograft rejection more accurately than expert pathologists (43). However, the algorithm was not fully automated—it relied on manual extraction of pathological features from histological slides. This method of data collection illustrates why AI did not find a more widespread application in transplant pathology sooner: collecting raw data is a prerequisite for downstream analysis. Commercial digital pathology slide scanners for high-throughput imaging have only recently become available (44, 45). Advances in computer performance, data storage and network speed enable increasingly efficient analysis. The I4.0 now provides us with the tools to fully exploit the potential of AI in transplant pathology. In a recent study, Hermsen et al., successfully implemented a deep learning algorithm to divide kidney biopsies from different centers into their anatomical components (46). The authors developed a convolutional neural network that classified each anatomical component. While the algorithm performed well in identifying healthy glomeruli, it struggled to identify more challenging structures such as sclerotic glomeruli or atrophic tubuli. Nevertheless, this study provides important groundwork and paves the way for further image analysis of kidney transplant biopsies. Most importantly, the authors proved that the same algorithm worked on histological samples from different centers, thereby addressing the issue of reproducibility.

In liver transplantation, quick and reliable assessment of liver steatosis during procurement still presents a challenge. Recently, several groups have developed deep learning algorithms to assess the degree of steatosis in liver biopsies (47–49). Perez-Sanz et al. developed a quick and easy workflow to quantify steatosis content in Sudan-stained frozen sections of procurement biopsies through machine learning. Their algorithm, available as an open-source interactive web platform (50), proved highly accurate in comparison with the assessment of an expert pathologist. This tool could be extremely valuable for the decision-making in remote procurement locations, where an expert pathologist is not readily available.

Automated image analysis, feature recognition, data extraction and deep learning models are everyday reality for the tech giants but have only partially reached precision pathology (51–53). Radiology is one step ahead and shows what is possible with the emerging field of radiomics—the extraction of data from radiograms to diagnose cancer, predict outcomes or guide therapy (54–56). Transplant pathology needs to follow this example with a concerted, multidisciplinary effort of pathologists, computational biologists and healthcare administrators. Challenges that lie ahead are the implementation of digital workflows to routinely scan histological slides, and collaboration between centers to establish image databases and bring the existing AI tools to transplant pathology (57).

CONCLUSION: AI CURRENT PITFALLS AND FUTURE PROMISES

The true potential of AI in healthcare has yet to be fully exploited and its application in solid organ transplantation is mostly under development. Some important limitations exist (58). Several algorithms have been developed in a single institution and still need an external validation to prove their robustness. Secondly, in some cases, the use of AI cannot provide significant improvements over current models (58–60). Moreover, the creation of a more comprehensive AI-based decision model (which includes characteristic of all organs as well patient-specific alternative therapeutic strategies) should be targeted. On the one hand, this could bring new insights to potentially enlarge the pool of transplantable organs and, on the other, improve patient outcomes. Implementing AI into daily clinical practice is an ongoing challenge and the best strategy forward is unclear. While most physicians are unconvinced that can AI play a weighty role in medicine, it is naive to think that this technology will not develop further. Moreover, while this manuscript focuses on the use of AI in transplantation, many other domains could benefit from it. Precision medicine (genetic-based solutions, drug discovery and development) (61), AI-assisted computer vision (62), augmented and virtual reality (63) and the AI-assisted integration and collection of patients' records (64, 65) are just few examples of how AI can be applied to medicine. AI is on a trajectory of exponential growth, and has the potential to improve how we experience our lives and to extend life itself.

AUTHOR CONTRIBUTIONS

AP conceived the idea of the manuscript. AA and PC had oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team. AP and BM wrote the first draft of the manuscript. VD, GO, AA, and PC critically reviewed and amended the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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The Role of Fc Gamma Receptors in Antibody-Mediated Rejection of Kidney Transplants

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For the past decades, complement activation and complement-mediated destruction of allograft cells were considered to play a central role in anti-HLA antibody-mediated rejection (AMR) of kidney transplants. However, also complement-independent mechanisms are relevant in the downstream immune activation induced by donor-specific antibodies, such as Fc-gamma receptor (FcγR)-mediated direct cellular activation. This article reviews the literature regarding FcγR involvement in AMR, and the potential contribution of FcγR gene polymorphisms to the risk for antibody mediated rejection of kidney transplants. There is large heterogeneity between the studies, both in the definition of the clinical phenotypes and in the technical aspects. The study populations were generally quite small, except for two larger study cohorts, which obviates drawing firm conclusions regarding the associations between AMR and specific FcγR polymorphisms. Although FcγR are central in the pathophysiology of AMR, it remains difficult to identify genetic risk factors for AMR in the recipient's genome, independent of clinical risk factors, independent of the donor-recipient genetic mismatch, and in the presence of powerful immunosuppressive agents. There is a need for larger, multi-center studies with standardised methods and endpoints to identify potentially relevant FcγR gene polymorphisms that represent an increased risk for AMR after kidney transplantation.

Keywords: kidney transplant, renal transplantation, antibody-mediated rejection, AMR, FcγR, FcγR polymorphism

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INTRODUCTION

Kidney transplantation remains the most cost-effective treatment for patients with end-stage kidney failure (1). Antibody-mediated rejection (AMR) has been identified as a main reason for this failure (2–5). The term "AMR" defines allograft rejections caused by donor-specific antibodies (DSAs), either against anti-human leukocyte antigens (HLA), blood group antigens, or endothelial cell antigens (6). AMR has been reported to occur in 3%–12% of kidney transplant patients (7) but can be as high as 50% in patients with HLA incompatible transplants (8–10).

Abbreviations: AMR, antibody-mediated rejection; APC, antigen-presenting cell; DSA, donor-specific antibodies; FcγR, Fc-gamma receptor; IFNγ, interferon gamma; HLA, human leukocyte antigen; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; MHC, major histocompatibility complex; NK, natural killer; PI3-K, phosphatidylinositol3-kinase; PLCy, phospholipase Cy; PKC, protein kinase C; SNP, single nucleotide polymorphism; TCMR, T-cell mediated rejection.

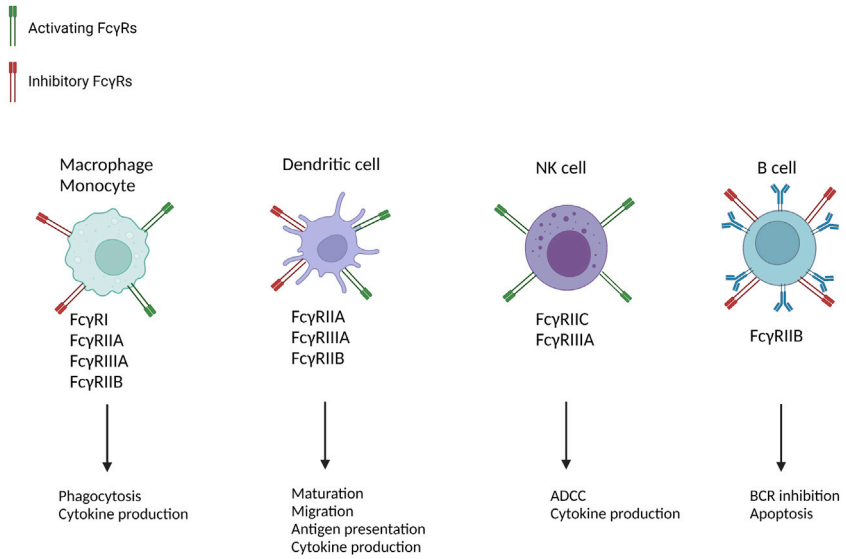

FIGURE 1 | Cellular distribution and function of FcγRs [Adapted from Castro-Dopico et al. (41)]. Multiple immune cells are implicated in AMR and express FcγRs. By promoting cell-type-specific immunological mechanisms they contribute to allograft rejection. B-cells only contain the inhibitory FcγRIIB, which is why they lead to BCR inhibition and apoptosis. NK-cells only express activating FcγRs which is why they only lead to activation of immunological mechanisms such as ADCC and cytokine production. Dendritic cells, macrophages and monocytes contain both activating and inhibitory FcγRs. ADCC, antibody-dependent cellular cytotoxicity; ROS, reactive oxygen species; NET, neutrophil extracellular traps; BCR, B-cell receptor.

Complement-mediated destruction of allograft cells induced by donor-specific anti-HLA antibodies (DSAs) is considered a key component to this pathophysiology of AMR, next to other mechanisms including alternative pathways of NK cell activation and antibody-dependent cellular cytotoxicity (11, 12). C1q binds to the antigen-antibody complexes on the graft endothelium. This activates the complement cascade which ultimately produces a membrane attack complex, initiating osmotic cell lysis. One of the complement split proteins (C4d) can covalently bind to the endothelium or basement membrane collagen. The presence of C4d in the allograft biopsy is therefore regarded as a marker of recent complement activation (13).

However, it was illustrated that graft survival is also impaired in patients with DSAs that are not complement-binding, when compared to patients without antibodies (14, 15). Furthermore, complement-inhibiting therapies did not effectively prevent AMR in all patients with non-complement binding DSAs (16–18). Finally, AMR cases often have no microvascular C4d deposition (19). Taken together, these findings suggest a role of complement-independent processes in antibody-mediated damage of kidney allografts.

Antibodies can also lyse target cells by complement-independent pathways, through the IgG Fc portion and FcγRs variably expressed at the surface of natural killer (NK) cells and of monocytes in a process known as antibody-dependent cell-mediated cytotoxicity (ADCC) (20–25). The antibody Fc region can bind to receptors on monocytes, macrophages, neutrophils, and NK cells. Through interaction between the Fc portion of the coating antibody and the Fc gamma receptor on NK cells, a signalling cascade is initiated that results in the release of cytotoxic granules (containing perforin, granzyme B) and production of cytokines (TNF-alpha and IFN-gamma), ultimately inducing apoptosis of the antibody-coated cell (22).

There are both inhibiting and activating FcγRs which differ in IgG affinity and signalling mechanisms. These signalling mechanisms can initiate various effector mechanisms including production of reactive oxygen species, cytokines and cytotoxins, immune cell recruitment and activation (**Figure 1**). Further evidence through histological appearances of FcγR expressing cells in AMR, transcriptomic signatures of FcγRIIIA transcripts in AMR and genetic association studies in transplantation that show a number of single nucleotide polymorphisms (SNPs) in FcγRs, have led to increasing evidence of the major role that FcγRs play in AMR (26–39).

Most SNPs or genetic polymorphisms have no effect on health or disease development, but some of them can act as biological markers by leading to variations in the amino acid sequence of a gene. This way, certain SNPs can be associated with certain diseases or a predisposition to develop a disease later. Several FcγR gene polymorphisms have been shown to change the functionality of FcγRs (29, 39, 40). Decreased immune cell activation, altered binding characteristics to immunoglobulins and altered receptor functions are some examples of how FcγRs can be influenced by certain SNPs. This article reviews the literature on the role of a complement-independent process via FcγRs in the pathophysiology of AMR, and the possible role of FcγR gene polymorphisms in the risk of rejection, AMR and ADCC. In 2016, Castro-Dopico et al reported on this topic (41). We re-evaluated the literature, including more recent references.

MATERIALS AND METHODS

A comprehensive literature search was performed by utilizing the following databases: PubMed, Embase and Web of Science core collection.

Our PubMed/MEDLINE search string consisted of the following terms: ("Receptors, IgG"[Mesh] OR "FcγR IIA" [Supplementary Concept] OR "FcγR IIB" [Supplementary Concept] OR "FcγR IIC" [Supplementary Concept] OR "FCΓR3A protein, human" [Supplementary Concept] OR "FCΓR3B protein, human" [Supplementary Concept] OR "FCΓR1A protein, human" [Supplementary Concept] OR "FcγR1 protein, mouse" [Supplementary Concept] OR "FcγR3 protein, mouse" [Supplementary Concept] OR "FcγR2a protein, rat" [Supplementary Concept] OR "Fcγ" OR "Fc gamma" OR "Fcgamma") AND ("Graft Rejection"[Mesh] OR [("transplant*" OR "graft*") AND "reject*")] AND ("kidney" OR "renal"). 55 hits were found on 14/04/2021.

Our Embase search string consisted of the following terms: "Fc receptor"/exp OR "Fc receptor": ti, ab, kw OR "Fc receptor IIa"/exp OR "Fc receptor Iib"/exp OR "Fc receptor Iic": ti, ab, kw OR "fc fragment receptor": ti, ab, kw OR "FcγR": ti, ab, kw OR "IgG fc receptor": ti, ab, kw OR "immunoglobulin fc fragment receptor": ti, ab, kw OR "immunoglobulin g fc receptor": ti, ab, kw OR "lymphocyte fc receptor": ti, ab, kw OR "FcγR": ti, ab, kw OR "FCΓR1A protein, human": ti, ab, kw OR "Fc gamma": ti, ab, kw OR "FCΓR3B protein, human": ti, ab, kw OR "Fcγ": ti, ab, kw AND "graft rejection"/exp OR "allograft rejection": ti, ab, kw OR "graft reaction": ti, ab, kw OR "allograft reaction": ti, ab, kw OR "transplant* rejection": ti, ab, kw AND "kidney"/exp OR "Renal": ti, ab, kw. 70 hits were found on 07 March 2021.

Our Web of Science core collection search string consisted of the following terms: TS=("Fc receptor" OR "Fc receptor IIa"/exp OR "Fc receptor IIb"/exp OR "Fc receptor IIc" OR "fc fragment receptor" OR "FcγR" OR "IgG fc receptor" OR "immunoglobulin fc fragment receptor" OR "immunoglobulin g fc receptor" OR "lymphocyte fc receptor" OR "FcγR" OR "FCΓR1A protein, human" OR "Fc gamma" OR "FCΓR3B protein, human" OR "Fcγ"). TS = ("graft rejection" OR "allograft rejection" OR "graft reaction" OR "allograft reaction" OR "transplant* rejection"). TS = ("kidney" OR "Renal"). 47 hits were found on 07 March 2021.

Study Selection

Articles from databases were identified and selected applying subsequent steps:

1) Identification of titles of records through database searching
2) Removal of duplicates
3) Screening and selection of abstracts. Abstracts had to contain information regarding both FcγRs and kidney transplant rejection (preferably AMR).
4) Judgement for eligibility through full-text articles; texts had to contain a thorough description of an FcγR polymorphism and AMR. They needed to report the incidence of the polymorphism comparing kidney transplant recipients with rejection to kidney transplant recipients without rejection.
5) Final inclusion in study.

After careful consideration, only five articles were included in the review. Multiple reviews and other articles were used to provide a framework and to refer to.

RESULTS

Fc-Gamma Receptor and Their Mechanisms of Action

FcγRs are glycoproteins that can be found on the surface of hematopoietic cells and bind to the Fc portion of IgG antibodies. This facilitates a link between the humoral and cellular immune systems (42). The family of FcγRs is involved in antigen presentation, regulation of B cell activation and initiation of intracellular signalling pathways which subsequently lead to immune cell activation and maturation (43). Classical FcγRs include an inhibitory receptor (FcγRIIB) and multiple activating receptors (FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA, and FcγRIIIB).

FcγRs have binding affinity for IgG and can recognize IgG-coated targets, such as opsonized pathogens or immune complexes. After cross-linking of activating FcγRs, tyrosine on the immunoreceptor tyrosine-based activation motif (ITAM) gets phosphorylated. Due note that cross-linking of FcγRs only occurs with aggregated IgG, such as opsonised cells or immune complexes, rather than monomeric IgG (44). Then both Src-kinases Lyn and subsequent recruitment of SH2-containing kinases are responsible for activating ITAM by phosphorylation. ITAMs are located either on the intracellular domain of the FcγRs (e.g., FcγRIIA) or in the associated common γ-chain (e.g., FcγRIIIA). ITAM-P leads to key recruitment of SH2 domain containing kinases, most notably spleen tyrosine kinase (SYK), and the subsequent activation of multiple downstream signalling mediators, including PI3K and PLCγ. All this leads to triggering protein kinase C (PKC) and initiating calcium flux (44, 45). The subsequent mechanisms differ between the different types of immune cells that express FcγR (**Figure 1**). Differences in these domains account for differences in function of FcγR. In contrast to activating FcγRs, FcγRIIB (inhibitory receptor) contains an intra-cellular immunoreceptor tyrosine-based inhibitory motif (ITIM). Cross-linking of FcγRIIB with activating FcγR leads to Src kinases phosphorylating ITIM and recruiting of inositol phosphatases to neutralise the activating signals (46). Therefore, the FcγRIIB can act as a supplementary regulatory mechanism and suppresses IgG-mediated inflammation (27).

Four different IgG subclasses in humans (IgG1-IgG4) are responsible for the action mechanism of FcγRs. The four IgG subclasses express different affinities to different receptors. IgG1 and IgG3 can efficiently activate the classical route of complement, while IgG2 and IgG4 do this less efficiently or only under certain conditions, as seen with IgG2. This can be explained by the reduced binding of C1q to IgG2 and IgG4 (47).

FcγRs are broadly expressed by hematopoietic cells such as natural killer (NK) cells, mast cells, macrophages, dendritic cells, neutrophils, monocytes, endothelial cells and B-cells (44). Cells can vary in the expression of different types of FcγRs and the

levels of expression of these FcγRs, allowing them to modulate the activation threshold when interacting with immune complexes (48). The activation state of FcγR-expressing cells is tightly controlled by the balance between activating and inhibitory FcγR, with the exception of NK cells (49). NK cells express only FcγRIIIA and no inhibitory FcγR. The distribution of the FcγRs across different cell types is illustrated in **Figure 1**. FcγR-ligated immune cells can directly activate the endothelium by binding to DSA and cause AMR through ADCC without interference of the complement-pathway.

Monocytes/Macrophages

Monocytes are innate immune cells that work as potent phagocytes and that can further differentiate into either macrophages or dendritic cells (50). Several studies suggest that monocyte infiltration is a key component of AMR after transplantation (34, 51, 52).

Macrophages express FcγRIIA, FcγRIIIA and FcγRIIB, with the activating FcγRs being more dominantly expressed. Activation of FcγRs leads to phagocytosis and cytokine release (TNF, IL6, IL-1alpha and neutrophil chemoattractants). These responses are counteracted by the inhibiting FcγRIIB (53). In dendritic cells this inhibiting FcγRIIB is dominantly expressed and suppresses immune-complex-mediated pro-inflammatory cytokine release, T-cell stimulation and migration (54–56).

Neutrophils

Human neutrophils express both FcγRI, FcγRIIA and FcγRIIIB. Activation of FcγRs on neutrophils leads to increased neutrophil adhesion to endothelial cells, cytokine and superoxide production, phagocytosis and neutrophil extracellular trap formation (NETosis) (57–61). When neutrophil infiltration in AMR is present, they are typically found in peritubular capillary lumens (62, 63).

Natural Killer Cells

NK cells primarily express activating FcγRIIIA and in some individuals a small fraction of NK cells may express FcγRIIC (64). As they do not express inhibitory FcγR, they could be the dominant effector cell in ADCC (65). When stimulated through their FcγR, they produce monocyte chemo-attractants CCL3, CCL4 and three effector cytokines; IFN-y, TNF and CSF2 (66).

B-Cells

The inhibitory FcγRIIB is the only FcγR expressed by B-cells. After crosslinking with B-cell receptors, the B-cell activation threshold will increase and suppress further antibody production (27).

Other Cell Types

Eosinophils express FcγRI, FcγRIIA, FcγRIIB and FcγRIIIB. Binding to antibodies induces degranulation. Platelets express FcγRIIA. Mast cells express FcγRIIB and FcγRIIIB. The role of eosinophils, platelets and mast cells seems limited in the process of AMR.

Different Fc-Gamma Receptor Polymorphisms Associated With Antibody-Mediated Rejection

Genetic variation in the genes of human FcγRs can alter receptor expression, function and affinity to IgG (27, 67). FcγR single nucleotide polymorphisms (SNPs) are now considered a hereditary risk factor for infectious and autoimmune diseases (68, 69). Also in allo-immune processes, genetic variations in FcγR genes could lead to different susceptibility to AMR. FcγRI has three non-synonymous SNP mutations (rs7531523, rs12078005, and rs142350980) but no studies investigating the association of these polymorphisms with AMR have been published (70). Furthermore, FcγRIIC has one SNP in intron 7 which has an effect on clearance of parasitaemia, but no studies have been published regarding the link with AMR (71). As there is currently no literature available on their association with AMR, they are not further discussed in this literature review.

FcγRIIA

FcγRIIA is a key FcγR for IgG-mediated responses in macrophages, monocytes or monocyte-derived dendritic cells (3, 72). FcγRIIA can also be found on the surface of neutrophils, platelets, basophils, eosinophils and other cells (73). The FcγRII gene is located on chromosome 1q23. Genetic variation in this gene locus is linked with several autoimmune and inflammatory diseases (68). The best-studied functionally relevant SNP, rs1801274, has been described in the extracellular domain of FcγRIIA, and exchanges adenine (A) to guanine (G) in the coding region in exon 4 of chromosome 1 (q23-24). As a result, histidine (H) is switched into an arginine (R) amino acid at position 131 in the immunoglobulin-like domain (H/R131), leading to altered receptor affinity and specificity (29). In contrast to FcγRIIIA, FcγRIIA polymorphisms seems to have less effect on AMR outcomes. This difference could be explained by the higher affinity of FcγRIIA for IgG1 instead of IgG3, opposite to the affinity observed in FcγRIIIA polymorphisms (74). The lack of inhibitory receptors on NK cells, who primarily express FcγRIIIA and lack inhibitory FcγRIIB expression, could contribute further to this observation (75).

Three studies investigated the association between the allelic frequency of this FcγRIIA H/R131 polymorphism in recipients with stable graft function compared to kidney transplant recipients with rejection (**Table 1**). First, Pawlik et al. conducted a case-control study in a population of 82 renal transplant recipients and found that the R/R131 genotype was associated with longer graft survival, which they hypothesized to be mechanistically explained by a lower affinity of this FcγR and less cytokine release, leading to a decreased immune response (39). The probability of graft survival over 7 years was 1.74-fold greater among subjects with the R/R131 polymorphism, compared to the H/H131 polymorphism. Next, and in contrast with their previous results, Pawlik et al. conducted another case-control study of 121 renal transplant recipients and found no significant differences in allele frequency between recipients with chronic rejection and recipients with

TABLE 1 | Distribution of the FcγRIIA genotypes and allele frequencies in patients with vs. without rejection. Numbers are noted as follows: X/Y (%). X = the number of patients with the specific polymorphism; Y = the total number of patients (study recipients or control population); % = the fraction is calculated to the percentage of people who carry the polymorphism; NS = not significant, X = the number of patients with the specific polymorphism. The *p*-value reflects the significance in differences of the allele frequencies between cases and controls.

	H/H131		H/R131		R/R131		*p*-value	Type of rejection
	Cases	Controls	Cases	Controls	Cases	Controls		
Yuan et al. (29) (Case-control study)	7/53 (13%) kidney transplant recipients with acute rejection	13/46 (28%) recipient non-rejectors	22/53 (42%)	24/46 (52%)	24/53 (45%)	9/46 (20%)	$p < 0.005$	Acute kidney rejection No DSA information present
Pawlik et al. (28) (Case-control study)	19/68 (27.9%) kidney transplant recipients with chronic allograft rejection	16/53 (30.2%) recipient non-rejectors	35/68 (51.5%)	26/53 (49.1%)	14/68 (20.6%)	11/53 (20.7%)	NS	Chronic kidney graft rejection No DSA information present
Wahrmann et al. (76) (Unselected cohort study)	55/229 (24%) kidney transplant recipients showing need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	206/781 (26.4%) kidney transplant recipients showing no need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	127/229 (55.5%)	412/781 (52.8%)	47/229 (20.5%)	163/781 (20.9%)	$p = 0.69$	Recipients treated for rejection within the first year after transplantation No DSA information present

stable graft function (28). However, Yuan et al., showed a significant positive association of the R/R131 genotype with acute kidney rejection (29). When homozygous, higher trends towards acute rejection were also observed. They noted that only 9 out of 46 (20%) non-rejectors had the FcγRIIA homozygote R/R131 polymorphism, compared to 24 out of 53 (45%) rejectors having the R/R131 polymorphism. The frequency of the R/R131 polymorphism was thus significantly higher in the rejector group compared to the non-rejector group. Finally, a recent large multicentre retrospective study with 1,940 kidney transplant recipients, found no association between the FcγRIIA H/R131 polymorphism and death-censored graft survival, graft function or requirement of rejection treatment (76). This study comprised an unselected cohort analysis with a patient cohort derived from the Collaborative Transplant Study (CTS, www.ctstransplant.org).

FcγRIIIA

FcγIIIA (CD16) is expressed on monocytes/macrophages, dendritic cells, and NK-cells. FcγRIIIA is the only human activating FcγR that has a preferential binding to IgG3. In kidney transplantation, it is suggested that IgG3- DSA positive recipients show more intense microvascular inflammation (77). These findings further suggest the key role of NK cells, monocytes and macrophages in orchestrating the inflammation observed in AMR and may also be, at least in part, the culprits behind the more damaging effects seen with complement-fixing HLA antibodies (15). This further contributes to our hypothesis that different effector mechanisms together lead to graft loss, and not complement-activation alone.

A functional SNP (rs396991) in the gene of FcγRIIIA substitutes a valine (V) to phenylalanine (P) amino acid at position 158 (V/F158), alters the affinity to IgG1 and IgG3 and thus influences immune cell activation (74, 78). For example, Arnold et al. described greater frequency of peritubular capillaritis when the FcγIIIA V158 allele was present due to greater immune cell recruitment in peritubular capillaries (79). Two studies discussed the association between the FcγRIIIA V/F158 polymorphism and AMR after kidney transplantation (**Table 2**). A case-control study by Litjens et al. linked the V-allele to an increased expression of FcγRIIIA on NK cells and to an increased glomerulitis score in a study of 141 kidney transplant patients (40). Confirming the earlier associations seen in Arnold et al. (79), they observed an association between V-allele and decreased kidney allograft survival after diagnosis of chronic AMR, but the 158V/V genotype itself did not appear to be a risk factor for the development of chronic AMR. Other than the positive association of this polymorphism and increased risk of graft failure after diagnosis of chronic AMR (40), also in heart and lung transplantation clinical associations of cardiac allograft vasculopathy and acute lung transplant rejection with FcγRIIIA polymorphisms have been observed (80, 81). This association between the V/F158 SNP in FcγRIIIA and increased risk of graft failure could be mediated by target cells opsonizing IgG antibodies to bind to FcγRIIIA on immune cells, followed by the release of cytotoxic granules which trigger apoptosis of the target cells. FCGR3A gene expression is also increased in biopsies diagnosed with AMR (36–38). Especially NK cells, which do not express the inhibitory FcγRIIB and thus cannot compensate for overactive FcγRIIIA signalling, could be major contributors to the deleterious effect of this polymorphism.

Despite these first suggestions of a significant association between the FcγRIIIA V/F158 polymorphism and AMR and outcome after kidney transplantation, a more recent and larger study included 1940 kidney transplant recipients (76). This study could however not confirm any association of the FcγRIIIA V/F158 polymorphism and impaired allograft function or increased need for rejection treatment within the first year after transplantation. Also in a subanalysis in 438 patients with higher risk of AMR, there was no association of Fc*γ*RIIIA polymorphisms with 10-year death-censored graft survival in this subgroup. We do note that Wahrmann et al. didn't specifically investigate different mechanisms responsible

TABLE 2 | Distribution of the FcγRIIIA genotypes and allele frequencies in patients with vs. without rejection. Numbers are noted as follows: X/Y (%). X = the number of patients with the specific polymorphism; Y = the total number of patients (study recipients or control population); % = the fraction is calculated to the percentage of people who carry the polymorphism; NS = not significant, X = the number of patients with the specific polymorphism. The *p*-value reflects the significance in differences of the allele frequencies between cases and controls.

	V/V158		V/F158		F/F158		*p*-value	Type of rejection
	Cases	Controls	Cases	Controls	Cases	Controls		
Litjens et al. (40) (Case-control study)	21/133 (15.8%) kidney transplant recipients with c-aAMR	17/116 (14.7%) recipient non-rejectors	59/133 (44.4%)	46/116 (48.7%)	53/133 (39.8%)	53/116 (45.7%)	$p = 0.65$	Chronic active AMR. DSA information present
Wahrmann et al. (76) (Unselected cohort study)	29/229 (12.7%) kidney transplant recipients showing need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	105/781 (13.4%) kidney transplant recipients showing no need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	104/229 (45.4%)	350/781 (44.8%)	96/229 (41.9%)	326/781 (37.4%)	$p = 0.85$	Recipients treated for rejection within the first year after transplantation No DSA information present

for allograft loss, like microvascular inflammation, whereas Litjens et al. did (40, 76).

FcγRIIIB

FcγRIIIB is expressed on neutrophils and eosinophils. The main function of FcγRIIIB is immune cell clearance of all cells that contain immunoglobulins recognized by FcγRIIIB. By triggering internalisation of captured immune complexes, degradation of antigen-antibody complexes can occur (44). Four amino acid substitutions lead to differences in glycosylation resulting in a FcγRIIIB NA1/NA2 polymorphism. NA1 is more efficient in binding to immune complexes containing IgG1 and IgG3 than NA2 and reduced binding affinity of NA2 genotype could potentially mean that clearance of immune complexes may be reduced (82–85). Furthermore, NA2/NA2 homozygotes show a lower capacity to mediate phagocytosis (86, 87). Because the expression of FcγRIIIB is limited to neutrophils and eosinophils, an association with FcγRIIIB polymorphisms and AMR is not expected. This is due to the fact that neutrophils are rarely observed in late AMR (79). Two studies investigated the difference in incidence of this polymorphism in FcγRIIIB between kidney transplant recipients with stable graft function and kidney transplant recipients with rejection (76, 88) (**Table 3**). First, a case-control study by Xu et al. showed that NA1/NA2 genotype frequency and allele frequency were not related to acute rejection vs. well-functional grafts in kidney transplant recipients. More recently, Wahrmann et al. confirmed the lack of association between the FcγRIIIB NA1/NA2 polymorphism and death-censored kidney graft survival, graft function or requirement of rejection treatment, in a large cohort of 1,940 kidney transplant recipients.

FcγRIIB

FcγRIIB is the only inhibitory FcγR and can be found on B cells, mast cells, macrophages, neutrophils, and eosinophils. The rs1050501 SNP induces a threonine to isoleucine substitution at position 232. Because this occurs within the transmembrane domain of the receptor, FcγRIIB I/T232 is responsible for the dysfunction of the receptor (89, 90). Dysfunction of this inhibitory receptor could theoretically lead to increased immune activation and associations with several autoimmune diseases have been found such as systemic lupus erythematosus, MS and ITP (87, 91–94). Murine studies previously showed associations between FcγRIIB I/T232 and outcomes on kidney allograft by raising the susceptibility to develop chronic AMR (95), but these results could not be replicated in a large human study by Clatworthy et al. (96). They conducted an analysis of three cohorts of 2,851 Caucasian transplant recipients, 570 Afro-Caribbean transplant recipients and 236 patients with a diagnosis of SLE derived from the CTS (96). No association could be found between presence of the FcγRIIB I/T232 polymorphism and differences in 10-year transplant survival. This contradiction could be explained by the observation that expression, structure, associated signalling molecules and most importantly, affinity for different IgG subclasses differ between murine and human FcγRs (97–99). They do however note that their failure to detect an association could be because their effect size of this SNP is smaller than estimated by their power calculations (96). An increased number of patients in a follow-up study could more accurately detect differences or further prove that no associations can be found.

DISCUSSION

Antibody-dependent cellular cytotoxicity is considered to play a major role in the pathophysiology of AMR after kidney transplantation, through the involvement of FcγRs. The mechanism of action and cellular expression of these receptors is well known. Several functional SNPs have been described in these FcγRs and could theoretically impact the risk of AMR after kidney transplantation. Although several studies have addressed this question, it remains however difficult to make conclusions about the role of FcγRs polymorphisms in the risk of AMR. Earlier and smaller studies (28, 30, 40, 88) described associations between FcγR polymorphisms and microcirculation inflammation. However, Wahrmann et al. did not confirm associations between these Fc*γ*R gene variants and early rejection, graft function, or long-

TABLE 3 | Distribution of the FcγRIIIB genotypes and allele frequencies in patients with vs. without rejection. Numbers are noted as follows: X/Y (%). X = the number of patients with the specific polymorphism; Y = the total number of patients (study recipients or control population); % = the fraction is calculated to the percentage of people who carry the polymorphism; NS = not significant, X = the number of patients with the specific polymorphism. The *p*-value reflects the significance in differences of the allele frequencies between cases and controls.

	FcγRIIIB (NA1/NA1)		FcγRIIIB (NA1/NA2)		FcγRIIIB (NA2/NA2)		*p*-value	Type of rejection
	Cases	Controls	Cases	Controls	Cases	Controls		
Xu et al. (88) (Case-control study)	9/85 (10.6%) kidney transplant recipients with AMR or cellular rejection	11/86 (11%) recipient non-rejectors	60/85 (70.6%)	61/86 (70.9%)	16/85 (18.8%)	14/86 (16.3%)	p = NS	Acute AMR or cellular kidney rejection No DSA information present
Wahrmann et al. (76) (Unselected cohort study)	30/229 (13.1%) kidney transplant recipients showing need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	87/781 (11.1%) kidney transplant recipients showing no need of rejection treatment during 1 year in a cohort of 1010 kidney transplant recipients	108/229 (47.2%)	349/781 (44.7%)	91/229 (39.7%)	345/781 (44.2%)	p = 0.20	Recipients treated for rejection within the first year after transplantation No DSA information present

term allograft failure (76). Even in patients who were sensitised and thus at higher risk for AMR, no associations were found with transplant outcomes.

The discrepancy between the studies are primarily explained by the wide heterogeneity in the choice and definition of the primary endpoints (graft dysfunction, acute and chronic rejection, graft survival time, …), which make comparisons between the studies complex. If for instance the rejection subtype is not evaluated, as was the case for Wahrmann et al. (76), it could be that potential associations between polymorphisms and subtypes of rejection are missed. Other sources of heterogeneity include demographic differences between the cohorts, differences in study design, different background immunological risk of the included patients, numbers of centres, era, etc. Study populations were overall rather small with the exception of the studies by Clatworthy et al. and Wahrmann et al. (76, 96). Also, when AMR is studied, detailed information on DSA is necessary, which is often not available (100). This is a major limitation of the literature on this topic, which importantly hampers making strong conclusions on the association of FcγR polymorphisms and AMR. This could explain why most studies, including Wahrmann et al., have failed to find any associations, while studies where detailed DSA information was available described significant associations between FcγR polymorphisms and the risk of prognosis of AMR. More systematic research on larger-scale collaborative cohorts, and detailed phenotyping of the cases are needed.

In conclusion, our literature review indicates a role of FcγRs in kidney transplant rejection, and the theoretical relevance of the FcγRs polymorphisms in AMR after kidney transplantation. However, in the absence of robust and sufficiently detailed and large-scale studies assessing the actual association of the polymorphisms with well-defined clinical events, we cannot make any robust conclusion on the clinical relevance of these polymorphisms. Furthermore, the two largest, multicenter studies, could not provide evidence for functional FcγR polymorphisms and therefore no impact on incidence of AMR. More systematic large and multi-center studies are needed to robustly determine the potential role of FcγR polymorphisms in the risk of AMR after kidney transplantation, independent of clinical risk factors and the donor-recipient genetic mismatch and in presence of potent immunosuppressive agents, but most importantly, with notion of DSA present.

AUTHOR CONTRIBUTIONS

BD performed the literature review and drafted the manuscript. EV and MN revised the manuscript critically for important intellectual content. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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META-ANALYSES AND SYSTEMATIC REVIEWS
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The Willingness to Donate Organs in Medical Students From an International Perspective: A Meta-Analysis

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Attitude toward organ donation mobilizes donation behavior and makes transplant surgery possible. As future health professionals, medical students will be a relevant generating opinion group and will have an important role in the organ requesting process. The goals of this meta-analysis were to obtain polled rates of medical students who are in favor, against, or indecisive toward cadaveric organ donation in the studies conducted around the world, and to explore sociocultural variables influencing the willingness to donate. Electronic search and revision of references from previous literature allowed us to locate 57 studies fulfilling the inclusion criteria. Data extraction and risk of bias assessment were performed by two independent investigators. Pooled estimations were computed assuming a random-effects model. Despite the fact that willingness to donate was elevated in medical students, estimated rates in studies from different geographical areas and sociocultural backgrounds exhibited significant differences. The age and the grade of the students also influenced the rate of students in favor. Donation campaigns should take into account cultural factors, especially in countries where certain beliefs and values could hamper organ donation. Also, knowledge and skills related to organ donation and transplant should be acquired early in the medical curriculum when a negative attitude is less resistant to change.

Keywords: meta-analysis, willingness, medical students, organ donation, cultural

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INTRODUCTION

Despite the advances in the field of organ donation and transplant, current rates of donation are still insufficient to cover minimum needs. The organ deficit is the main cause of death in waitlisted patients (1). There are several factors involved in the process of requesting and donating organs for transplants. Sociocultural factors are one of the main sources of variability among studies on the attitudes toward donation. First, geographical area influences the willingness to donate. Differences in organ donation systems and organ requesting protocols in each country mean that even people from similar cultural backgrounds (e.g., Latin) and living in different geographic areas could exhibit different levels of disposition to donate (2). Second, attitudes to donation are dependent on the local cultural and socioeconomic background. Death conceptions, religion, and values must be considered

by the organ donation system in each country for transplantation programs to be successful (3, 4). Finally, sociodemographic factors such as age, gender, and educative level have also been shown to have influence on attitudes toward donation and transplant.

Health professionals have an important role in the successful development of the organ donation process (5). In the community context, they are one of the most relevant opinion-generating groups. Moreover, negative attitudes based on information provided by professionals are more resistant to change since they are supported by experts (6). Medical students are the new generation of clinicians, and therefore, the future link between donors and recipients.

Obtaining knowledge about attitudes toward cadaveric organ donation in medical students has been considered of particular importance and exists in a wide range of scientific literature. Research has been conducted in different countries and cultural backgrounds, has examined different dimensions of organ donation attitudes (awareness, willingness, registration, etc.), and has used a variety of methodological procedures. As a consequence, the results reported a high heterogeneity across studies. Despite its extension, the literature has not been systematically integrated and factors behind the heterogeneity of findings have not been explored yet. Meta-analytical procedures could contribute to reaching well-established conclusions about the intention of medical students to donate their organs after death.

Following the PICOS strategy to formulate questions in meta-analyses, the current study intended to answer the following question: what is the rate of medical students (participants) who are in favor (outcome) of donating their organs after death (intervention) in observational studies (study design)? From this question, two goals were considered: 1) to obtain the polled estimated rate of medical students who were in favor, against, or indecisive toward cadaveric organ donation; and 2) to explore sociocultural variables influencing the willingness to donate. We expected that the elevated pooled rate of medical students in favor of cadaveric donation would be superior to rates of students against and indecisive. It is likely that rates of students willing to donate were influenced by potential moderators, such as geographical area, grade of students, and gender.

MATERIALS AND METHODS

This meta-analysis was performed following the PRISMA 2020 Guideline for Reporting Meta-analyses (7) and the MOOSE Checklist for Meta-analyses of Observational Studies (8). See **Supplementary Data Sheet S1**.

Inclusion and Exclusion Criteria

To be included in the meta-analysis studies had to fulfill the following eligibility criteria: 1) assess willingness to donate organs after death; 2) report necessary statistics to compute the proportion of participants who are willing to donate (events and sample size); 3) participants were medical students; 4) observational designs without experimental manipulations; and 5) published in English, Spanish, or Portuguese. Studies examining attitudes toward living donation, donation of specific organs, studies that did not report results for medical students separately from samples of other populations (e.g., non-medical students, general public, etc.), and studies sharing samples (totally or partially) with other included studies were excluded. Studies in languages other than English, Spanish, or Portuguese could not be included due to the language limitations of researchers.

Search Strategy

An electronic search was conducted in PubMed, CINALH Complete, PsycInfo, and Psychology and Behavioral Sciences Collection until February 2021. English and Spanish keywords were organ donation AND (attitude OR willingness OR perceptions OR beliefs OR opinions) AND medical students. References of previous meta-analyses (9–11) and studies collected were also screened. Finally, the most prolific authors in the field were contacted to request potential unpublished data. **Figure 1** shows the search and eligibility processes in the PRISMA flow diagram.

The electronic search yielded 403 outputs, and 28 references were located from previous publications. After deleting duplicates, the title and abstract of 357 papers were reviewed. After excluding a further 229, the full text of 128 articles was reviewed to assess their potential inclusion; 73 articles were rejected due to reasons shown in **Figure 1**. Finally, 54 papers (12–65) including 56 separate samples fulfilled the inclusion criteria.

Data Extraction

A data extraction protocol including statistics and potential moderator variables was elaborated and applied by two independent investigators to each selected study. Variables concerning participants were: 1) gender (percentage of men); 2) rate of men in favor; 3) rate of women in favor; 4) mean age; 5) the percentage of students in each grade; 6) proportion of first-grade students in favor; 7) proportion of students in the last grade in favor; 8) country of participants; 9) continent; 10) cultural background in the country of participants; and 11) the percentage of participants of each religion. Variables related to the methodology of studies were: 1) year of survey; 2) completion rate; 3) type of measure (interview or self-report); 4) administration modality (face-to-face, online, or both); and 5) methodological quality of the study (rated from 0 to 5, see Quality Assessment section).

Risk of Bias Assessment

To assess the risk of bias in individual studies, a five-item checklist was elaborated based on the STROBE Checklist for cross-sectional studies (66). Items were rated as follows: 1) setting: whether the study provided information about locations, setting, and dates of data collection (1 yes, 0 no); 2) sample size: whether the study explained how the sample size was arrived at (1 yes, 0 no); 3) participants: whether the study reported eligibility criteria and methods of selection of participants (1 yes, 0 no); 4) completion rate: whether the study reported the percentage of distributed surveys that were retrieved (1 yes, 0 no); and 5) outcome: whether the study employed a validated outcome measure or conducted a pilot study prior to its administration

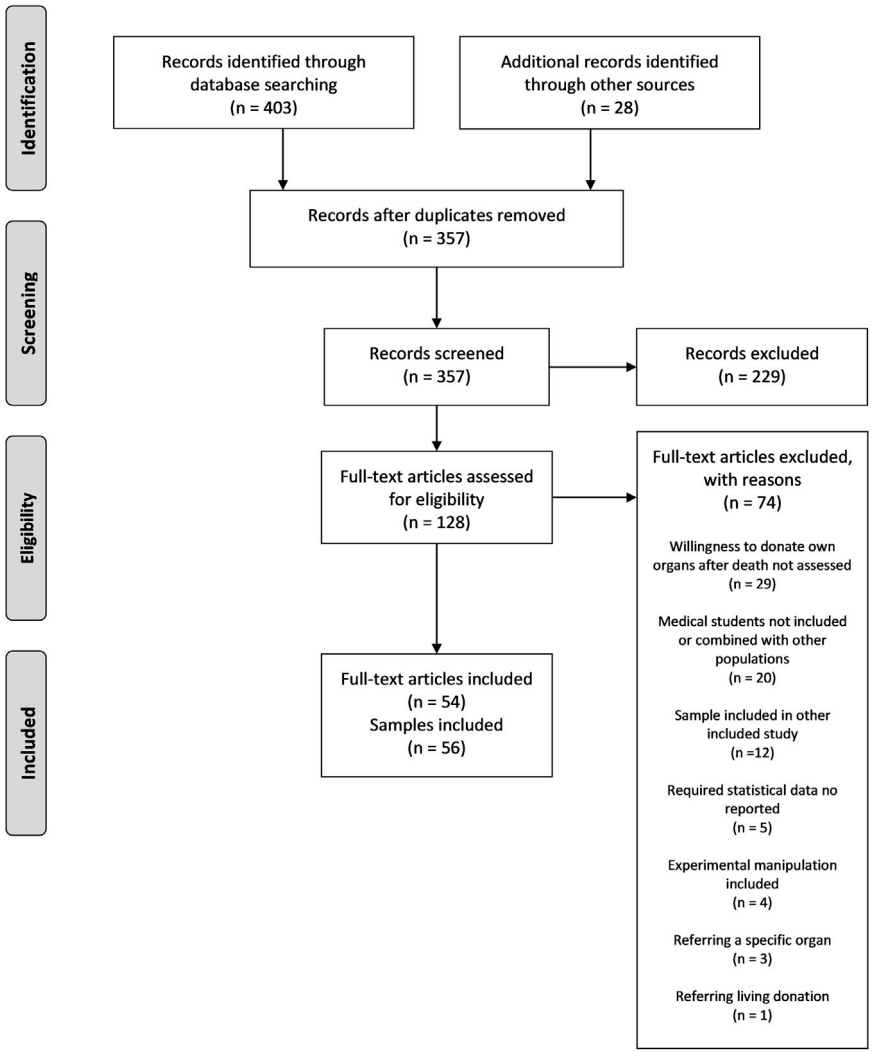

FIGURE 1 | PRISMA flow diagram.

(1 yes, 0 no). A methodological quality score was computed as the sum of the five items.

Statistical Analysis

The primary outcome was the pooled estimate rate (proportion) of medical students who were willing to donate organs after death. Rates of students against and indecisive were also extracted as secondary outcomes. Under the assumption that samples of selected studies could be representative of different populations, pooled rates were computed assuming a random-effects model, where each individual proportion was pondered by its precision. Heterogeneity was examined by computing Q statistics and the percentage of the observed variance between studies' I^2. To analyze the effect of potential moderator variables on the primary outcome (rate of students in favor), ANOVAs with Q_B statistics and meta-regression models with Q_R statistics were computed for categorical and continuous variables, respectively. The percentage of explained variance was assessed by R^2 index (67). Publication bias analysis included the Egger test and the construction of a funnel plot implementing the trim-and-fill method (68). All data analyses were conducted in Comprehensive Meta-Analysis (CMA) 3.0 (69).

RESULTS

Study Characteristics and Risk of Bias

Table 1 shows the main characteristics of the 56 independent studies included in the meta-analysis. Studies were conducted in 25 different countries between 1999 and 2020. The total sample included 33,536 medical students with mean ages between 17.60 and 26.35 years. The percentage of men ranged from 16.6% to 93.8%. The completion rate reported by the studies ranged from 32% to 100%. Concerning the risk of bias, the mean methodological quality was 2.18, with 35.1% of studies having scores ≥3 See **Supplementary Table S1**.

TABLE 1 | Summary of the included studies.

Study	Year of survey	Country	No. of participants	Completion rate, %	Quality, range 1–5	Age, mean	Men, %	In favor, %	Against, %	Indecisive, %
Akkas et al. (12)	2013	Turkey	100	66.80	3	17.60	43.0	54.00	16.00	30.00
Akkas et al. (12)	2013	Turkey	100	66.80	3	24.20	56.0	70.00	14.00	16.00
Ali et al. (13)	2011	Pakistan	158	81.02	3	20.00	36.7	44.94	—	—
Alnajjar et al. (14)	2019	Saudi Arabia	113	74.83	5	20.04	93.8	55.75	8.85	35.40
AlShareef et al. (15)	2016	Saudi Arabia	225	36.12	2	22.77	68.0	38.22	19.11	42.67
Anwar et al. (16)	2019	Bangladesh	100	—	1	—	—	28.00	16.00	48.00
Ashfaq et al. (17)	2017	Pakistan	400	—	3	20.98	50	61.25	—	—
Atamañuk et al. (18)	2016	Argentina	1012	96.80	3	21.40	35.5	81.92	—	—
Bilgel et al. (19)	—	Turkey	409	80.50	2	20.30	49.9	58.44	22.74	18.83
Burra et al. (20)	—	Italy	100	51.30	1	23.70	29.0	88.00	—	—
Cahill & Ettarh (21)	2007	Ireland	187	87.00	2	—	—	63.64	7.49	28.88
Chung et al. (22)	2006	China	655	94.00	2	21.00	58.0	85.04	—	—
Dahlke et al. (23)	—	Germany	165	—	1	21.50	35.2	56.36	—	—
Dahlke et al. (23)	—	Japan	99	—	1	22.40	72.7	52.53	—	—
Dahlke et al. (23)	—	United States	66	—	1	23.90	48.5	65.15	—	—
Dibaba et al. (24)	2019	Ethiopia	320	—	2	23.48	57.8	58.12	—	—
Dutra et al. (25)	2002	Brazil	779	77.82	2	21.90	59.5	69.06	30.68	—
Edwards et al. (26), Essman (29)	2005	United States	500	93.00	3	24.00	50.0	82.40	5.00	9.00
El-Agroudy et al. (27)	2017	Bahrein	376	75.20	2	22.10	39.1	71.81	18.88	11.97
Englschalk et al. (28)	2015	Germany	181		2	23.10	37.6	82.32	7.18	9.94
Figueroa et al. (30)	2011	Holland	506	84.00	3	20.76	26.6	79.84	5.73	14.03
Galvao et al. (31)	—	Brazil	347	32.00	3	—	—	89.91	10.09	—
Goz et al. (32)	—	Turkey	213	36.91	2	—	—	56.81	—	—
Hamano et al. (33)	2018	Japan	702	100.00	2	25.00	—	54.70	13.96	31.05
Hasan et al. (34)	2019	Pakistan	157	82.00	2	20.60	16.6	41.40	—	—
Inthorn et al. (35)	2009	Germany	466	95.10	2	—	—	63.52	—	—
Jamal et al. (36)	2017	Pakistan	150	88.50	4	—		61.33	—	—
Jung et al. (37)	—	Romania	140	—	0	20.50	30.0	81.43	3.57	15.00
Kirimlioglu et al. (38)	—	Turkey	214	71.30	2	20.00	45.8	22.43	27.10	—
Kobus et al. (39)	—	Poland	203	—	0	21.80	-	94.58	—	—
Kocaay et al. (40)	2013	Turkey	88	—	1	—	—	60.23	—	—
Kozlik et al. (41)	2012	Poland	400	—	2	21.80	37.3	90.50	3.00	6.50
Lei et al. (42)	2016	China	284	—	2	—		15.14	—	—
Lima et al. (43)	2007	Brazil	300	85.70	3	—	51.0	62.00	—	—
Liu et al. (44)	2019	China	1363	90.90	2	21.5	39.5	62.73	37.27	
Marques et al. (45)	2008	Puerto Rico	227	76.70	3	—	49.1	88.55	11.01	—
Marván et al. (46)	2018	Mexico	205	—	3	—	48.3	91.71	—	—
Mekahli et al. (47)	2006	France	571	—	1	18.50	34.5	81.09	13.49	5.43
Naçar et al. (48)	2014	Turkey	464	94.70	1	20.90	48.9	50.00	5.82	44.18
Najafizadeh et al. (49)	2006	Iran	41	—	1	22.80	44.0	87.80	4.88	—
Ohwaki et al. (50)	2004	Japan	388	100.00	2	—	74.0	59.02	15.98	21.91
Ríos et al. (51)	2011	Spain	9275	95.70	5	21.00	28.2	79.53	1.66	18.91
Rydzewska et al. (52)	—	Poland	569	—	0	21.77	25.8	92.97	2.46	4.57
Sağiroğlu et al. (53)	2012	Turkey	356	71.80	2	20.40		49.44	16.85	33.71
Sahin and Abbasoglu (54)	2013	Several countries	1541	—	2	21.80	41.0	94.35	1.36	4.28
Sampaio et al. (55)	—	Brazil	518	49.01	1	—	25.9	84.94	1.35	13.71
Sanavi et al. (56)	2008	Iran	262	97.00	1	22.10	32.0	85.11	—	—
Sayedalamin et al. (57)	2014	Saudi Arabia	481	—	2	21.39	48.0	31.81	68.19	—
Sebastián-Ruiz et al. (58)	2015	Mexico	3056	—	2	20.30	53.3	73.99	26.01	—
Tagizadieh et al. (59)	2016	Iran	400	—	2	26.35	59.0	85.00	15.00	—
Tuesca et al. (60)	1999	Colombia	993	84.27	5	25.00	52.6	84.79	6.65	8.56
Tumin et al. (61)	2014	Malaysia	264	88.00	4	—	—	72.73	—	—
Verma et al. (62)	—	India	1463	73.00	3	-	44.9	65.62	34.38	—
Wu et al. (63)	—	China	264	88.00	3	20.25	29.5	39.77	42.05	18.18
Zahmatkeshan et al. (64)	2012	Iran	340	—	3	—	—	79.12	9.41	11.47
Zhang et al. (65)	—	China	199	—	1	—	43.2	32.16	27.14	40.70

TABLE 2 | Pooled estimated rates, confidence intervals, and heterogeneity indexes for study outcomes.

Outcome	*K*	*Q*	I^2	p_+	95% C.I.	
					I_l	I_u
Students in favor	56	3144.31***	98.25	0.692	0.647	0.734
Students against	36	2978.40***	98.82	0.117	0.084	0.161
Indecisive students	27	973.39***	97.33	0.177	0.140	0.220

C.I., confidence interval; k, number of studies; Q, heterogeneity statistic; I^2, heterogeneity index; p_+*, pooled estimated rate,* I_l *and* I_u*, lower and upper confidence limits.*
***p < *0.001.*

Pooled Rates of Medical Students in Favor, Against, and Indecisive

Table 2 shows combined estimated proportions and confidence intervals for each outcome in the meta-analysis. In the primary outcome, a combined percentage of 69.2% (95% CI: 64.7%–73.4%) of medical students was willing to donate their organs after death. Significant and high heterogeneity was observed (I^2 = 98.25%). Regarding secondary outcomes, the pooled estimation of students against donating, including 36 studies, was 11.7% (95% CI: 8.4%–16.1%) and the pooled estimation for indecisive students, including 27 studies, was 17.7% (95% CI: 14%–22%). Heterogeneity tests showed significant and high variability among studies in both against (I^2 = 98.82%) and indecisive (I^2 = 97.33%) participants.

Factors Influencing the Willingness to Donate

Participant-Related Variables

Continent, Culture, and Religion

Significant differences were observed depending on the continent where the study was conducted (Q_3 = 27.13, p <0.000). The highest pooled rates of students in favor were obtained by the studies conducted in North America (k = 2, p_+ = 0.753, 95% CI [0.554, 0.882]), Latin America (k = 9, p_+ = 0.820, 95% CI [0.767,

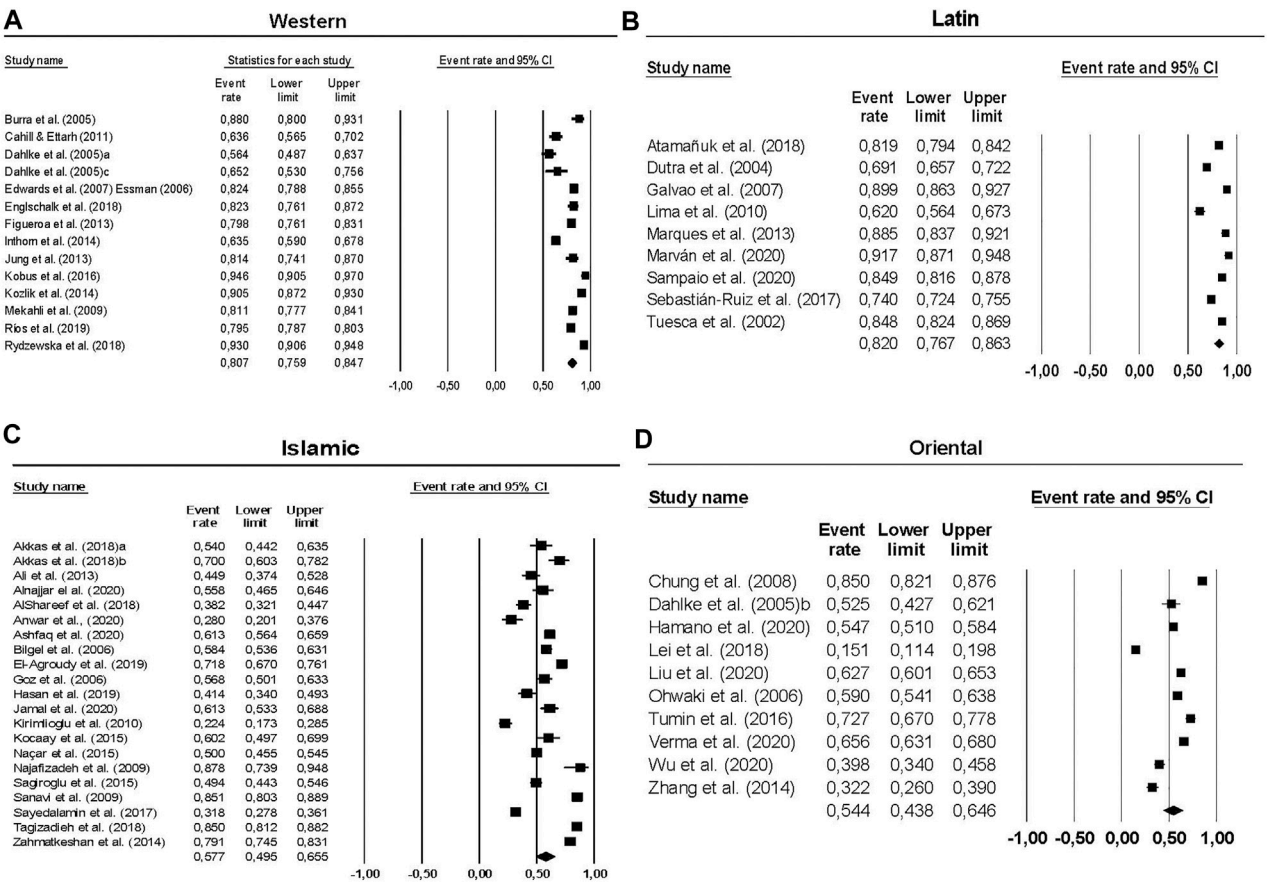

FIGURE 2 | Forest plots of individual rates and confidence intervals for each study (squares) and pooled estimations and confidence intervals for each cultural background (diamonds). **(A)** Forest plot of individual and pooled rates of students willing to donate in Western countries. Individual rates vary from 0.564 to 0.940. The pooled estimated rate by the random-effects model was 0.807. **(B)** Forest plot of individual and pooled rates of students willing to donate in Latin countries. Individual rates vary from 0.620 to 0.917. The pooled estimated rate by the random-effects model was 0.820. **(C)** Forest plot of individual and pooled rates of students willing to donate in Islamic countries. Individual rates vary from 0.224 to 0.878. The pooled estimated rate by the random-effects model was 0.577. **(D)** Forest plot of individual and pooled rates of students willing to donate in Oriental countries. Individual rates vary from 0.151 to 0.850. The pooled estimated rate by the random-effects model was 0.544.

0.863]), and Europe (k = 20, p = 0.718, 95% CI [0.642, 0.784]) which were significantly superior to the pooled rate for studies in Asia (k = 23, p_+ = 0.580, 95% CI [0.503, 0.654]). Given these results, and to obtain a more accurate view of differences, we considered grouping studies by predominant culture in the country of participants. **Figure 2** shows forest plots of pooled estimations for each cultural background and individual rates for each study. Cultural background significantly influenced the willingness to donate (Q_3 = 49.850, p < 0.000). Higher rates were observed for studies in countries with Latin (k = 9, p_+ = 0.820, 95% CI [0.767, 0.863]) and Western (k = 14, p_+ = 0.807, 95% CI [0.760, 0.850]) cultural backgrounds, finding significant differences with Islamic (k = 21, p_+ = 0.577, 95% CI [0.495, 0.655]) and Oriental (k = 10, p_+ = 0.544, 95% CI [0.438, 0.646]) countries. Regarding religion, the percentage of Catholic students showed a positive and significant relationship with the proportion of students in favor (k = 15, b_j = 0.02, Q_1 = 28.09, p <0 .000, R^2 = 0.44) whereas the percentage of Muslim students was not related to the rate of students in favor (k = 10, b_j = −0.01, Q_1 = 2.13, p = 0.144, R^2 = 0.00). The influence of the percentage of students affiliated with other religions could not be analyzed due to the reduced number of studies that reported these data.

Age and Grade of Participants

The mean age of participants showed a significant and positive relationship with the proportion of students in favor of donating (k = 39, b_j = 0.16, Q_1 = 4.85, p = 0.024, R^2 = 0.10) explaining 10% of the variance. Results of meta-regression analyses showed that percentages of students in 2nd, 3rd, 4th, 5th, and 6th grade included in the studies, were not significant predictors of the willingness to donate (p >0 .05). Only the percentage of first-grade students showed a significant and negative relationship with the proportion of students in favor of donation (k = 25, b_j = -0.01, Q_1 = 4.75, p = 0.029, R^2 = 0.06) with 6% of the accounted variance. There were marginally significant differences between first-grade (k = 13, p_+ = 0.65, 95% CI [0.55, 0.73]) and sixth-grade students (k = 10, p_+ = 0.79, 95% CI [0.67, 0.87]) according to the subgroup analysis (Q_1 = 3.79, p = 0.052).

Gender

The percentage of men was not a significant predictor of the willingness to donate (k = 43, b_j = −0.02, Q_1 = 2.56, p = 0.11, R^2 = 0.00). Similarly, subgroup analysis did not yield significant differences (Q_1 = 1.487, p = 0.223) in the proportion of men (k = 9, p_+ = 0.61, 95% CI [0.52, 0.69]) and women (k = 9, p_+ = 0.68, 95% CI [0.59, 0.77]) in favor.

Methodological Variables

Meta-regression analysis revealed that the completion rate (k = 34, b_j = 0.00, Q_1 = 0.02, p = 0.900, R^2 = 0.00) and the methodological quality score (k = 56, b_j = −0.03, Q_1 = 0.06, p = 0.810, R^2 = 0.00) were not significantly associated with the proportion of students willing to donate. Only the year of survey (k = 41, b_j = −0.07, Q_1 = 8.79, p = 0.003, R^2 = 0.08) was negatively associated with the rate of students in favor. There were not significant differences between face-to-face (k = 48, p_+ = 0.68, 95% CI [0.64, 0.73]) and online (k = 6, p_+ = 0.68, 95% CI [0.46, 0.85]) administration (Q_1 = 0.000, p = 0.997).

Publication Bias Analysis

First, results from Egger's test were not significant (b_0 = −2.89; t [54] = 1.60, p = 0.115), supporting the absence of publication bias. Second, after the implementation of the trim-and-fill method, it was not necessary to introduce imputed values into the funnel plot to reach symmetry (**Figure 3**), with the pooled proportion of adjusted values equal to the pooled proportion of observed values.

DISCUSSION

This is the first meta-analysis on the willingness to donate in medical students. Similarly, this is the first work analyzing cultural and individual variables as potential explaining factors of the variability of results reported by studies around the world. Results have revealed a pooled rate of close to 70% of students willing to donate their organs after death. This is higher than the observation in studies conducted with the general public in different countries (10, 70–72) supporting that medical students have a heightened awareness of organ donation, similar to students from other health disciplines (32, 73, 74).

However, results in primary studies exhibited high heterogeneity, pointing to the presence of factors influencing willingness to donate. Both geographical area (continent) and cultural background had significant effects on the rate of students in favor. Studies conducted in countries with Latin (82%) and Western (70.6%) cultures obtained the greatest percentages, followed by Islamic countries (57.7%) and studies in countries with an Oriental culture (54.4%) which obtained the lowest percentage. These results are in line with previous literature. The meta-analysis by Mekkodathil et al. (10), including studies with the general public from Islamic countries, reported a pooled percentage of favorable attitude toward donation of less than 50%. Also, studies conducted with Asian populations have reported reduced rates of donation intention and registration among students, health workers, and the general public (75).

Sociocultural background includes social, spiritual, religious, and family beliefs and values that affect the decision-making process about donation. Regarding medical students in Islamic countries, motives related to body preservation after death were reported by students against donating their organs in some included studies (15, 19, 49, 54, 65). Conversely, the percentage of students worried about the mutilation of the body after death was considerably low in studies conducted in Western (30, 52) and Latin (59) cultural backgrounds. As in Western (26, 30, 40, 52, 76) or Latin countries (31, 59, 61) religious motives against donation were reported by reduced percentages of medical students in studies conducted in Turkey (32, 39, 49, 54). However, knowing the attitude toward donation and transplant promoted by participants' own religion can influence individual attitudes. In some included studies conducted in Saudi Arabia, Turkey, and Iran, about 30% of medical students ignored whether religion was in favor of donation and transplant (15, 41, 60). By contrast, in countries

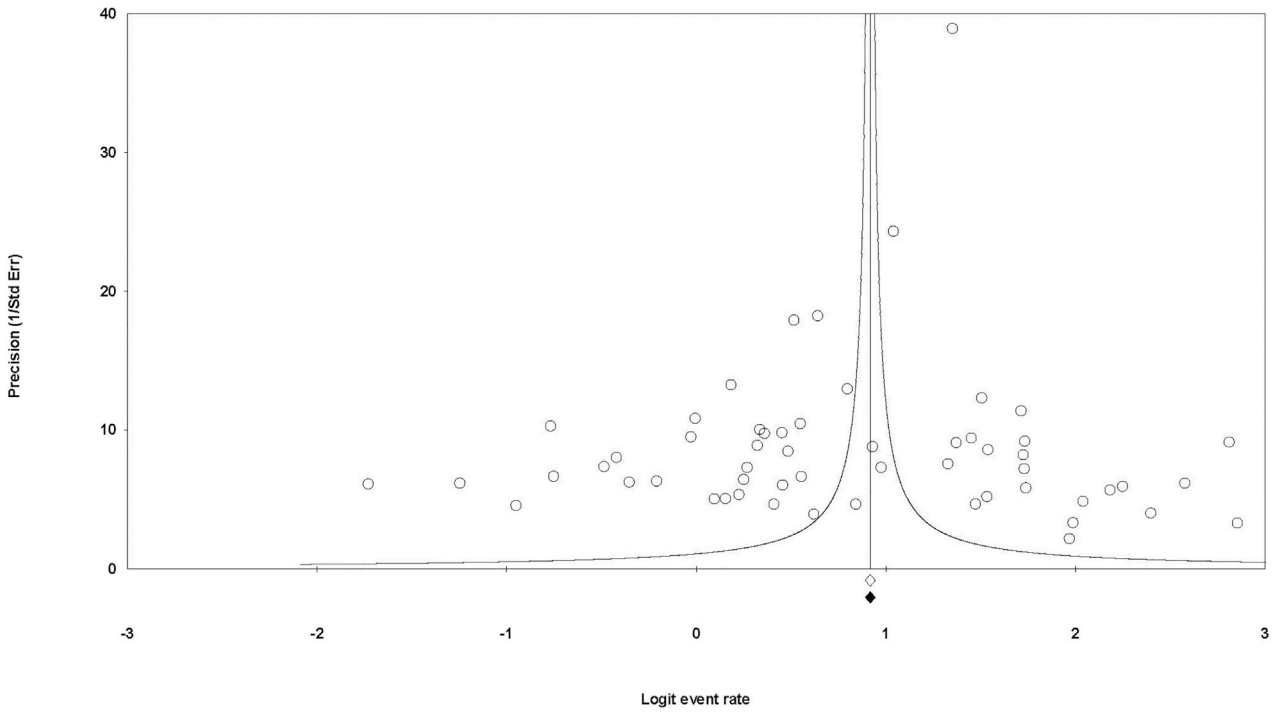

FIGURE 3 | Funnel plot of the individual observed rates for each study (circles) and observed (white diamond) and adjusted (black diamond) pooled rates of students willing to donate. The absence of imputed values to achieve symmetry in the dots' distribution and the equivalence between observed and adjusted pooled rates allow for us to discard publication bias.

with high predisposition rates such as Spain, only 12% of medical students did not know their religion's posture on donation and transplant (52).

In countries with a predominant Oriental culture, family opinion about donation seemed to be of particular importance. In the study by de Ohwaki et al. (51), more than 65% of medical students stated that their families would disagree with organ donation. Similarly, Lei et al. (43) observed that 95.5% of the students with no favorable attitude believed that their family was against donation. Oriental culture confers to family a relevant role in the life of individuals. Traditional values emphasized family interests over the individual's ones (43). Although in a Western or Latin cultural context, family's opinion influences the willingness to donate (52), the percentages of students who had discussed donation with their family (60%–70%) were considerably elevated (18, 26, 52, 59). Also in these countries, it has been reported that elevated proportions of medical students think that their parents' opinion is favorable (52, 59). Therefore, the family would play a beneficial role to promote favorable attitudes in Western and Latin cultural contexts. The importance of body preservation is another factor that affects the intention to donate after death in Asian medical students. A high percentage of students recognized concerns about body mutilation in the organ extraction process in some studies (22, 43). The Confucian heritage that promotes the idea of body care as a way of respect to parents, together with beliefs related to life after death, contributes to the importance of body preservation after death in Oriental cultures (75). As commented, the importance of body preservation was not a relevant reason against donation in cultural contexts with high rates of willingness to donate, being more rated than other motives such as the lack of information (26, 52, 59) and fear of trafficking or fair organ allocation (26, 52, 59).

According to the reports from the Global Observatory on Donation and Transplantation (77) in 2020, cultural differences observed in willingness to donate could be reflected by the rates of deceased donors in the countries of studies included in this meta-analysis. Using the same classification by cultural background, the highest mean of deceased donors per million population was observed in Western countries (16.38), followed by the mean in Latin (7.40), Islamic (3.86), and Oriental (1.69) countries. As it can be seen, the trend was similar to the observed willingness to donate, except for Latin countries, in which despite having an elevated rate of students in favor in this meta-analysis, the rates of deceased donors were discrete and lower than in Western countries. Possible explanations for this difference are that medical students were not representative of the general population in Latin America and that in addition to the attitudes, there were other variables (economic, related to donation system, etc.) influencing the factual deceased donor rates.

Age was positively related to the rate of students in favor. Given that the population studied in this meta-analysis was medical students, whose level of knowledge rises yearly, it is highly probable that the change in their perspective would be due to the educational level more than to the age effect itself. In fact,

the percentage of first-grade students included had a negative impact on the proportion of students in favor. Moreover, the subgroup analysis revealed differences between first- (65%) and sixth-grade students (79%). Taken together, these results may support the positive influence of years of training received by the students on their willingness to donate. It has been demonstrated that knowledge about aspects related to donation and transplant has a positive impact on attitudes toward donation (30, 52, 78). In addition, students in more advanced grades could have more opportunities for contact with transplant patients and donors or have attended campaigns or workshops to promote awareness toward donation. These experiences have also shown beneficial effects on the attitude to donation (18, 52).

In this meta-analysis, gender was not significantly related to the rate of students in favor, whereas individual studies have shown contradictory findings: existing studies where women exhibited a more favorable attitude (19, 32, 52) and studies where significant differences were not observed (27). Despite the fact that our findings revealed a higher rate for women (68%) than for men (61%), the reduced number of subgroups included in the analysis could explain the absence of significant differences.

Regarding methodological variables, the completion rate did not affect the rate of students willing to donate. Percentage of response could be a risk of bias indicator in attitudinal studies since higher participation could be associated with greater interest in the topic, or even with a more favorable attitude. As a consequence, it would be desirable that at least 75% of spread surveys could be included in the analysis (78). In this meta-analysis, 80% of studies that reported the completion rate showed percentages over 70%. This fact could explain the absence of significant effects on the willingness to donate. Remarkably, 39% of the included studies did not report the completion rate. The modality of administration of surveys (face-to-face vs. online) also affected the rate of students in favor, when taking into account that only six studies used online surveys. Finally, the year in which the survey was conducted showed an inverse association with the rate in favor, pointing to the absence of an increasing trend in the willingness to donate through the years.

The findings of this meta-analysis must be interpreted attending to some limitations. First, some of the studies included presented low scores in methodological quality assessment. The absence of sample size estimation procedures, the absence of random sampling, and the use of non-validated measures were the main weaknesses in the included studies. This could lead to bias in sample representativeness, and variability in the measurement of the willingness to donate. Despite this, it is remarkable that neither the risk of bias nor other methodological variables had a significant impact on the rate of students in favor. Second, all studies used self-report measures. Therefore, inherent disadvantages to self-reports in attitudinal studies (e.g., the trend to answer in a socially desirable way) could affect our results. Third, relevant variables such as discussing organ donation with family, contact with patients and donors, and frequency of other altruistic activities could not be analyzed as influencing factors because they were not reported by enough studies.

Despite these limitations, these results suggest practical implications for medical curriculum design. According to our findings, medical students present a high willingness to donate their organs, improving their attitudes as they progress in their medical careers. However, the percentage of students against and indecisive is still considerable. This picture is heterogeneous around the world, in which there are remarkable differences depending on the sociocultural background which students are immersed. This meta-analysis has evidenced that countries with Oriental and Islamic cultures showed the lowest rates of medical students willing to donate their organs after death. As commented, these studies have shown that the major reasons behind poor donation rates are cultural-related myths, lack of information, and religious misconceptions. In recent years, some countries in these cultural backgrounds have made efforts to include organ donation and transplantation contents in the medical curriculum. However, these modifications have been mainly focused on the acquisition of knowledge (brain death concept, organ donation system functioning, waitlists, etc.) ignoring the approach to sociocultural and religious issues (79). In order to address cultural issues in the medical curriculum, the following aspects are considered of particular importance: 1) promoting the discussion of the topic with family, 2) providing information about the local religion's attitude to donation, 3) discussing cultural-related death conceptions, and 4) providing reliable information about body manipulations in the donation process. Besides addressing cultural barriers, the possibility of taking advantage of certain cultural values to promote organ donation has been highlighted, for example, the Confucian values of helping others and positive life attitude in Chinese society (80). Knowledge and skills related to organ donation and transplant should be addressed early (first years) in the medical curriculum. This allows for saving resources from campaigns in medical professionals whose negative attitude is more resistant to change (6).

Given that the development of culture-specific campaigns and study plans implies being aware of beliefs, values, and practices of different population groups, future research should examine more deeply culture-bound conceptualizations of death, organ donation, and other related aspects. Moreover, recommendations for the medical curriculum could be extrapolated to other relevant population targets, especially in educative contexts. This would be the case for adolescents, who are immersed in the development of their own system of values and attitudes.

AUTHOR CONTRIBUTIONS

MI-S: Conception and design, study search and data extraction, statistical analysis, interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and final approval of the version to be

published. AL-N: Study search and data extraction, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and final approval of the version to be published. PG: Critical revision of the manuscript for important intellectual content, obtaining funding for this project or study, and final approval of the version to be published. PR: Critical revision of the manuscript for important intellectual content and final approval of the version to be published. AR: Conception and design, study search and data extraction, interpretation of data, drafting of the manuscript, and final approval of the version to be published.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10446/full#supplementary-material

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ORIGINAL RESEARCH
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Recipient Comorbidities for Prediction of Primary Graft Dysfunction, Chronic Allograft Dysfunction and Survival After Lung Transplantation

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Since candidates with comorbidities are increasingly referred for lung transplantation, knowledge about comorbidities and their cumulative effect on outcomes is scarce. We retrospectively collected pretransplant comorbidities of all 513 adult recipients transplanted at our center between 1992–2019. Multiple logistic- and Cox regression models, adjusted for donor-, pre- and peri-operative variables, were used to detect independent risk factors for primary graft dysfunction grade-3 at 72 h (PGD3-T72), onset of chronic allograft dysfunction grade-3 (CLAD-3) and survival. An increasing comorbidity burden measured by Charleston-Deyo-Index was a multivariable risk for survival and PGD3-T72, but not for CLAD-3. Among comorbidities, congestive right heart failure or a mean pulmonary artery pressure >25 mmHg were independent risk factors for PGD3-T72 and survival, and a borderline risk for CLAD-3. Left heart failure, chronic atrial fibrillation, arterial hypertension, moderate liver disease, peptic ulcer disease, gastroesophageal reflux, diabetes with end organ damage, moderate to severe renal disease, osteoporosis, and diverticulosis were also independent risk factors for survival. For PGD3-T72, a BMI>30 kg/m2 was an additional independent risk. Epilepsy and a smoking history of the recipient of >20packyears are additional independent risk factors for CLAD-3. The comorbidity profile should therefore be closely considered for further clinical decision making in candidate selection.

Keywords: lung transplantation, primary graft dysfunction, recipient selection, comorbidities, Charlson-Deyo-Index, chronic allograft dysfunction

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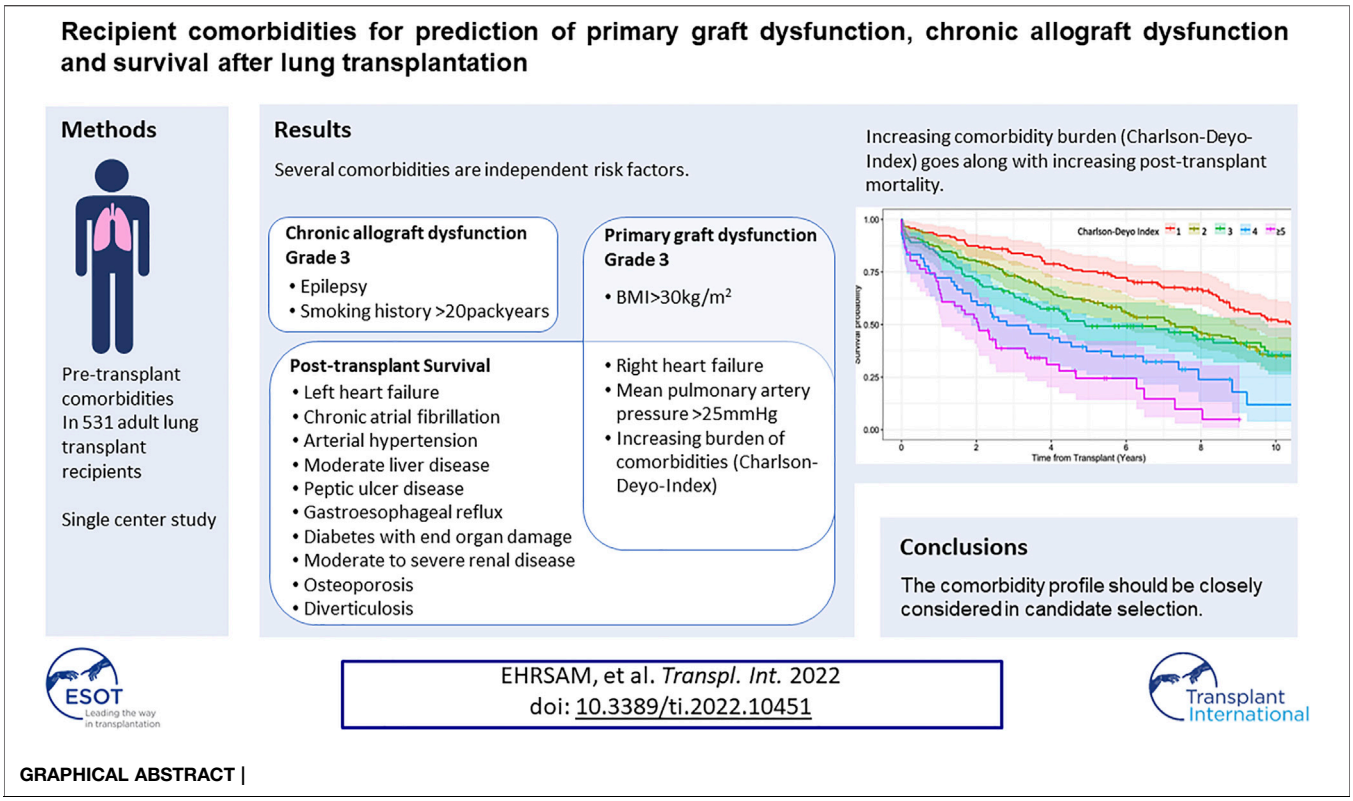
Abbreviations: CDI, Charlson-Deyo-Index; CLAD, chronic lung allograft dysfunction; ISHLT, International Society for Heart and Lung Transplantation; mPAP, mean pulmonary artery pressure; UNOS, United Network for Organ Sharing; ZDS, Zurich Donor Score.



GRAPHICAL ABSTRACT |

INTRODUCTION

Comorbidities in lung transplant candidates have increasingly been accepted over the last decades in parallel with steadily increasing numbers of lung transplantation procedures over time. This broadening of acceptable candidates was partly supported by the International Society for Heart and Lung Transplantation (ISHLT) consensus report for the selection of lung transplant candidates, published in 1998 and updated in 2006, 2014 (1) and 2021 (2). However, these consensus reports are based mainly on expert opinion. Strong evidence about comorbidities and their impact on primary graft dysfunction (PGD), chronic allograft dysfunction (CLAD), and survival are still missing. Moreover, almost nothing is known about the cumulative effect of comorbidities in a potential lung transplant candidate. In other fields of medicine, the cumulative effect of comorbidities for prognostic assessment has been extensively studied. One of the most commonly used comorbidity models is the Charlson-Comorbidity-Index introduced in 1987 (3). This index is based on comorbid conditions with varying assigned weights, resulting in a composite score. As increasing age was shown to be more an expression of accumulation of comorbidities than an actual risk factor per se, an age independent version, the Charlson-Deyo-Index (CDI)(4) was proposed. Among transplant patients, the CDI and its derivates has shown to be predictive in recipients undergoing renal transplantation (5, 6) and liver transplantation (7, 8).

In the era of organ shortage, it is of paramount importance to know which patient and at which time point will benefit from lung transplantation for an extended time period. We investigated the impact of a large variety of pretransplant comorbidities among our recipients transplanted at our center in respect to PGD, CLAD and survival. For cumulative comorbidity conditions, we additionally evaluated the CDI for the same outcomes.

METHODS

We systematically, retrospectively collected data from medical records of all adult recipients and their corresponding donors transplanted at the University Hospital of Zurich between 11/1992 and 12/2019, with last follow-up in 01/2022. Recipient selection was based on a liberal use of the updated ISHLT consensus document (1). All comorbidity variables were based on the most immediate pretransplantation data. Follow-up of the recipients was performed in our outpatient department or in close quarterly to half-yearly exchange with other institutions.

Definition of the Charlson-Deyo-Index

This index (4) is age independent and estimates the impact of multiple comorbidities. It considers 19 comorbid conditions (ranging from 1 to 6 points), of which 1 point was always reserved by the chronic pulmonary disease in each of our recipients. All included comorbidities and their assigned points

TABLE 1 | Pre-transplant recipient characteristics for survival.

	N = 513	Univariable analysis			Multivariable analysis			
		HR	95% CI	*p*	Model	HR	95% CI	*p*
Recipient Characteristics								
Age (median, range)	49 (18–70)	1.02	1.02–1.03	0.000	A, B, C, D	1.01	1.00–1.02	0.004
Sex male	270 (52.6%)	1.14	0.92–1.41	0.220				
Diagnosis								
Cystic fibrosis	156 (30.4%)	0.57	0.45–0.73	0.000				
Idiopathic pulmonary arterial hypertension	27 (5.2%)	1.33	0.86–2.07	0.205				
Emphysema	155 (30.2%)	1.19	0.95–1.49	0.133				
Idiopathic pulmonary fibrosis	111 (21.6%)	1.54	1.21–1.97	0.001				
Other	64 (12.5%)							
Smoking (pack years) (median, range)	0 (0–120)	1.01	1.00–1.01	0.006				
>20py	187 (36.5%)	1.34	1.08–1.66	0.009				
Waitlist (days) (median, range)	150 (0–1965)	1.00	1.00–1.00	0.525				
Recipient Comorbidities								
Any coronary artery disease	58 (11.3%)	1.71	1.23–2.37	0.001				
Myocardial infarction[a](1pt)	7 (1.4%)	2.44	1.01–5.93	0.048				
Postinterventional coronary disease (stent)	16 (3.1)	1.47	0.82–2.61	0.194				
Coronary disease mild	43 (8.4%)	1.68	1.16–2.44	0.006				
Congestive heart failure[a](1pt)	267 (52.0%)	2.13	1.71–2.64	0.000	A	1.91	1.53–2.40	0.004
Right heart failure	262 (51.1%)	2.04	1.65–2.53	0.000	C	1.81	1.45–2.28	0.000
mPAP (median, range)	28 (17–82)	1.02	1.01–1.03	0.000	B, C	1.64	1.31–2.06	0.000
>25 mmHg	264 (51.5%)	1.91	1.54–2.37	0.000				
Left heart failure	12 (2.3%)	3.62	1.97–6.64	0.000	C	2.07	1.11–3.87	0.023
Chronic atrial fibrillation	26 (5.1%)	3.33	2.10–5.29	0.000	B	2.10	1.31–3.38	0.002
Systemic hypertension	138 (26.9%)	2.02	1.60–2.56	0.000	B, C	1.33	1.03–1.72	0.028
Peripheral vascular disease[a](1pt)	18 (3.5%)	1.86	1.06–3.25	0.030				
Peripheral artery disease grade I	12 (2.3%)	1.24	0.58–2.62	0.579				
Aortic dissection	3 (0.6%)	5.82	1.86–18.26	0.003				
Aortic ectasia	4 (0.8%)	2.92	1.08–7.86	0.034				
Cerebrovascular disease[a](1pt)	11 (2.1%)	0.97	0.46–2.04	0.927				
Hemiplegia[a](2pt)	0							
Epilepsy	6 (1.2%)	1.08	0.45–2.61	0.866				
Dementia[a](1pt)	0							
Connecstive tissue disease[a](1pt)	22 (4.3)	0.89	0.52–1.53	0.683				
Rheumatoid arthritis	10 (1.9%)	1.66	0.82–3.36	0.156				
Scleroderma	6 (1.2%)	0.44	0.14–1.39	0.163				
Liver disease mild[a](1pt)	78 (15.2%)	1.17	0.85–1.60	0.350				
Liver disease moderate[a](3pt)	12 (2.3%)	1.49	1.19–1.87	0.000	A, B, C	1.41	1.12–1.77	0.004
Peptic ulcer disease[a](1pt)	18 (3.5%)	2.49	1.48–4.19	0.001	A, B, C	1.78	1.00–3.24	0.040
Gastroesophageal reflux	147 (28.7%)	1.67	1.32–2.12	0.000	A, B, C	1.28	1.00–1.65	0.023
Barret oesophagus	17 (3.3%)	1.44	0.81–2.57	0.217				
Chronic pulmonary disease[a](1pt)	513 (100.0%)							
Diabetes mild[a](1pt)	90 (17.5%)	0.85	0.64–1.13	0.262				
Diabetes end-organ damage[a](2pt)	8 (1.6%)	1.45	1.01–2.07	0.043	A, B, C	1.59	1.11–2.28	0.012
Moderate or severe renal disease[a](2pt)	61 (11.9%)	1.64	1.41–1.92	0.000	A, B, C	1.38	1.18–1.62	0.000
BMI (median, range)	20.8 (13.1–38.1)	1.05	1.03–1.07	0.000				
30.0–34.9	28 (5.5%)	1.42	0.92–2.19	0.112				
≥35	4 (0.8%)	3.09	1.15–8.31	0.025				
<18.5	142 (27.7%)	0.77	0.61–0.98	0.031				
Osteoporosis	178 (34.7%)	1.52	1.22–1.89	0.000	A, B, C	1.52	1.21–1.92	0.000
Diverticulosis	65 (12.7%)	2.02	1.48–2.75	0.000	A, B, C	1.42	1.01–2.00	0.043
Morbus Crohn/Colitis ulcerosa	6 (1.2%)	1.21	0.39–3.78	0.743				
Cholecystolithiasis	30 (5.8%)	1.23	0.78–1.93	0.373				
Pre-transplant critical situation (e.g., MV, ECMO, ICU)	56 (10.9%)	1.53	1.08–2.17	0.017				
Pre-transplant ECMO	34 (6.6%)	1.51	0.97–2.35	0.071				
Lymphoma[a](2pt)	6 (1.2%)	0.75	0.43–1.33	0.331				
Leukemia[a](2pt)	1 (0.2%)	2.38	0.89–6.38	0.085				
Tumor[a](2pt)	24 (4.7%)	1.18	0.92–1.50	0.198				
Metastatic solid tumor[a](6pt)	0							
AIDS[a](6pt)	0							
[a]Charlson-Deyo-Index pt (median, range)	2 (1–8)	1.37	1.26–1.48	0.000				
1	142 (27.7%)				D	Ref		
2	166 (32.4%)					1.56	1.18–2.05	0.002
3	100 (19.5%)					1.65	1.19–2.30	0.003

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TABLE 1 | (*Continued*) Pre-transplant recipient characteristics for survival.

	N = 513	Univariable analysis			Multivariable analysis			
		HR	95% CI	*p*	Model	HR	95% CI	*p*
4	54 (10.5%)					3.08	2.11–4.50	0.000
≥5	51 (9.9%)					4.10	2.76–6.09	0.000
Transplant and Donor Characteristics								
Era 1992–2000 vs. 2001–2019	98 (19.1%)	1.31	1.00–1.71	0.051				
Era 1992–2008 vs. 2009–2019	247 (48.1%)	1.22	0.97–1.54	0.093				
Unilateral Transplantation	36 (7.0%)	2,01	1.41–2.87	0.000	A, B, C, D	2.68	1.85–3.87	0.000
Re-Transplantation	23 (4.5%)	2.41	1.53–3.80	0.000				
Intra-operative ECMO use	241 (47.0%)	1.40	1.14–1.73	0.002				
CMV high risk	131 (25.5%)	1.01	0.79–1.28	0.961				
Zurich Donor Score, median (range)	3 (0–12)	1.13	1.09–1.18	0.000	A, B, C, D	1.10	1.06–1.15	0.000
DCD	28 (5.5%)	0.90	0.50–1.61	0.718				
EVLP	10 (1.9%)	0.78	0.32–1.88	0.575				
PGD3 at T72	79 (15.4%)	2.07	1.58–2.70	0.000				

[a]*Variables and points (pt) of Charlson-Deyo-Index.*

Abbreviations: AIDS, acquired immune deficiency syndrome; BMI, body mass index; CI, confidence interval; CMV, cytomegalo virus; DCD, lung donation after circulatory death; HR, hazard ratio; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation; EVLP, ex vivo *lung perfusion; FEV1, forced expiratory volume in 1 s; ICU, intensive care unit; mPAP, mean pulmonary artery pressure; OR, odds ratio; PGD, primary graft dysfunction py, pack years.*

are listed in **Tables 1–3**. An increasing score of points represents an increasing category of risk.

Definition of Comorbidities

The comorbidities in the CDI were defined by relying mostly on the original publication (4). In our selection program, all candidates with risk factors for coronary artery disease or aged ≥50 years old were evaluated by coronary angiogram. Congestive heart failure contains right or left heart failure or a combination of both. Right heart failure was defined as a mean pulmonary artery pressure (mPAP) >25 mmHg combined with echocardiographic evidence of right ventricular dysfunction (ventricular hypertrophy, moderate valve insufficiency, pericardial effusion) and/or signs of secondary liver or kidney dysfunction; left heart failure as having a reduced left ventricular ejection fraction <40%. Peripheral vascular disease includes aortic aneurysm, aortic ectasia and peripheral arterial disease grade I-IV. Cerebrovascular disease is defined as history of stroke with residual neurological deficit or transient ischemic attack. Connective tissue disease includes diagnosis of systemic lupus, rheumatoid arthritis, scleroderma, or seronegative spondyloarthropathy. Mild diabetes mellitus is type 1 and type 2 requiring medication, excluding dietary-controlled diabetes. For diabetes with end-organ damage renal, ophthalmic or neurological manifestations are required. Mild liver disease is defined as no portal hypertension with elevated liver enzymes more than three times the upper limit of normal. Moderate liver disease includes forms of fibrosis or cirrhosis causing portal hypertension with elevated liver enzymes. Moderate or severe renal disease includes glomerular filtration rate (eGFR) ≤60 ml/min/1.73 m^2 or acute renal replacement therapy. Tumor means a history of malignancy, excluding non-melanoma skin cancer. Further comorbidities were selected based on the 2014 and 2021 ISHLT consensus statement (1) and availability. Thereby, systemic hypertension was defined as without treatment ≥140/90 mmHg; critical or unstable condition such as mechanical ventilation (MV), extracorporeal membrane oxygenation (ECMO) or other reasons requiring pre-operative ICU; and osteoporosis as bone density with T-score below −2.5. To screen for diverticulosis and other colon disorders, candidates ≥50 years of age (for cystic fibrosis ≥40 years) were evaluated by colonoscopy. Gastroscopy was performed in all candidates with history of gastrointestinal symptoms or age ≥50 years. Gastroesophageal reflux disease was diagnosed predominantly on symptoms or endoscopic or radiological evidence, rarely on manometry and pH-metry testing.

Outcomes

The outcomes were PGD Grade-3 at 72 h, CLAD Grade-3 and survival after lung transplantation. PGD3-T72 is defined as PaO_2/FiO_2-ratio <200 mmHg and the presence of diffuse parenchymal infiltrates in the allograft on chest radiograph at 72 h after transplantation (9). As the definition was established in 2005, earlier cases were retrospectively analyzed by X-ray, ventilation curve and arterial blood gases. CLAD-3 is defined as a persistent decline of forced expiratory volume in 1 s (FEV1) ≤50% from baseline and an obstructive or restrictive physiology after exclusion of other causes (10).

Definition of Donor and Era Variables

To consider the impact of donor factors, the Zurich-Donor-Score (11) was used. This score estimates the quality of donor lungs, based on 5 extended donor criteria: age, diabetes mellitus, smoking history, pulmonary infection, and ratio of partial pressure of arterial oxygen to inspired oxygen fraction. Due to change in induction and immunosuppression (Anti-thymocyte globuline to Basiliximab) therapy in 2000, this era effect was tested. Other arbitrary defined models splitting in two or three different eras of similar case size or years of transplant did not show any significant differences in survival.

Statistical Methods

Statistical analysis was performed with IBM SPSS version 26 (SPSS IBM, Armonk, New York, USA) and R (Version 4.0.5,

TABLE 2 | Pre-transplant recipient characteristics for PGD3 on day 3.

	N = 79/507	Univariable analysis			Multivariable analysis			
		OR	95% CI	*p*	Model	OR	95% CI	*p*
Recipient Characteristics								
Age (median, range)	48 (18–68)	1.01	0.99–1.02	0.586				
Sex male	38 (48.1%)	0.81	0.50–1.31	0.398				
Diagnosis								
Cystic fibrosis	18 (22.8%)	0.62	0.35–1.09	0.096				
Idiopathic pulmonary arterial hypertension	14 (17.7%)	6.36	0.29–13.97	0.000				
Emphysema	8 (10.1%)	0.22	0.10–0.46	0.000				
Idiopathic pulmonary fibrosis	28 (35.4%)	2.35	1.40–3.96	0.001				
Other	18 (22.8%)							
Smoking (pack years) (median, range)	0 (0–80)	0.98	0.97–1.00	0.015				
>20py	21 (26.6%)	0.58	0.34–1.00	0.048				
Waitlist (days) (median, range)	39 (11–88)	1.00	1.00–1.00	0.274				
Recipient Comorbidities								
Any coronary artery disease	5 (6.3%)	0.48	0.19–1.24	0.128				
Myocardial infarction[a](1pt)	1 (1.3%)	0.90	0.11–7.59	0.924				
Postinterventional coronary disease (stent)	2 (2.5%)	0.83	0.18–3.75	0.808				
Coronary disease mild	3 (3.8%)	0.38	0.12–1.27	0.117				
Congestive heart failure[a](1pt)	64 (81.0%)	5.00	2.76–9.06	0.000	A	4.28	2.34–7.83	0.000
Right heart failure	63 (79.7%)	4.79	2.68–8.57	0.000	C	2.47	1.28–4.80	0.007
mPAP (median, range)	35 (20–80)	1.04	1.03–1.06	0.000	B	2.15	1.12–4.15	0.022
>25 mmHg	62 (78.5%)	4.32	2.44–7.62	0.000				
Left heart failure	4 (5.1%)	3.21	0.92–11.23	0.068				
Chronic atrial fibrillation	6 (7.6%)	1.87	0.72–4.87	0.199				
Systemic hypertension	25 (31.6%)	1.31	0.78–2.20	0.315				
Peripheral vascular disease[a](1pt)	2 (2.5%)	0.67	0.15–2.97	0.597				
Peripheral artery disease grade I	0	—						
Aortic dissection	1 (1.3%)	2.73	0.25–30.48	0.414				
Aortic ectasia	1 (1.3%)	1.82	0.19–17.69	0.607				
Cerebrovascular disease[a](1pt)	0	—						
Hemiplegia[a](2pt)	0	—						
Epilepsy	0	—						
Dementia[a](1pt)	0	—						
Connective tissue disease[a](1pt)	7 (8.9%)	2.68	1.06–6.79	0.038				
Rheumatoid arthritis	3 (3.8%)	2.37	0.60–9.38	0.218				
Scleroderma	3 (3.8%)	5.59	1.11–28.22	0.037				
Liver disease mild[a](1pt)	14 (17.7%)	1.25	0.66–2.36	0.495				
Liver disease moderate[a](3pt)	2 (2.5%)	1.07	0.64–1.79	0.810				
Peptic ulcer disease[a](1pt)	1 (1.3%)	0.31	0.04–2.36	0.258				
Gastroesophageal reflux	22 (27.8%)	0.99	0.58–1.69	0.973				
Barret oesophagus	0	—						
Chronic pulmonary disease[a](1pt)	79 (100.0%)	—						
Diabetes mild[a](1pt)	12 (15.2%)	0.83	0.43–1.61	0.580				
Diabetes end-organ damage[a](2pt)	2 (2.5%)	1.48	0.65–3.40	0.352				
Moderate or severe renal disease[a](2pt)	11 (13.9%)	1.12	0.79–1.59	0.532				
BMI (median, range)	22.8 (14.7–36.0)	1.09	1.04–1.15	0.001				
≥30.0	13 (16.5%)	5.42	2.47–11.91	0.000	A, B, C, D	4.27	1.88–9.68	0.001
≥35	2 (2.5%)	5.53	0.77–39.87	0.090				
<18.5	19 (24.1%)	0.80	0.46–1.40	0.441				
Osteoporosis	33 (41.8%)	1.43	0.88–2.33	0.153				
Diverticulosis	15 (19.0%)	1.81	0.96–3.43	0.067				
Morbus Crohn/Colitis ulcerosa	0	—						
Cholecystolithiasis	3 (3.8%)	0.59	0.17–1.98	0.390				
Pre-transplant critical situation (e.g., MV, ECMO, ICU)	14 (17.7%)	2.15	1.11–4.18	0.024				
Pre-transplant ECMO	10 (12.7%)	2.81	1.27–6.22	0.011				
Lymphoma[a](2pt)	1 (1.3%)	1.04	0.35–3.07	0.941				
Leukemia[a](2pt)	0	—						
Tumor[a](2pt)	3 (3.8%)	0.90	0.48–1.67	0.732				
Metastatic solid tumor[a](6pt)	0	—						
AIDS[a](6pt)	0	—						
[a]Charlson-Deyo-Index pt (median, range)	2 (1–6)	1.22	1.04–1.45	0.017				
1					D	Ref		
2						3.42	1.54–7.57	0.002
3						2.45	1.01–5.91	0.047
≥4						3.75	1.60–8.77	0.002

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TABLE 2 | (*Continued*) Pre-transplant recipient characteristics for PGD3 on day 3.

	N = 79/507	Univariable analysis			Multivariable analysis			
		OR	95% CI	*p*	Model	OR	95% CI	*p*
Transplant and Donor Characteristics								
Era 1992–2000 vs. 2001–2019	11 (13.9%)	1.58	0.80–3.11	0.188				
Era 1992–2008 vs. 2009–2019	44 (55.7%)	0.71	0.44–1.15	0.166				
Unilateral Transplantation	3 (3.8%)	0.49	0.15–1.64	0.245				
Re-Transplantation	1 (1.3%)	0.26	0.04–1.98	0.194				
Intra-operative ECMO use	60 (75.9%)	1.52	2.61–7.84	0.000	A, B, C	2.93	1.56–5.53	0.001
CMV high risk	17 (21.5%)	0.76	0.42–1.35	0.341				
Zurich Donor Score, median (range)	3 (0–11)	1.14	1.04–1.24	0.003	A, B, C, D	1.11	1.01–1.21	0.028
DCD	3 (3.8%)	0.64	0.19–2.16	0.469				
EVLP	2 (2.5%)	0.73	0.15–3.52	0.698				

[a]*Variables and points (pt) of Charlson-Deyo-Index.*

Abbreviations: AIDS, acquired immune deficiency syndrome; BMI, body mass index; CI, confidence interval; CMV, cytomegalo virus; DCD, lung donation after circulatory death; HR, hazard ratio; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation; EVLP, ex vivo *lung perfusion; FEV1, forced expiratory volume in 1 s; ICU, intensive care unit; mPAP, mean pulmonary artery pressure; OR, odds ratio; PGD, primary graft dysfunction py, pack years.*

Vienna, Austria). Continuous data were compared using the Mann–Whitney test and categorical variables compared using the v^2 test or the Fisher's exact test for expected frequencies <5. Kaplan-Meier method was used to estimate survival as well as time to CLAD-3. The log-rank test compared survival curves. Cox regression was used to assess risk factors for mortality. Cox regression for CLAD-3 was adjusted for the competing factor of death by the Fine Gray methodology. Logistic regression was used to assess factors for PGD3-T72. First, every variable was checked with a univariate (enter) model. Variables with a *p*-value < 0.2 (12) were tested in a multivariate stepwise backward Cox regression model or linear regression model, respectively. The number of factors introduced into the final multivariable model was calculated by considering sample size and number of occurring events (13). To confirm that variables show a stable significance, they had to be frequent in number. Linear regression was used to test collinearity between variables. A variance inflation factor >5 and a tolerance <0.2 was defined as indicating a collinearity problem. Different final multivariate models are provided to bypass variables with statistical or clinical collinearity. In general, a *p*-value < 0.05 was considered to be the threshold for statistical significance.

The local research ethics review committee approved the study (KEK-Nr.2019-00873).

RESULTS

In our study population, there were 513 adult recipients who underwent lung transplantation between 1992 and 2019. Of these, 353 recipients (68.8%) died, 266 (51.9%) developed CLAD-3 and 79 (15.4%) PGD3-T72. Median follow-up time was 12.7 years. No loss to follow-up occurred. Half of the transplants were performed in the era 1992–2008 and showed a trend of better survival than the era 2009–2019 (median survival 8.4 vs. 5.9 years, respectively, log-rank = 0.092). The same was observed for onset of CLAD-3 (median 7.6 vs. 5.5 years, respectively, log-rank = 0.121). In line with these trends, donor marginality measured by ZDS (mean 2.8 vs. 4.0 points, $p < 0.001$) and the recipient comorbidity burden measured by CDI (mean 2.2 vs. 2.7 points, $p < 0.001$) increased significantly in the second era. **Figure 1** shows the detailed increase of the CDI score burden over the study period. In the earlier era a trend of more PGD3-T72 occurred (17.8% vs. 13.2%, respectively, $p = 0.144$).

Seventy two percent of the recipients had at least one comorbidity represented in the CDI, beside of the always present underlying chronic pulmonary disease which accounts for an extra point. As illustrated in the Kaplan-Meier survival curve of **Figure 2A**, an increasing number or severity of comorbidities in the CDI was associated with significantly poorer survival, except that a score of 2 points was comparable to a score of 3 points (log-rank = 0.776). The median survival for a CDI score of 1, 2, 3, 4 and ≥5 points was 10.5, 7.3, 4.9, 2.8, and 2.1 years, respectively.

For the overall population, detailed descriptive statistics of recipient-, donor-, intra-operative characteristics are shown in **Table 1**. The most frequent underlining diseases were cystic fibrosis (30%) and emphysema (30%). The most frequent comorbidity was congestive heart failure (52%) including in 98% of these cases right heart failure all with an mPAP >25 mmHg. The next most frequent comorbidities were osteoporosis (35%), gastroesophageal reflux (29%), systemic hypertension (27%), mild diabetes (18%), mild liver disease (15%), diverticulosis (13%) and moderate to severe renal disease (12%).

Risk Factors for Survival

All comorbidities listed in **Table 1** were assessed in univariable and if applicable in multivariable risk analysis. In multivariable Cox regression (**Table 1**, Model A), moderate liver disease, peptic ulcer disease, gastroesophageal reflux, diabetes with end-organ damage, moderate to severe renal disease, osteoporosis, diverticulosis, and congestive heart failure were independent risk factors for mortality, beside of increasing age, increasing ZDS and unilateral lung transplantation. The subgroups of left heart failure and right heart failure as well as mPAP >25 mmHg,

TABLE 3 | Pre-transplant recipient characteristics for onset of CLAD-3.

	N = 266/513	Univariable analysis			Multivariable analysis			
		HR	95% CI	*p*	Model	HR	95% CI	*p*
Recipient Characteristics								
Age (median, range)	51 (18–70)	1.01	1.00–1.02	0.002				
Sex male	143 (53.8%)	1.11	0.88–1.41	0.380				
Diagnosis								
Cystic fibrosis	69 (25.9%)	0.69	0.53–0.91	0.007				
Idiopathic pulmonary arterial hypertension	13 (4.9%)	0.82	0.48–1.40	0.470				
Emphysema	86 (32.3%)	1.17	0.91–1.50	0.230				
Idiopathic pulmonary fibrosis	64 (24.1%)	1.38	1.03–1.86	0.031	A, B, D	1.44	1.07–1.95	0.017
Other								
Smoking (pack years) (median, range)	4 (0–120)	1.01	1.00–1.01	0.001	A, B, C, D	1.48	1.16–1.91	0.002
>20py	112 (42.1%)							
Waitlist (days) (median, range)	150.5 (0–1378)	1.00	1.00–1.00	0.820				
Recipient Comorbidities								
Any coronary artery disease	32 (12.0%)	1.33	0.89–1.98	0.160				
Myocardial infarction[a](1pt)	2 (0.8%)	0.57	0.12–2.66	0.470				
Postinterventional coronary disease (stent)	8 (3.0%)	0.95	0.41–2.18	0.900				
Coronary disease mild	24 (9.0%)	1.48	0.95–2.30	0.080				
Congestive heart failure[a](1pt)	142 (53.4%)	1.30	1.03–1.64	0.030	A	1.27	1.00–1.16	0.053
Right heart failure	140 (52.6%)	1.31	1.04–1.66	0.023	B	1.24	0.98–1.58	0.078
mPAP (median, range)	32 (20–80)	1.01	1.00–1.02	0.038	C	1.23	0.97–1.57	0.092
>25 mmHg	141 (53.0%)							
Left heart failure	6 (2.3%)	1.10	0.41–2.93	0.850				
Chronic atrial fibrillation	9 (3.4%)	0.67	0.33–1.38	0.280				
Systemic hypertension	74 (27.8%)	1.29	0.97–1.70	0.077				
Peripheral vascular disease[a](1pt)	5 (1.9%)	0.50	0.20–1.27	0.140				
Peripheral artery disease grade I	2 (0.8%)	0.29	0.07–1.19	0.085				
Aortic dissection	1 (0.4%)	0.61	0.07–5.50	0.660				
Aortic ectasia	3 (1.1%)	2.13	0.62–7.27	0.230				
Cerebrovascular disease[a](1pt)	6 (2.3%)	1.32	0.62–2.81	0.460				
Hemiplegia[a](2pt)	0							
Epilepsy	5 (1.9%)	1.92	0.90–4.07	0.089	A, B, C, D	2.34	1.06–5.19	0.036
Dementia[a](1pt)	0							
Connective tissue disease[a](1pt)	15 (5.6%)	1.44	0.87–2.39	0.150				
Rheumatoid arthritis	8 (3.0%)	2.24	1.07–4.72	0.033				
Scleroderma	4 (1.5%)	1.10	0.46–2.67	0.830				
Liver disease mild[a](1pt)	29 (10.9%)	0.74	0.49–1.10	0.130				
Liver disease moderate[a](3pt)	4 (1.5%)	0.87	0.62–1.22	0.410				
Peptic ulcer disease[a](1pt)	6 (2.3%)	0.73	0.28–1.85	0.500				
Gastroesophageal reflux	71 (26.7%)	1.05	0.80–1.38	0.740				
Barret oesophagus	9 (3.4%)	1.10	0.57–2.12	0.780				
Chronic pulmonary disease[a](1pt)	266 (100.0%)							
Diabetes mild[a](1pt)	41 (15.4%)	0.82	0.59–1.13	0.230				
Diabetes end-organ damage[a](2pt)	5 (1.9%)	1.17	0.72–1.90	0.520				
Moderate or severe renal disease[a](2pt)	25 (9.4%)	0.91	0.72–1.14	0.400				
BMI (median, range)	21.1 (13.1–36.0)	1.05	1.02–1.08	0.000				
≥30.0	18 (6.8%)							
≥35	2 (0.8%)							
<18.5	52 (27.2%)							
Osteoporosis	94 (35.3%)	1.15	0.89–1.49	0.270				
Diverticulosis	36 (13.5%)	1.27	0.89–1.82	0.190				
Morbus Crohn/Colitis ulcerosa	1 (0.4%)	0.35	0.05–2.51	0.300				
Cholecystolithiasis	13 (4.9%)	0.80	0.47–1.36	0.410				
Pre-transplant critical situation (e.g., MV, ECMO, ICU)	20 (7.5%)	0.68	0.42–1.09	0.110				
Pre-transplant ECMO	11 (4.1%)	0.64	0.33–1.36	0.180				
Lymphoma[a](2pt)	2 (0.8%)	0.70	0.36–1.36	0.290				
Leukemia[a](2pt)	0							
Tumor[a](2pt)	11 (4.1%)	0.95	0.70–1.29	0.730				
Metastatic solid tumor[a](6pt)	0							
AIDS[a](6pt)	0							
[a]Charlson-Deyo-Index pt (median, range)	2 (1–6)	0.96	0.88–1.05	0.330				
1	76 (28.6%)				D	Ref		
2	99 (37.2%)					1.29	0.98–1.71	0.074
3	50 (18.8%)					1.04	0.72–1.49	0.840

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TABLE 3 | (*Continued*) Pre-transplant recipient characteristics for onset of CLAD-3.

	N = 266/513	Univariable analysis			Multivariable analysis			
		HR	95% CI	*p*	Model	HR	95% CI	*p*
4	20 (7.5%)					0.80	0.53–1.20	0.270
≥5	21 (7.9%)					1.28	0.95–1.72	0.100
Transplant and Donor Characteristics								
Era 1992–2000 vs. 2001–2019	50 (18.8%)	1.43	1.08–1.90	0.011	D	1.28	0.95–1.72	0.100
Era 1992–2008 vs. 2009–2019	146 (54.9%)	1.01	0.80–1.28	0.920				
Unilateral Transplantation	15 (5.6%)	0.71	0.41–1.23	0.220				
Re-Transplantation	7 (2.6%)	0.52	0.23–1.19	0.120				
Intra-operative ECMO use	126 (47.4%)	1.23	0.97–1.56	0.092				
CMV high risk	76 (28.6%)	1.27	0.98–1.66	0.075	A, B, C, D	1.32	1.01–1.74	0.026
Zurich Donor Score, median (range)	3 (0–11)	1.06	1.02–1.11	0.007	A, B, C, D	1.05	1.00–1.10	0.048
DCD	11 (4.1%)	0.95	0.51–1.77	0.880				
EVLP	4 (1.5%)	0.95	0.31–2.93	0.930				
PGD3 at T72	43/(16.2%)	1.19	0.84–1.68	0.340				

[a]*Variables and points (pt) of Charlson-Deyo-Index.*

Abbreviations: AIDS, acquired immune deficiency syndrome; BMI, body mass index; CI, confidence interval; CMV, cytomegalo virus; DCD, lung donation after circulatory death; HR, hazard ratio; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation; EVLP, ex vivo *lung perfusion; FEV1, forced expiratory volume in 1 s; ICU, intensive care unit; mPAP, mean pulmonary artery pressure; OR, odds ratio; PGD, primary graft dysfunction py, pack years.*

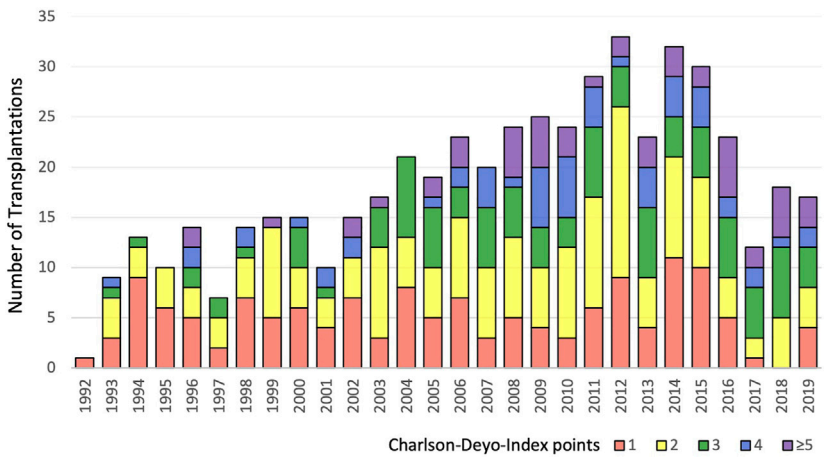

FIGURE 1 | Distribution of the recipient comorbidity burden over the study period, measured by the Charlson-Deyo-Index. The first scoring point accounts for the always present underlying lung disease.

chronic atrial fibrillation and systemic hypertension were also multivariate risk factors for mortality when independently analyzed from congestive heart failure (**Table 1**, Model B, C). Of note, the underlying lung diseases were no multivariable risk factors in the models, after introducing comorbidities. The same effect was found for re-transplantation, pre-transplant critical situation, ECMO as bridge to transplantation and intraoperative ECMO use.

The accumulation of comorbidities with CDI in the multivariable model (**Table 1**, Model D) showed an even better performance for survival estimates than the unadjusted Kaplan-Meier curves (**Figure 2A**).

Risk Factors for PGD3-T72

Recipient-, donor-, intra-operative characteristics for those transplantations where PGD3-T72 occurred are listed in **Table 2**. In this subpopulation, the underlying diagnosis of idiopathic pulmonary fibrosis (35%, $p = 0.001$) and idiopathic pulmonary arterial hypertension (18%, $p < 0.001$) were significantly higher represented. The percentage of congestive heart failure (81%, $p < 0.001$), a mPAP >25 mmHg (79%, $p < 0.001$), ECMO as bridge to transplantation (13%, $p = 0.019$), intraoperative ECMO use (76%, $p < 0.001$), CDI ($p = 0.006$) and ZDS ($p = 0.011$) were also significantly higher than in the overall population.

In multivariable logistic regression congestive heart failure, a BMI>30kg/m2, an increasing ZDS and intraoperative ECMO use were independent risk factors for PGD3-T72 (**Table 2**, Model A). Additional analyses excluding congestive heart failure revealed, that mPAP >25 mmHg and right heart failure were also factors for PGD3-T72 (**Table 2**, Model B, C). The accumulation of comorbidities in the CDI was associated with the risk of

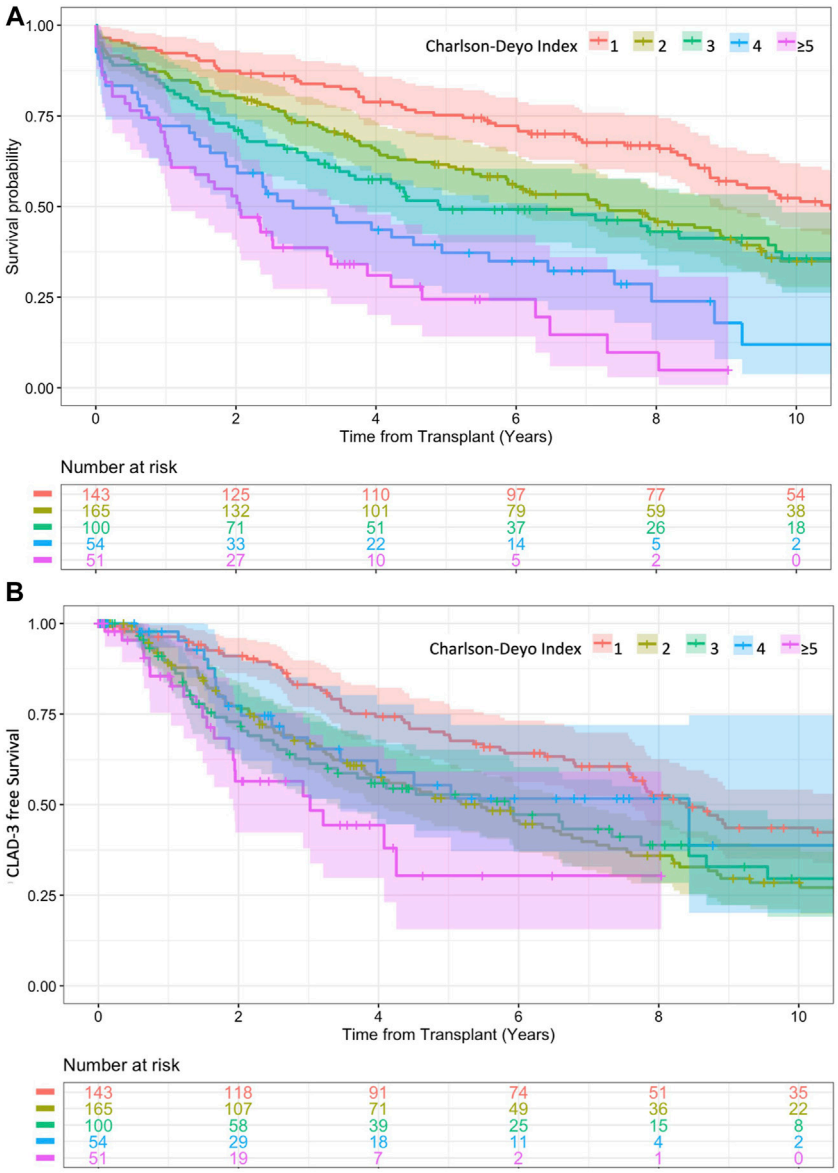

FIGURE 2 | (A) Kaplan-Meier survival curves for different comorbidity burdens in the Charlson-Deyo-Index. 1 vs. 2 log-rank = 0.004, 2 vs. 3 log-rank = 0.776, 3 vs. 4 log rank = 0.020, 4 vs. ≥5 log rank = 0.045. **(B)** Kaplan-Meier curve for onset of CLAD-3 for different comorbidity burdens in the Charlson-Deyo-Index. 1 vs. 2 log-rank = 0.001, 2 vs. 3 log-rank = 0.927, 3 vs. 4 log rank = 0.537, 4 vs. ≥5 log rank = 0.059.

PGD3-T72 but not in a linear increasing way with increasing scoring points (**Table 2**, Model D), likely due to the small sample size.

Risk Factors for Onset of CLAD-3

For the subpopulation of CLAD-3, recipient-, donor-, intra-operative characteristics are listed in **Table 3**. The CLAD-3 subpopulation was comparable to the overall population with respect to the underlying disease and variables of intraoperative procedure, but showed a trend to more marginal donor lungs in the ZDS (p = 0.097) and a significantly higher comorbidity burden in the CDI (p = 0.018).

Multivariate Cox regression revealed that the underling diagnosis of idiopathic pulmonary fibrosis, a smoking history of the recipient of >20 packyears, epilepsy, CMV high-risk constellation and an increasing ZDS were independent risk factors for onset of CLAD-3 (**Table 3**, Model A, B, C). Congestive heart failure, right heart failure and mPAP >25 mmHg were borderline risk factors (**Table 3**, Model A, B, C). The change in induction and immunosuppression in 2000 from Anti-thymocyte globuline to Basiliximab was a borderline risk factor (**Table 3**, Model D). Recipient age and PGD-3 were no risk factors for developing CLAD-3.

Moreover, the comorbidity burden estimated by CDI was not a multivariable risk factor for developing CLAD-3 (**Table 3**, Model D). This is in line with the Kaplan-Meier estimate, where onset of CLAD-3 was not gradually reduced by an increasing CDI (**Figure 2B**). The median time until onset of CLAD-3 for a CDI score of 1, 2, 3, 4 and ≥5 points was 8.4, 5.5, 5.9, 8.4, and 3.0 years, respectively.

DISCUSSION

This study is the first detailed analysis of association between recipient comorbidities prior to transplantation and survival, PGD3-T72 and onset of CLAD-3 after lung transplantation. We show that several recipient comorbidities and their accumulation have a strong impact on post-transplant survival, and that some comorbidities also affect the development of PGD3-T72 and CLAD-3.

It is paramount to define the right time of listing and transplanting a candidate. On one hand, a limited life expectancy due to the lung disease is required to justifying the benefit over the risk of a lung transplantation. On the other hand, a prolonged time span until transplantation is often associated with developing a more extensive comorbidity profile. This problem is further aggravated by a demographic shift toward older candidates, who are per se more likely to be multi-morbid.

While lung transplantation may improve previously poor organ oxygenation and consecutively slow down the progression of many comorbidities, surgical complications and the side effects of the immunosuppression regime may worsen comorbidities considerably and even create new comorbidities over time.

In addition to respecting the ISHLT consensus document (1) for absolute contraindications, our center has been fairly liberal in the acceptance of candidates with reasonable comorbidities. Estimated by the CDI, 72% of our recipients had at least one comorbidity in addition to the underlying lung disease, providing ideal conditions for a thorough analysis.

Factors Associated With Survival

Among pretransplant recipient comorbidities, we identified right heart failure as an important risk factor affecting survival, PGD3-T72 and partially also CLAD-3. It was the most frequent comorbidity found in half of our cohort. Right heart failure and especially its approximative surrogate of pulmonary hypertension >25 mmHg were also risk factors for mortality in a single center study (14) and in the United Network for Organ Sharing (UNOS) Database in 3105 emphysema patients (15). Even though right heart failure may be partially to fully reversible after lung transplantation, pulmonary hypertension requires sometimes peri-operative extracorporeal membrane oxygenation (ECMO) implantation to avoid reperfusion edema which goes along with a variety of factors that can increase morbidity (16). One of the morbidities is PGD attributed to the systemic inflammatory response associated with the machine as well as its systemic anticoagulation requirements (17). In our cohort, intraoperative ECMO use was also an independent risk factor for developing PGD3-T72.

In our study, the few cases of left heart failure were also strongly associated with mortality. Previous reports about left heart failure are lacking, likely as it is widely considered a contraindication for transplantation (2).

Systemic hypertension was present in one fourth of our cohort. It was a risk factor for mortality, in line with a previous report in 821 pulmonary fibrosis recipients (18). Pretransplant systemic hypertension may aggravate differently after transplant because of the side effects of immunosuppression treatment with calcineurin inhibitors than in previously non-hypertensive recipients. This might lead to earlier end organ damage. Moreover, a meta-analysis (19) has shown that systemic hypertension was a risk factor for postoperative atrial arrhythmias and therefore had prognostic implications for length of hospital stay and overall survival.

Pretransplant atrial fibrillation increased the risk of adverse cardiovascular outcomes and longer hospital stay in a single-center study (20). In our study, pretransplant chronic atrial fibrillation was even an independent risk factor for mortality.

We identified diabetes mellitus with end-organ damage but not mild diabetes as a risk factor for mortality. This is in line with the findings of the University of Melbourne study (21) for poorly controlled glycemic controlled candidates. The ISHLT report even lists any stage of diabetes as a risk factor for 10-year mortality (22), including diabetes without end organ damage.

Renal disease may further aggravate in the peritransplant period mainly due to the immunosuppression regimen and fluid shifts after transplantation. Moderate to severe renal disease was an independent risk factor for mortality in our cohort. An eGFR of 60 ml/min/1.73m2 or less was also an independent risk factor for 1-year survival using UNOS data (23). And the ISHLT report lists recipient with a pre-transplant dialysis condition as a risk factor for 10-year mortality (22).

Currently, the impact of moderate liver disease is poorly understood because it has hardly been investigated so far. Although we found moderate liver disease to be a risk factor for mortality in our cohort, liver cirrhosis with or without portal hypertension did not have a negative impact on 5-year survival in 6 matched cystic fibrosis recipients in a previous study (24).

Gastroesophageal reflux was suggested to be associated with secondary aspiration contributing to acute rejection, pulmonary infection and CLAD and consecutive mortality (25). However, even though gastroesophageal reflux was an independent risk factor for mortality in our cohort, no risk association was found for development of PGD3-T72 and CLAD-3. A reason might be that several asymptomatic recipients were insufficiently screened in our program (26), preventing a correlation to PGD and CLAD. Another reason may be that we universally teach patients about anti-reflux measures (27).

Peptic ulcer disease was also a risk for mortality in our study. It is reported from small series to occur and reoccur after transplantation and may lead to intestinal perforation (28, 29).

The rate of developing acute diverticulitis from preexisting diverticulosis in immunosuppressed patients is significantly higher than in the general population (30). At our center, we

reported an overall rate of diverticulitis of 4.5% after lung transplantation (31).

The prevalence of osteoporosis affected one third of our cohort and it was a significant risk factor for survival. Osteoporosis is in part reflected by preoperative steroid use which was a risk factor for 1-year survival in a study using UNOS data (23).

Neither mild nor post-interventional coronary disease were independent risk factors in our cohort, which is in line with previous studies (32, 33). Our cases with a history of myocardial infarction might have been too few in number or too highly selected to become an independent risk.

Multiple reports on other solid organ transplantations indicate that the presence of symptomatic peripheral vascular disease is one of the strongest predictors of mortality (34-36). In our study, a mild peripheral artery disease grade I seems to have minor impact on post-transplant survival. Previous aortic dissection and aortic ectasia appeared to be associated with post-transplant mortality in univariable analysis, but the limited number in our cohort did not justify further analysis.

We noted, that the underlying lung disease, a preoperative critical situation, and re-transplantation lost their strength as risk factors for mortality, when analyzed along with comorbidities. These variables may consecutively be regarded as surrogates for the comorbidity burden. For an optimal candidate selection, the focus should therefore lie on the comorbidity profile.

Factors Mainly Associated With PGD3-T72

In addition to right heart failure and mPAP >25 mmHg, described above, a BMI>30 kg/m^2 was a strong risk factor for developing PGD3-T72 in our study. Pulmonary hypertension and BMI >25 were also reported as independent risk factors for PGD in a cohort of 7322 recipients (37) and in a meta-analysis (38). The mechanism of adipositas on PGD is not yet fully understood. It is likely caused by comorbidities associated with adipositas. This would also explain why adipositas was not a multivariable risk factor for mortality in our study.

Factors Mainly Associated With CLAD-3

This study is the first to detect epilepsy as a risk factor for CLAD-3. Some anti-epileptic medication show side effects on respiratory depression, increase oral and pulmonary secretions and even interstitial lung disease (39). Moreover, epilepsy might go along with an increased risk of aspiration leading to pneumonia, inflammation, and consecutive fibrotic alterations of the lung allograft. An additional risk for CLAD-3 was a previous smoking history of more than 20 packyears. We do not believe that the systemic damages caused by previous smoking is responsible for this effect, but the increased likelihood of being still exposed to a smoking environment or even due to smoking resumption (40). Another important aspect is the underlying disease in particular idiopathic pulmonary fibrosis. It was an independent factor for developing CLAD. The process may be due to the re-occurrence of the underlying disease in the allograft.

PGD was repeatedly associated with the risk of developing CLAD (41). However, we could not find such a correlation in our cohort. The detected borderline risk of a pre-transplant mPAP >25 mmHg might occasionally have caused *de novo* pulmonary hypertension and chronic lung edema and fibrosis of the lung allograft.

Charlson-Deyo-Index

An increasing comorbidity burden, estimated by the CDI, was well associated with an increasing risk for mortality. We showed that already one proportionally mild comorbidity in the CDI bears a significant risk on survival outcome. This should emphasize that a very careful selection of candidates considering comorbidities is crucial. However, we can not provide a recommendation based on our single-center analysis.

Our finding of CDI as a good predictor for survival is in line with multiple studies of other solid organ transplants (5-8). However, the Pittsburgh group (42) calculated the original Charlson Index for 748 lung transplant recipients and neither detected an association with in-hospital post-transplant complications nor an association with survival in a multivariate model. This might be due to an incomplete assessment of comorbidities, incomplete adjustment for confounders, and incorporation of recipient age in the score.

We detected several other comorbidities beyond the 18 comorbidity conditions represented in the CDI as important risk factors for survival. Thus, the addition of other comorbidities, a different weighing or sub-categorization may even improve the prediction of the CDI in the context of lung transplantation. This would have to be determined and proven in future studies.

The association of the comorbidity burden in the CDI was weaker for PGD3-T72 than for survival. Only one comorbidity of the CDI, congestive heart failure, was independently associated with onset of PGD3-T72 and borderline associated with onset of CLAD-3. Mechanisms of developing PGD and especially CLAD appear to rely more on a limited number of specific comorbidities, rather than on their quantity.

Limitations

This study has several limitations. It is a retrospective single-center study over more than 2 decades. The pre- and posttransplant treatment of some comorbidities might have changed over time. However, we could not detect an era effect in univariable and multivariable analyses. Some comorbidities might have been underrepresented in our study, which would have otherwise been important risk factors.

CONCLUSION

Our study identified several comorbidities that were associated with post-transplant survival, onset of PGD and CLAD. Based on our findings we consider the comorbidities mentioned in the current ISHLT-consensus document (2) as relative contraindications as valid risk factors for mortality after lung transplantation. The CDI may potentially be used for a more refined evaluation of multimorbid candidates.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because Potential conflict with the Swiss Privacy Act, a federal law. The data that support the findings of this study are only available from the corresponding author, upon reasonable request. Requests to access the datasets should be directed to II, ilhan.inci@usz.ch.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the local research Ethics Review Committee (KEK-ZH-Nr.2019-00873). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JE and II participated in conception and design of study. JE and II participated in acquisition of data. JE and ML participated in analysis of data. JE, MS, and II participated in interpretation of data. JE drafted the article. MS, IO, and II participated in revising the article critically. All authors approved to the version of the article to be published.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Reduced Rates of Post-Transplant Recurrent Hepatocellular Carcinoma in Non-Alcoholic Steatohepatitis: A Propensity Score Matched Analysis

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Non-alcoholic steatohepatitis (NASH)-related hepatocellular carcinoma (HCC) has become the second leading cause of HCC-related liver transplantation in the United States. This study investigated post-transplant recurrence and survival for patients transplanted for NASH-related HCC compared to non-NASH HCC etiologies. Retrospective review of the United Network for Organ Sharing (UNOS) Organ Procurement and Transplantation Network (OPTN) database identified 7,461 patients with HCC—1,405 with underlying NASH and 6,086 with non-NASH underlying diseases. After propensity score matching (PSM) to account for patient- and tumor-related confounders 1,175 remained in each group. Primary outcomes assessed were recurrence rate and recurrence-free survival. Recurrent malignancy at 5 years post-transplant was lower in NASH compared to non-NASH patients (5.80 vs. 9.41%, $p = 0.01$). Recurrence-free survival, however, was similar at 5 years between groups. Patients with NASH-related HCC were less likely to have post-transplant recurrence than their non-NASH counterparts, although recurrence-free survival was similar at 5 years.

Keywords: United Network for Organ Sharing, hepatocellular carcinoma, non-alcoholic steatohepatitis, recurrence, Organ Procurement and Transplantation Network

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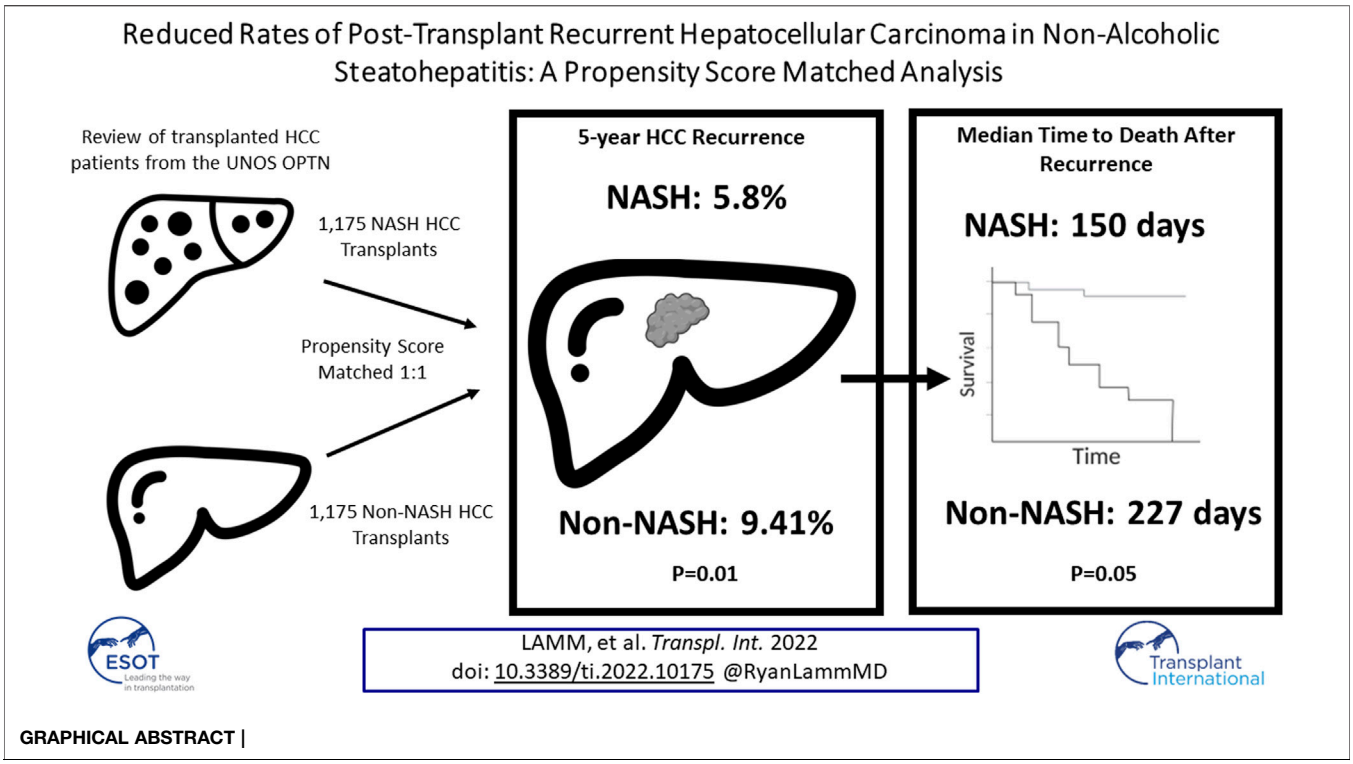
Abbreviations: AFP, alpha feto-protein; BMI, body mass index; CIT, cold ischemia time; CNS, central nervous system; CVA, cerebrovascular accident; DAA, direct-acting antiviral; DCD, donation after cardiac death; ESLD, end-stage liver disease; EtOH, alcohol; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICU, intensive care unit; LDRI, liver donor risk index; MELD, model for end stage liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OPTN, Organ Procurement and Transplantation Network; PSM, propensity score matched; PVT, portal vein thrombosis; TACE, trans-arterial chemoembolization; TARE, trans-arterial radioembolization; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for the fourth most cancer-related deaths in the United States (US) (1). Despite a recent national decline in the incidence of HCC cases, HCC secondary to non-alcoholic steatohepatitis (NASH) has become the fastest growing cause of HCC amongst liver transplant registrants in the US (2). This correlates to the increased rates of transplantation for NASH, currently representing the most common indication for liver transplantation in females and the second most common overall (3). As the obesity epidemic continues, it is becoming increasingly important to understand the outcomes associated with this subset of the HCC cohort.

HCC develops through progressive hepatocellular inflammation, leading to fibrosis, cell death, and aberrant regeneration which results in tumor formation (4). Different underlying etiologies uniquely impact gene regulation and cellular function leading to disease progression (4). World-wide, viral hepatitides (hepatitis C virus [HCV] and hepatitis B virus [HBV]) remain the most frequent etiologies of HCC; however, in the United States the burden of viral hepatitis-related HCC has been reduced by preventative treatment including the HBV vaccine and direct-acting antiviral (DAA) therapies for HCV (5–7). In contrast to viral hepatitis, as the obesity epidemic and prevalence of metabolic syndrome increases, non-alcoholic fatty liver disease (NAFLD) has become a progressively more common cause of end-stage liver disease (ELSD) (8). NAFLD currently afflicts 25% of the US population, with 20% of these patients demonstrating hepatocellular ballooning, inflammation, and steatohepatitis characteristic of NASH (9, 10).

Owing to the underlying metabolic syndrome often associated with NASH, these patients carry higher rates of concomitant cardiovascular and endocrine comorbidities than non-NASH ESLD population (11). Despite this, previous studies evaluating transplantation for NASH have consistently demonstrated similar post-transplant outcomes compared to patients with non-NASH liver failure (11, 12). Few studies, however, have assessed transplantation for NASH-related HCC which has increased in prevalence every year since 2002 (13). Specifically, little is known regarding recurrence rates and post-transplant survival in these patients compared to their non-NASH counterparts. This study sought to assess post-transplant recurrence rates and survival for NASH compared to non-NASH populations, as well as investigate survival patterns in patients with recurrent HCC after transplant.

METHODS AND PATIENTS

Patient Population

We performed a retrospective review of the Organ Procurement and Transplantation (OPTN) database for all adult (≥18-year-old) deceased donor liver transplant recipients in the United States diagnosed with HCC in the setting of known underlying liver disease. Our study population included transplants from 4 November 2012 to 6 December 2020, with the initiation date coinciding to the date OPTN began tracking tumor characteristics on transplant hepatectomy specimens. Recipients were first classified by diagnosis of NASH (NASH: 1,405, non-NASH: 6,086; **Figure 1**). Non-NASH patients with a primary HCC and no precipitating liver disease (i.e., HCV, alcoholic cirrhosis, HBV) were excluded, as were

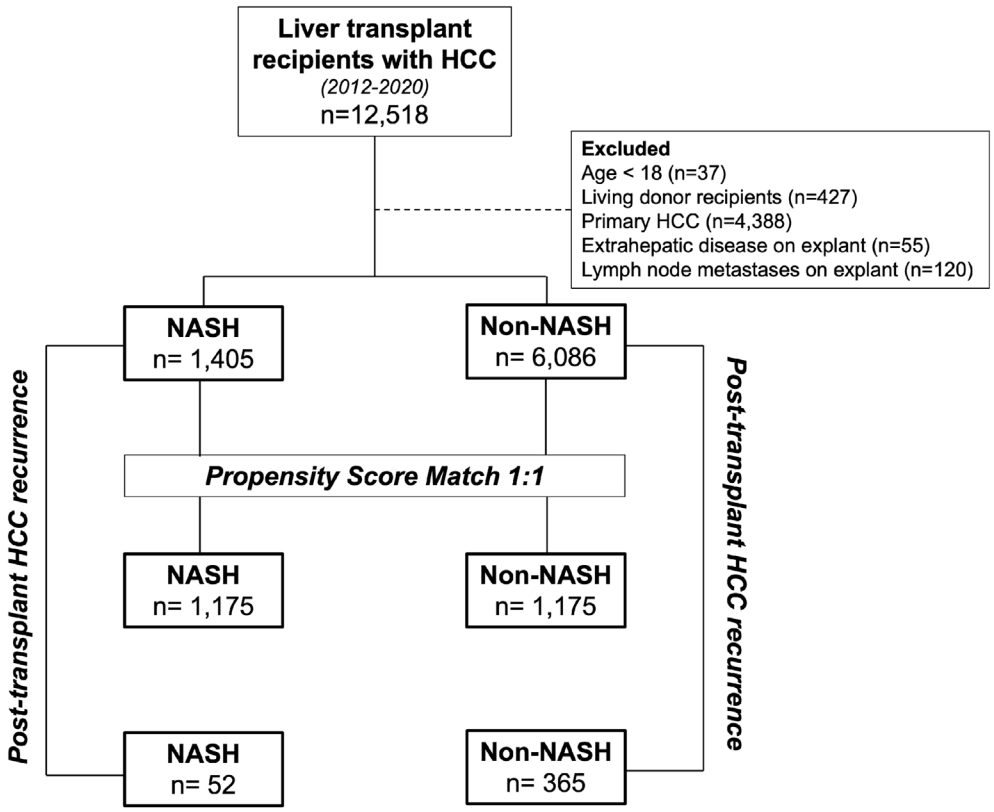


FIGURE 1 | Study design. NASH transplant recipients with HCC were first compared to non-NASH recipients with HCC. These patients were then propensity matched and further compared. Additional analysis was performed on the unmatched populations to compare with post-transplant HCC recurrence and post-recurrence survival between NASH and non-NASH populations.

those with evidence of extrahepatic spread or lymph node metastases on explant. To account for the high rate of undiagnosed NASH in patients with cryptogenic cirrhosis (14, 15), those with cryptogenic cirrhosis and underlying diabetes or BMI ≥30 were included in the NASH population, consistent with the methodology of previously validated, published studies (16–18). Patients were then stratified by post-transplant HCC recurrence, with cases of recurrent HCC identified through malignancy follow-up data (19). Here, NASH and non-NASH populations with recurrent malignancy were compared (NASH: 52, non-NASH: 365). Approval to conduct this analysis was obtained from the Thomas Jefferson University Institutional Review Board.

Assessing Post-Transplant Hepatocellular Carcinoma Recurrence Rate in NASH and Non-NASH Recipients

We first set out to assess post-transplant HCC recurrence rate in NASH vs non-NASH patients. We defined recurrence rate as a post-transplant HCC-related death or a diagnosis of HCC recurrence, derived from a validation study showing reliability of HCC recurrence data in the UNOS OPTN database (19). To reduce confounding bias associated with recipient cohorts of interest, non-NASH patients were propensity score matched (PSM) to NASH patients (**Supplementary Figure S1**). Both unmatched and PSM cohorts were compared with respect to baseline recipient, donor, and transplant characteristics. Tumor characteristics on transplant hepatectomy were also compared.

As most cases of recurrent HCC occur within 5 years (20), primary analysis focused on 5-year post-transplant recurrence rates. Secondary outcomes included median time to recurrence for those with recurrent HCC following transplant, and overall survival in NASH and non-NASH patients.

Evaluating Survival After Post-Transplant Recurrence

We then assessed survival patterns in NASH and non-NASH patients who developed post-transplant recurrence. Here, patients with recurrent HCC after transplant were again divided by underlying diagnosis (NASH: 52, non-NASH: 365). Baseline recipient, donor, and transplant characteristics were compared, as were tumor characteristics on transplant hepatectomy. The primary outcome assessed was survival after recurrence.

To evaluate differences between NASH and non-NASH patient cohorts' overall survival after transplant with and without recurrence, and to verify any trends seen only in the recurrence population, overall survival was reported in all four of those subgroups.

TABLE 1 | Propensity score matched baseline characteristics between NASH and non-NASH recipients with HCC.

	NASH	Non-NASH	*p*-value
Number	1,175	1,175	
Median followup (days)	1,070 (382–1,809)	1,243 (688–1,903)	
Recipient characteristics			
Age	64 (60–68)	64 (60–67)	0.55
Female sex	378 (32.17%)	384 (32.68%)	0.83
Ethnicity			0.67
White	882 (75.06%)	865 (73.62%)	
Black	11 (0.94%)	14 (1.19%)	
Other	282 (24.00%)	296 (25.19%)	
BMI	31.79 (28.20–35.53)	27.75 (24.64–31.66)	<0.01
Pre-exception MELD	12 (9–16)	12 (9–16)	0.48
AFP			0.98
<100 ng/ml	1,097 (93.36%)	1,094 (93.11%)	
100–399 ng/ml	62 (5.28%)	65 (5.53%)	
≥400 ng/ml	16 (1.36%)	16 (1.36%)	
Locoregional therapy			
TACE	752 (64.00%)	759 (64.60%)	0.79
TARE	132 (11.23%)	140 (11.91%)	0.65
Ablation	384 (32.68%)	365 (31.06%)	0.43
Other	11 (0.94%)	13 (1.11%)	0.84
Number of locoregional treatments			0.69
0	126 (10.72%)	116 (9.87%)	
1	727 (61.87%)	747 (63.57%)	
2	254 (21.62%)	238 (20.26%)	
≥3	68 (5.79%)	74 (6.30%)	
Disabled functional status	165 (14.04%)	184 (15.66%)	0.29
Diabetes mellitus	818 (71.57%)	328 (28.20%)	<0.01
Portal vein thrombosis	189 (16.11%)	203 (17.32%)	0.44
Hemodialysis	10 (0.85%)	19 (1.62%)	0.13
Previous abdominal surgery	626 (53.28%)	610 (51.91%)	0.53
Multiorgan	20 (1.70%)	23 (1.96%)	0.76
Primary diagnosis			—
NASH	1,175 (100.00%)	0 (0.0%)	
HCV	0 (0.0%)	63 (5.41%)	
HBV	0 (0.0%)	759 (65.15%)	
EtOH	0 (0.0%)	251 (21.55%)	
Other[a]	0 (0.0%)	92 (7.90%)	
Donor characteristics			
Age	46 (30–58)	45 (31–59)	0.80
Female sex	492 (41.87%)	499 (42.47%)	0.80
BMI	27.46 (23.74–32.34)	27.65 (23.56–31.96)	0.76
Diabetes mellitus	159 (13.53%)	165 (14.04%)	0.76
Macrosteatosis (%)	5 (0–10)	5 (0–10)	0.08
Inotrope support	566 (48.17%)	556 (47.32%)	0.71
LDRI	1.58 (1.28–1.92)	1.60 (1.28–1.94)	0.22
Cause of death			0.36
Anoxia	420 (35.74%)	459 (39.06%)	
CVA	391 (33.28%)	391 (33.28%)	
Head trauma	337 (28.68%)	302 (25.70%)	
CNS tumor	8 (0.68%)	5 (0.43%)	
Other	19 (1.62%)	18 (1.53%)	
DCD	84 (7.15%)	83 (7.06%)	0.99
Transplant details			
CIT (hours)	5.90 (4.60–7.25)	5.93 (4.50–7.55)	0.43

Values are listed as number (percentage) or median ± interquartile range unless otherwise stated.

BMI, body mass index; NASH, non-alcoholic steatohepatitis; AFP, alpha fetoprotein; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; HCV, Hepatitis C Virus; EtOH, alcohol; CVA, cerebrovascular accident; LDRI, Liver Donor Risk Index; CNS, central nervous system; DCD, donation after cardiac death; CIT, cold ischemia time.

[a]*Includes metabolic, autoimmune and cholestatic diseases.*

Statistical Analysis

Continuous variables were evaluated for normality using the Shapiro Wilk test. Non-normally distributed variables were compared with a Wilcoxon rank-sum test and were represented as median interquartile range (IQR). Categorical variables were compared using a chi-square or Fisher's exact test and were represented as numbers (percentage of population).

TABLE 2 | Propensity score matched tumor characteristics in transplant hepatectomy specimens.

	NASH	Non-NASH	*p*-value
Number	1,175	1,175	
No tumor on explant	71 (6.04%)	76 (6.47%)	0.73
Number of tumors			0.83
1	548 (46.64%)	524 (44.60%)	
2	268 (22.81%)	269 (22.89%)	
3	128 (10.89%)	130 (11.06%)	
≥4	160 (13.62%)	176 (14.98%)	
Largest tumor size (cm)	2.5 (1.5–3.5)	2.4 (1.5–3.5)	0.59
Tumor differentiation[a]			0.75
Complete necrosis	296 (25.19%)	276 (23.49%)	
Well	274 (23.32%)	270 (22.98%)	
Moderate	532 (45.28%)	555 (47.23%)	
Poor	73 (6.21%)	74 (6.30%)	
Vascular invasion			0.86
Microvascular	125 (10.64%)	134 (11.40%)	
Macrovascular	21 (1.79%)	21 (1.79%)	
Satellite lesions	59 (5.02%)	61 (5.19%)	0.93

Values are listed as number (percentage) or median ± interquartile range unless otherwise stated.
[a]*Differentiation of worst tumor.*

PSM of non-NASH to NASH patients was completed using 1:1 nearest-neighbor matching with a caliper width of 0.2. Covariates matched in propensity score models were identified *a priori* or by regression analysis as recipient, tumor explant, and donor characteristics predictive of graft survival. Appropriate matching was confirmed through histogram analysis of propensity score distributions and by Rubin's Bias and Ratio tests comparing matched cohorts. Full details regarding the PSM, including covariates used in the match, can be found in **Supplementary Figure S1**.

Post-transplant HCC recurrence rates were assessed using a competing risk-regression model with non-cancer-related death used as a competing outcome. Cumulative incidence of HCC recurrence was evaluated using Fine-Gray proportional sub distribution hazard ratio (SHR) models in NASH and non-NASH recipients. Post-transplant survival and survival after diagnosis of recurrence, as defined above, were reported via Kaplan-Meier curves with statistical significance assessed using Log-rank tests. Recurrence rates were compared using Cox Proportional Hazard regression modeling. These data were remained unadjusted as attempts at adjusted analyses yielded underpowered results. `For all comparisons two-sided statistical significance was set *a priori* at $p < 0.05$. All statistical analyses were performed using Stata/MP 16.1 (Statacorp, College Station, TX).

RESULTS

Post-Transplant Recurrence Rates in NASH and Non-NASH Patients

Baseline Characteristics of Hepatocellular Carcinoma Patients by Diagnosis of NASH

Prior to propensity matching, 1,405 patients had NASH-related HCC compared to 6,086 with non-NASH diagnoses (**Supplementary Tables S1, S2**). Median follow-up was 924 days (IQR: 365–1,707) in the NASH cohort and 1,366 days (IQR 678–1,898) for the non-NASH cohort. Underlying diseases in the non-NASH population were as follows: HCV (66.44%), HBV (6.34%), EtOH (21.30%) and "Other," which included metabolic, cholestatic and autoimmune conditions (5.92%).

PSM resulted in 1,175 matched pairs with largely similar profiles (**Tables 1**, **2**). Median follow-up was 1,070 days for NASH (IQR: 382–1,809) and 1,243 days (IQR 668–1,903) for non-NASH. In the PSM non-NASH group, HCV was the underlying diagnosis in 65.15% of patients (n = 759), while 5.41% (n = 63) had HBV, 21.55% (n = 251) EtOH and 7.90% (n = 92) other. No significant differences were observed in recipient or transplant profiles, or in tumor explant characteristics.

Outcomes of Hepatocellular Carcinoma Patients by Diagnosis of NASH

Comparing NASH to non-NASH transplant recipients, we observed reduced post-transplant HCC recurrence rate in NASH patients. After PSM, recurrence rates at 5 years were 5.80% in the NASH group and 9.41% in non-NASH patients (SHR: 0.61, 95% CI: 0.42–0.89, $p = 0.01$; **Table 3** and **Figure 2A**). For patients with post-transplant HCC recurrence, however, we could not show significant differences between median time to recurrence (426 vs. 400 days, $p = 0.59$). Additionally, while

TABLE 3 | Propensity score matched transplant outcomes by diagnosis of NASH.

	NASH	Non-NASH	HR/SHR	95% CI	*p*-value
Number	1,175	1,175			
Acute Rejection within 1 year	77 (8.85%)	62 (7.17%)	—	—	0.78
Recurrent Malignancy			(SHR)		
5-year	5.80%	9.41%	0.61	0.42–0.89	0.01
Median time to recurrence[a]	426 (213–752)	400 (195–796)	—	—	0.59
Post-transplant survival			(HR)		
Overall	—	—	0.87	0.71–1.07	0.20
1-year	92.98%	94.06%	—	—	0.32
3-year	86.35%	84.34%	—	—	0.38
5-year	80.71%	78.40%	—	—	0.30

Values are listed as percent, number (percentage) or median ± interquartile range unless otherwise stated.
[a]*For patients with recurrent HCC only.*

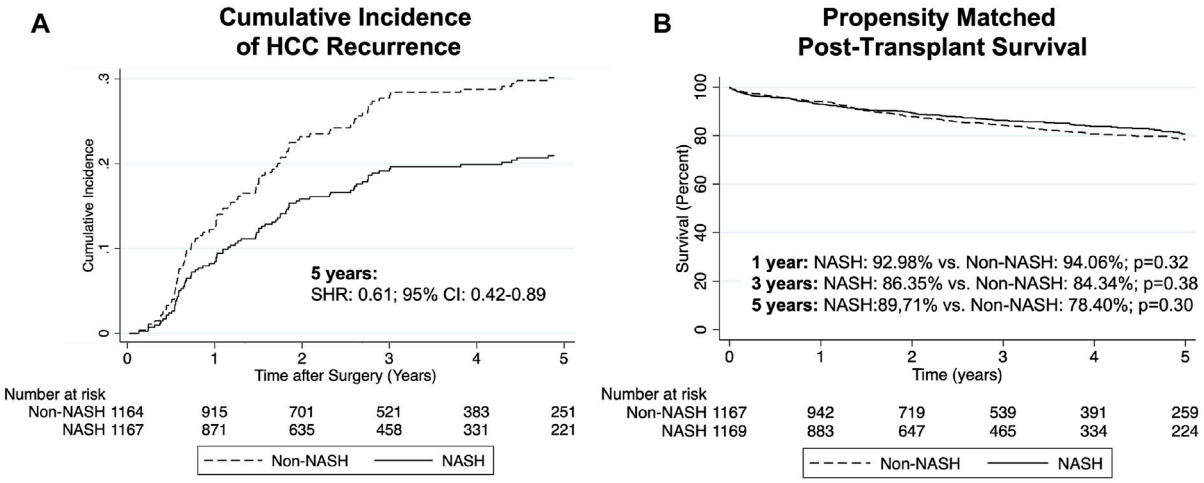


FIGURE 2 | Cumulative incidence of post-transplant HCC recurrence **(A)** and Kaplan-Meier curves comparing survival **(B)** in NASH vs PSM non-NASH patients.

recurrent rates were reduced in NASH patients, overall survival was not statistically significantly different (HR: 0.87, 95% CI: 0.71–1.07, p = 0.20, **Figure 2B**). At 1 year, survival in NASH patients was 92.98% and in non-NASH patients 94.06% (p = 0.32); at 3 years, survival was 86.35% vs. 84.34% (p = 0.38), and at 5 years, 80.71% vs. 78.40% (p = 0.30), thus all non-significant.

Assessing Survival Following Post-Transplant HCC Recurrence in NASH and Non-NASH Populations

Baseline Characteristics of Patients With Recurrent Hepatocellular Carcinoma by Diagnosis of NASH

We next assessed only patients with recurrent HCC after transplant. In this cohort, median follow-up for NASH patients was 2,059 days (IQR: 1,003–2,157) and 2,132 days (IQR: 1,445–2,409) for non-NASH patients. As shown in **Table 4**, we found that NASH patients were older (65 vs. 61 years old, $p < 0.01$), more frequently female (36.54% vs. 17.53%, $p < 0.01$), and comprised different ethnicities. Again, they also carried higher BMI (32.39 vs. 27.40, $p < 0.01$) along with increased incidence of diabetes (62.00% vs. 26.52%, $p < 0.01$) and PVT (25.00% vs. 12.36%, p = 0.02). No significant differences were noted in pre-transplant locoregional therapies, donor characteristics or transplant details. Additionally, tumor explant characteristics, were similar between NASH and non-NASH patients with recurrent HCC (**Table 5**).

Outcomes in Patients With Recurrent Hepatocellular Carcinoma by Diagnosis of NASH

We then compared outcomes in patients with recurrent malignancy. Here, we found no statistically significant differences in survival from time of recurrence in NASH compared to non-NASH patients (**Figure 3**; **Table 6**). At 6 months, survival was 53.99% vs. 67.02, p = 0.10; at 1 year, survival was 45.95% vs. 46.71% (p = 0.63), and at 18 months 29.03% vs. 34.43% (p = 0.45). Further, when measuring median time to death from date of recurrence in those patients with recurrence who had died, time was substantially shorter in NASH patients (150 vs. 227 days, p = 0.05), however this finding was not statistically significant (**Table 6**).

DISCUSSION

In this study we compared NASH-related and non-NASH HCC transplant populations, specifically looking at recurrence rates as well as survival post recurrence. NASH patients were found to have a lower HCC recurrence rate at 5 years while post-transplant survival remained similar between the two groups.

Previous studies comparing NASH to non-NASH populations have provided conflicting results to date with regards to HCC outcomes. Billeter et al. utilized propensity-score matching to compare NASH-related and non-NASH HCC patients in 34 NASH patients receiving liver resection in a single institution and found no differences in 1-, 3-, or 5-year recurrence-free survival (21). Furthermore, in a 60 patient cohort, Sadler et. al. noted no difference in overall survival in NASH-related and non-NASH patients receiving liver transplant for HCC (22). Additionally, they observed that meeting Milan criteria did not impact recurrence for NASH-related HCC patients, suggesting that even advanced HCC in NASH may have favorable outcomes (22). While these studies suggested no difference in outcomes for NASH-related HCC, Weinmann et. al. reported decreased overall survival in NASH patients undergoing transplant; however recurrence free survival was not reported (23). Finally, several studies, similarly limited by data on recurrence, have suggested improved overall survival in NASH patients (11, 24, 25). To provide clarity to the conflicting data, our study utilized the largest available national dataset of liver transplant recipients with HCC and found a significantly lower rate of post-transplant HCC recurrence, as well as worse post-recurrence outcomes in the NASH patient population.

TABLE 4 | Baseline characteristics in NASH and non-NASH recipients with HCC recurrence after transplant.

	NASH	Non-NASH	*p*-value
Patients with recurrent HCC	52	365	
Median followup (days)	2,058 (1,002–2,156)	2,133 (1,444–2,503)	
Recipient characteristics			
Age	65 (62–67)	61 (57–65)	<0.01
Female sex	19 (36.54%)	64 (17.53%)	<0.01
Ethnicity			0.01
White	37 (71.15%)	232 (63.56%)	
Black	0 (0.0%)	50 (13.70%)	
Other	15 (28.85%)	83 (22.74%)	
BMI	32.39 (29.21–35.39)	27.40 (24.27–31.32)	<0.01
Pre-exception MELD	12 (9–16)	11 (8–15)	0.66
AFP			0.65
<100 ng/ml	41 (80.39%)	271 (75.70%)	
100-399 ng/ml	6 (11.76%)	61 (17.04%)	
≥400 ng/ml	4 (7.84%)	26 (7.26%)	
Locoregional therapy			
TACE	38 (73.08%)	264 (72.33%)	0.99
TARE	6 (11.54%)	24 (6.58%)	0.24
Ablation	17 (32.69%)	98 (26.85%)	0.41
Other	0 (0.00%)	4 (1.10%)	0.99
Number of locoregional treatments			0.99
0	6 (11.54%)	41 (11.24%)	
1	27 (51.92%)	194 (53.15%)	
2	14 (26.92%)	94 (25.75%)	
≥3	5 (9.62%)	36 (9.86%)	
Disabled functional status	6 (11.54%)	60 (16.44%)	0.42
Diabetes mellitus	31 (62.00%)	96 (26.52%)	<0.01
Portal vein thrombus	13 (25.00%)	45 (12.36%)	0.02
Hemodialysis	0 (0.00%)	6 (1.64%)	0.99
Previous abdominal surgery	22 (42.31%)	154 (42.19%)	0.99
Multiorgan recipient	0 (0.00%)	7 (1.92%)	0.60
Primary diagnosis			—
NASH	52 (100.00%)	0 (0.0%)	
HCV	0 (0.0%)	245 (67.68%)	
HBV	0 (0.0%)	18 (4.97%)	
EtOH	0 (0.0%)	83 (22.93%)	
Other[a]	0 (0.0%)	16 (4.42%)	
Donor characteristics			
Age	42 (26–56)	44 (30–56)	0.73
Female sex	22 (42.31%)	151 (41.37%)	0.99
BMI	27.23 (23.99–31.65)	27.27 (23.13–31.44)	0.71
Diabetes mellitus	5 (9.62%)	47 (12.88%)	0.66
Macrosteatosis	5 (5–18)	5 (0–10)	0.06
Inotrope support	26 (50.00%)	182 (49.86%)	0.99
LDRI	1.53 (1.23–1.87)	1.54 (1.27–1.87)	0.83
Cause of death			0.98
Anoxia	20 (38.46%)	132 (36.16%)	
CVA	18 (34.62%)	127 (34.79%)	
Head trauma	14 (26.92%)	100 (27.40%)	
CNS tumor	0 (0.00%)	2 (0.55%)	
Other	0 (0.001%)	4 (1.10%)	
DCD	5 (9.62%)	25 (6.85%)	0.40
Transplant details			
CIT (hours)	6.05 (4.25–8.26)	5.95 (4.66–7.58)	0.58

Values are listed as number (percentage) or median ± interquartile range unless otherwise stated.

BMI, body mass index; NASH, non-alcoholic steatohepatitis; AFP, alpha fetoprotein; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; HCV, Hepatitis C Virus; EtOH, alcohol; CVA, cerebrovascular accident; LDRI, liver donor risk index; CNS, central nervous system; DCD, donation after cardiac death; CIT, cold ischemia time.

[a]*Includes metabolic, autoimmune and cholestatic diseases.*

Understanding the biology of HCC in NASH-related and non-NASH patients is critical to understanding tumor behavior as well as response to transplantation and adjuvant treatment modalities. Unlike HCC secondary to non-NASH diseases, NASH-related HCC pathogenesis is uniquely affected by a cascade of insulin resistance which causes oxidative stress, inflammation, and fibrosis-stimulating cytokines (4, 26). Additionally, AFP is a frequently used biomarker in screening for HCC associated

TABLE 5 | Tumor characteristics in transplant hepatectomy specimens in patients with recurrent HCC after transplant.

	NASH	Non-NASH	*p*-value
Patients with recurrent HCC	52	365	
No tumor on explant	0 (0.00%)	9 (2.47%)	0.61
Number of tumors			0.13
1	21 (40.38%)	138 (37.81%)	
2	8 (15.38%)	80 (21.92%)	
3	11 (21.15%)	35 (9.59%)	
≥4	12 (23.08%)	103 (28.22%)	
Largest tumor size (cm)	3.2 (2.1–4.6)	2.8 (1.7–4.3)	0.09
Tumor differentiation[a]			0.50
Complete necrosis	6 (11.54%)	39 (10.68%)	
Well	4 (7.69%)	48 (13.15%)	
Moderate	29 (53.85%)	207 (56.71%)	
Poor	14 (26.92%)	71 (19.45%)	
Vascular invasion			0.11
Microvascular	12 (23.08%)	113 (30.96%)	
Macrovascular	6 (11.54%)	18 (4.93%)	
Satellite lesions	5 (9.62%)	38 (10.41%)	0.99

Values are listed as number (percentage) or median ± interquartile range unless otherwise stated.
[a]*Differentiation of worst tumor.*

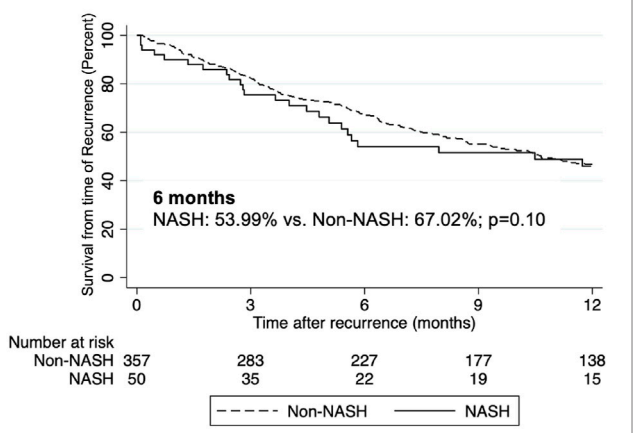


FIGURE 3 | Kaplan-Meier curves comparing survival following recurrence in NASH vs. non-NASH patients.

with tumor aggressiveness since it is produced during times of sustained liver injury and regeneration (27). Studies have found that NASH-related HCC patients have lower levels of AFP and have hypothesized that this may suggest a less aggressive tumor biology (28, 29). Our study similarly noted lower AFP levels in NASH-related HCC patients. Mittal et. al. showed a potential clinical significance of the less aggressive phenotype by noting that NASH-related HCC patients were less likely to be screened for HCC within 3-years of their diagnosis compared to HCV-related, and thus presented at a more advanced stage (28). Despite this, NASH-related HCC patients demonstrated similar 1-year survival to non-NASH patients (28). These findings may help explain the lower recurrence rate we observed in the NASH-related HCC cohort. Ultimately, further studies investigating the biology of post-transplant recurrent HCC and its clinical impact will be critical to define these observations.

Another important difference between NASH-related and non-NASH patients are tumor characteristics at time of surgical treatment. Utilizing the UNOS OPTN database, Lewin et. al. found that NASH patients receiving liver transplantation for HCC were less likely to have tumors with vascular invasion and/or poor differentiation upon explant and were less likely to have evidence of metastasis compared to other HCC etiologies (30). This could support the theory that NASH HCC may be less aggressive at time of surgical intervention, leading to less overall recurrence, but warrants further study.

While we observed lower recurrence rates in NASH HCC patients, those who did recur had shorter median survival than non-NASH patients. Some emerging data may help explain that by highlighting differences in NASH-related HCC response to adjuvant therapies. Locoregional therapy, namely TACE, has been shown to have lower complete response, more progression of disease, higher rates of residual disease, and more recurrence in 1-2-month follow-up imaging in the obese population (31). Wu et. al. attributed this finding to the chronic low level of inflammation associated with obesity which they believed to incite a pro-inflammatory and, thus, tumorigenic metabolic milieu potentially contributing to increased recurrence (31). In addition, resistance to sorafenib, a widely used systemic treatment for late-stage HCC, is observed in patients on chronic metformin therapy as these drugs work on similar downstream pathways (32, 33). Some studies suggest Sorafenib delays time to HCC

TABLE 6 | Outcomes in patients with recurrent HCC after transplant by diagnosis of NASH.

	NASH	Non-NASH	HR	95% CI	*p*-value
Patients with recurrent HCC	52	365			
Median time to death after recurrence (days)[a]	150 (73–375)	227 (97–484)	—	—	0.05
Survival after recurrence					
Overall	—	—	1.06	0.73–1.53	0.75
6 months	53.99%	67.02%	—	—	0.10
1 year	45.95%	46.71%	—	—	0.63
18 months	29.03%	34.43%	—	—	0.45

Values are listed as percent, number (percentage) or median ± interquartile range unless otherwise stated.
[a]*For mortalities only.*

recurrence and in a small study, Kang et. al. found just over a 7-month survival benefit in a heterogenous population of post-transplant HCC patients with recurrence (34, 35). With a majority of NASH-related HCC patients being obese and having diabetes these findings could provide insight into why we observed that NASH-related HCC patients with recurrence had a significantly shorter survival, although we are limited by the data source. Clearly, further investigation using more a detailed data source is required to explain the recurrent tumor biology associated with the NASH.

Our study suffers several limitations which include but are not limited to the retrospective nature of a large, federally maintained database. It should be noted that HCC outcomes in this database lack granular details regarding some tumor and treatment characteristics. A recent study, however, showed that the UNOS OPTN observed HCC recurrence rate was not significantly lower than the expected rate, validating the use of the OPTN database in evaluating outcomes related to transplantation for HCC (19). Moreover, while we sought to evaluate tumor specific outcomes between NASH-related and non-NASH recipients, we cannot definitively comment on the "biology" of the tumor itself, but can draw attention to the series of comparisons we made between NASH and non-NASH groups of HCC post-transplant patients. As such, future studies should focus their attention on the tumor-specific behavior which contributes to the diversion of these two distinct populations. In addition, our study inclusion period started prior to the widespread use of DAAs, possibly affecting the HCC recurrence rate in non-NASH patients. However, a recent review compiling multiple observational studies reported that while, in fact, early studies warned of a higher HCC recurrence rate in HCV-related HCC patients, there is actually no significant change in recurrence linked to DAA treatment (36). We performed an unreported subanalysis of our own patient cohort removing patients diagnosed in the years 2012–2014 (prior to the widespread use of DAAs) which showed similar results, but all of which were underpowered. Another limitation of our study is the potential bias due to timing of HCC recurrence detection. The median survival post-recurrence will have some bias based on when the diagnosis is made which we could not account for given the dataset. Also, while most HCC recurrence post-transplant occur within 2 years, another limitation of the study is the relatively short median follow up at 3.4 years, which may miss late HCC recurrence. Additionally, the number of recurrences is relatively small leading to potential for bias in our subanalysis of overall survival. Unfortunately, we also did not have access to all the data surrounding reason for death within the database. However, of the available data, 72% of non-NASH and 69% of NASH deaths after recurrence were recorded as being secondary to malignancy with the remainder of causes of death being <10% for both cohorts except in the "Other" category. Additionally, many patients with recurrence decline and have a different reported ultimate cause of death despite the decline resulting from the recurrence. Finally, follow-up time for non-NASH patients in our study was 1,366 days (versus 924 for NASH patients). Unreported subanalysis was performed to remove non-NASH patients with longer follow-up and results were similar, but, again, underpowered.

Currently, increased early detection of HCC and surgical treatment offers the best therapeutic opportunity for HCC patients with any etiology (37). This study highlights, however, that differences do exist within the heterogeneous HCC patient population. These differences, likely linked to underlying etiology-specific tumor biology, should be the focus of future investigations to elucidate how we can exploit them and directly improve HCC outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Thomas Jefferson University Hospital Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RL, PA, and AB participated in research design, writing of the paper, performance of the research, and data analysis. KP, OS, AA, JC, WM, AF, CR, JG, AS, and HD participated in writing of the paper.

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AUTHOR DISCLAIMER

The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10175/full#supplementary-material

Supplementary Figure S1 | The propensity score match. **(A)** demonstrates covariates selected for use in the propensity score model. **(B)** demonstrates bias across each covariate between non-NASH and NASH recipients. These were compared before and after matching. On the bottom two graphs **(C)**, propensity score distribution from NASH (top) and non-NASH (bottom) populations are compared before [left, **(Ci)**] and after [right, **(Cii)**] matching.

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ORIGINAL RESEARCH
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Estimation of Early Graft Function Using the BETA-2 Score Following Clinical Islet Transplantation

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Little is known about how early islet graft function evolves in the clinical setting. The BETA-2 score is a validated index of islet function that can be calculated from a single blood sample and lends itself to frequent monitoring of graft function. In this study, we characterized early graft function by calculating weekly BETA-2 score in recipients who achieved insulin independence after single transplant (group 1, n = 8) compared to recipients who required a second transplant before achieving insulin independence (group 2, n = 7). We also determined whether graft function 1-week post-transplant was associated with insulin independence in individuals who received initial transplant between 2000–2017 (n = 125). Our results show that graft function increased rapidly reaching a plateau 4–6 weeks post-transplant. The BETA-2 score was higher in group 1 compared to group 2 as early as 1-week post-transplant (15 + 3 vs. 9 + 2, p = 0.001). In an unselected cohort, BETA-2 at 1-week post-transplant was associated with graft survival as defined by insulin independence during median follow up of 12 months (range 2–119 months) with greater survival among those with BETA-2 score >10 (p < 0.001, log-rank test). These findings suggest that primary graft function is established within 4–6 weeks post-transplant and graft function at 1-week post-transplant predicts long-term transplant outcomes.

Keywords: islet transplantation, graft survival, graft function, engraftment, BETA-2 score

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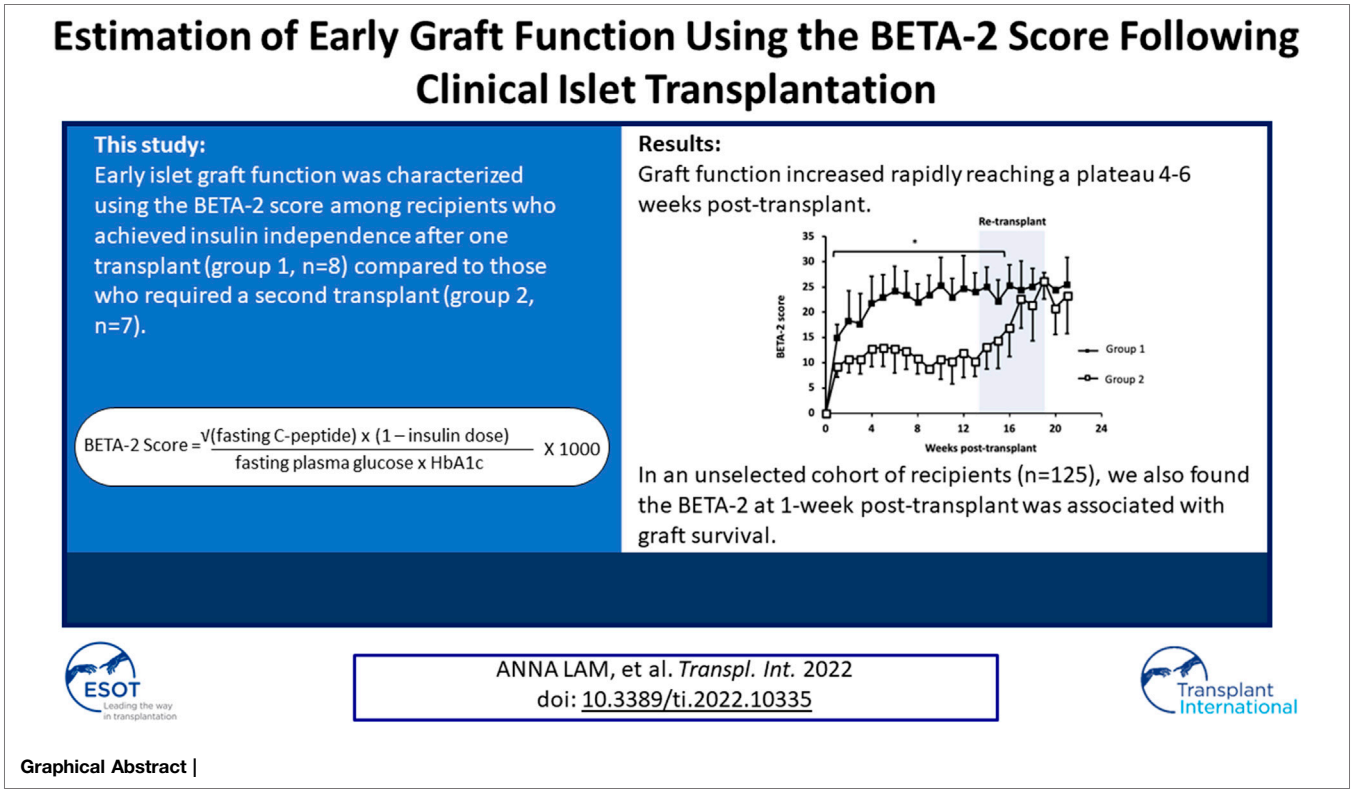
Abbreviations: BMI, body mass index; CP/G, C-peptide/glucose ratio; HbA1c, hemoglobin A1c; HOMA2-B%, homeostasis model assessment index of beta cell function; IE, islet equivalents; SD, standard deviations; SUITO, Secretory Unit of Islet Transplant Objects; TEF, transplant estimated function.



Graphical Abstract |

INTRODUCTION

Advances in clinical islet transplantation including in islet processing and immunosuppression protocols have led to improved outcomes with increased rates of insulin independence and longer-lasting graft function (1). However, most recipients will require at least two islet transplants to achieve insulin independence and will have declining graft function over time with less than 50% of recipients maintaining insulin independence at 3 years post-transplant (2).

Optimization of early islet graft function remains an important target for improving long-term islet transplant outcomes. More than 50% of transplanted islets are lost in the first few days post-transplant (3, 4) and peri-transplant interventions limiting inflammation and islet stress have been shown to promote insulin independence and long-term islet survival (5, 6). Primary graft function at one-month post-transplant has been associated with long-term islet graft function (7), however, it remains unknown how primary graft function evolves in the first weeks to months after transplant.

One of the major challenges in this area has been the inability to closely monitor islet function. Formal stimulation tests measuring insulin or C-peptide response to stimuli such as glucose or arginine provide precise information on graft function, but the metabolic stress, as well as the time and labor-intensive nature of these tests, make them impractical for frequent monitoring in the clinical setting. Taking advantage of the BETA-2 score, a validated measure of islet function that can be calculated from a single fasting blood sample (8, 9), we characterized graft function in the first-weeks post-transplant and determined whether graft function as early as 1-week post-transplant is associated with long-term transplant outcomes.

METHODS

Recipients

All subjects provided informed consent, and the analysis of data was approved by the University of Alberta Health Research Ethics Board. We performed a retrospective single-center analysis of individuals newly transplanted with allogeneic islets between 2009 and 2014. To characterize the establishment of islet graft function, BETA-2 score was calculated weekly in two selected groups representing distinct transplant outcomes: 1) subjects who achieved and maintained insulin independence for at least 12 months after a single islet infusion (group 1), and 2) subjects who only became insulin-independent (which was sustained beyond 12 months) after they received a second islet infusion after 3–6 months because they had not achieved insulin independence after their first infusion (group 2). Insulin independence was defined by no exogenous insulin use and no more than 2 self-monitored blood glucose levels >10.0 mmol/L during a 7-day period (10). A cohort of islet transplant recipients newly transplanted between 2000 and 2017 who had available lab

TABLE 1 | Baseline characteristics.

	All patients (*n* = 15)	Group 1 (*n* = 8)	Group 2 (*n* = 7)	*p*
Sex (male/female)	5/10	2/6	3/4	0.61
Age (years)	55.6 ± 9.9	56.8 + 9.4	54.3 ± 11.1	0.64
Diabetes duration (years)	34.9 ± 13.6	32.8 + 13.4	37.3 ± 14.4	0.54
Weight (kg)	68.5 ± 10.8	64.1 + 8.1	73.4 ± 11.9	0.10
BMI (kg/m^2)	25.4 ± 2.6	23.9 + 1.9	27.0 ± 2.3	0.01
HbA1c (%)	8.6 ± 1.1	9.2 + 0.9	8.0 ± 0.9	0.03
Fasting blood glucose (mmol/L)	12.3 ± 5.4	13.5 + 4.9	11.0 ± 6.2	0.41
Insulin dose (units/kg per day)	0.5 ± 0.1	0.5 + 0.1	0.5 ± 0.1	0.96
First transplant				
IEQ	525,364 ± 274,102	624,189 + 348,429	412,422 ± 75,944	0.14
IEQ/kg	7,669 ± 3,626	9,476 + 4,205	5,603 ± 846	0.03
Second transplant				
IEQ			519,886 ± 176,138	
IEQ/kg			7491 ± 2,312	
Total IEQ	767,978 ± 328,107	624,189 + 348,429	932,308 ± 224,685	0.07
Total IEQ/kg	11,164 ± 3,935	9,476 + 4,205	13,094 ± 2711	0.07

BMI, body mass index; IEQ, islet equivalents; IEQ/kg, islet equivalents per recipient body weight. Data are expressed as mean ± SD and n (%).

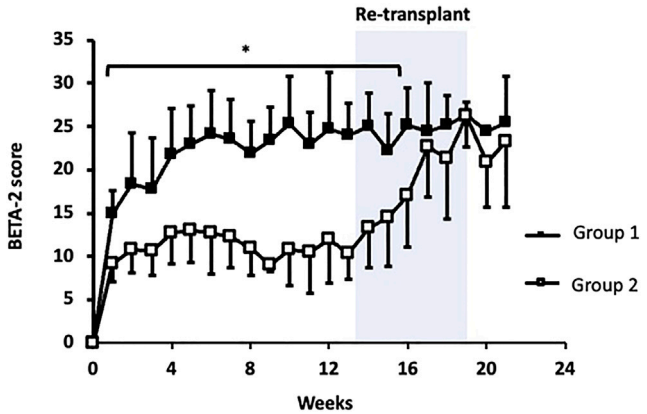


FIGURE 1 | BETA-2 score in the first 6 months post initial islet transplant. Group 1 (closed squares). Group 2 (open squares). Shaded area indicates when group 2 received their second transplant. *$p < 0.05$, group 1 vs. group 2.

results and insulin records at 1-week post-transplant were evaluated to determine whether BETA-2 score at 1-week post-transplant is associated with long-term transplant outcomes. The indications for islet transplantation, islet preparation, transplant procedure, and monitoring have been previously described (11, 12). Immunosuppression consisted of induction with alemtuzumab, thymoglobulin, daclizumab or basiliximab, and maintenance with tacrolimus and sirolimus or mycophenolate mofetil.

Clinical Assessment

All subjects were seen weekly in-clinic during the first month post-transplant and then every 3–6 months in the first year post-transplant. Subjects were asked to self-monitor blood glucose and insulin usage. No specific protocol for insulin titration was used; post-transplant insulin doses were adjusted to avoid hyper- and hypo-glycemia (i.e., target glucose 4–10 mmol/L). Insulin dose (unit/kg) was calculated based on reported insulin dose divided by body weight measured at the most recent clinical assessment. Unfortunately, data on insulin delivery method was not available for this analysis. Blood work including fasting C-peptide and fasting glucose were measured every 1–2 weeks during the first 6 months post-transplant. HbA1c (as a percentage) was measured every 1–3 months post-transplant. For fasting blood work, patients were advised not to eat or drink after midnight the night before blood work was drawn with no specific instructions regarding insulin doses.

Assays

Fasting plasma glucose concentrations were determined by the glucose oxidase method. C-peptide concentrations were measured using a commercial assay (Roche Elecsys; Roche Diagnostics, Indianapolis, IN). The lower limit of sensitivity for C-peptide in our laboratory was 0.02 nmol/L and the inter-assay coefficient of variation was 3.5%. HbA1c was measured by the Bio-Rad Variant II kit (Hercules, CA).

Calculation of BETA-2 Score

BETA-2 scores were calculated weekly post-transplant. Derivation and validation of the BETA-2 score have previously been described (10). The BETA-2 is generated based on fasting C-peptide (nmol/L), daily insulin dose (units/kg), fasting plasma glucose (mmol/L), and HbA1c (%) as follows:

$$\text{BETA-2 Score} = \frac{\sqrt{(\text{fasting C-peptide})} \times (1 - \text{insulin dose})}{\text{fasting plasma glucose} \times \text{HbA1c}} \times 1000$$

Other Indices of Islet Graft Function

Alternative simple indices of graft function were calculated at 1-week post-transplant as detailed below.

TABLE 2 | Baseline characteristics of individuals newly transplanted between 2000–2017.

	All patients	BETA-2 score at 1-week post-transplant			*p*
		<10	10–14	≥15	
n	125	61	45	19	
Sex (male/female)	55/70	29/32	16/29	10/9	0.33
Age (years)	48.3 ± 9.8	46.5 ± 10.7	49.6 ± 8.7	51.0 ± 9.0	0.12
Diabetes duration (years)	32.7 ± 10.7	31.0 ± 10.2	33.2 ± 11.1	36.7 ± 10.7	0.12
Weight (kg)	74.0 ± 12.3	73.8 ± 13.4	73.5 ± 11.3	75.5 ± 11.5	0.84
BMI (kg/m^2)	25.9 ± 3.4	25.9 ± 3.7	25.9 ± 3.1	26.2 ± 3.6	0.92
HbA1c (%)	8.3 ± 1.2	8.2 ± 1.3	8.3 ± 1.1	8.7 ± 1.3	0.25
Insulin dose (units/kg/day)	0.56 ± 0.16	0.60 ± 0.16	0.54 ± 0.16	0.50 ± 0.13	0.02
IEQ	465,565 ± 143,129	437,523 ± 147,628	483,862 ± 126,893	512,582 ± 152,524	0.08
IEQ/kg	6291 ± 1,581	5906 ± 1,482	6603 ± 1,532	6787 ± 1774	0.03

BMI, body mass index; IEQ, islet equivalents; IEQ/kg, islet equivalents per recipient body weight.
Data are expressed as mean ± SD and n (%).

C-peptide/glucose ratio (CP/G) was calculated from C-peptide (ng/ml) and fasting plasma glucose (mg/dl) levels (13).

$$\text{CP/G} = \frac{\text{fasting C-peptide}}{\text{fasting plasma glucose}} \times 100$$

The homeostasis model assessment index of beta-cell function (HOMA2-B%) was calculated from fasting C-peptide (nmol/L) and plasma glucose (mmol/L) using the HOMA calculator (www.dtu.ox.ac.uk/homacalculator).

The Secretory Unit of Islet Transplant Objects (SUITO) index was also calculated from fasting plasma glucose (mmol/L) and C-peptide (nmol/L) (14, 15).

$$\text{SUITO index} = \frac{250 \times \text{fasting C-peptide}}{\text{fasting plasma glucose} - 3.43}$$

Transplant estimated (TEF) was calculated from the daily insulin requirement (DIR; units/kg/24 h) and HbA1C (%) as previously described (16).

$$\text{TEF} = \left(\text{DIRpreTx} + \frac{\text{HbA1cpreTx}}{5.43}\right) - \left(\text{DIR} + \frac{\text{HbA1c}}{5.43}\right)$$

Statistics

Statistical analyses were performed using Stata version 14.1 (StataCorp, College Station, TX). Descriptive statistics are expressed as mean ± standard deviation (SD). Two-tailed t-test, Chi-square test, one-way ANOVA, and Tukey test were used to compare groups as appropriate. Receiver operating characteristic curves were constructed for recipients' BETA-2 score, CP/G, HOMA2-B%, SUITO index, and TEF at 1-week post-transplant based on insulin independence. The association between BETA-2 score and insulin independence was evaluated by multiple logistic regression adjusted for pre-transplant BMI, HbA1C, and insulin dose, as well as islet equivalents per recipient body weight (IEQ/kg), transplanted. Survival analysis for the duration of insulin dependence was generated using the Kaplan-Meier method and analyzed using the Mantel-Cox log-rank test. A p-value < 0.05 was considered statistically significant and all p-values were reported as two-sided. To compare the differences in survival between groups, Bonferroni-adjusted posthoc pairwise comparisons were conducted with an adjusted p-value <0.017 considered statistically significant.

RESULTS

Baseline Characteristics

The BETA-2 score was calculated on a weekly basis for the first 6 months post-transplant in 1) recipients who achieved insulin independence after a single transplant (n = 8, group 1) and 2) recipients who achieved insulin independence after having a second islet transplant 3–6 months from their first transplant (n = 7, group 2). Baseline characteristics were similar between both groups except for HbA1c which was higher in group 1 and BMI which was higher in group 2 (**Table 1**). Group 1 subjects received significantly higher islet equivalents per recipient body weight (IEQ/kg) with their first transplant compared to group 2 subjects (9476 ± 4205 IEQ/kg vs. 5603 ± 846 IEQ/kg, p = 0.03), however, there was no significant difference in total IE/kg after

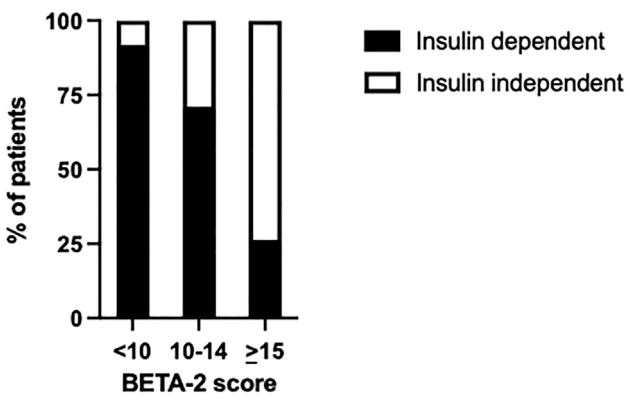


FIGURE 2 | Percentage of transplant recipients who achieved insulin independence or remained insulin-dependent according to BETA-2 score at 1-week post-transplant p < 0.001.

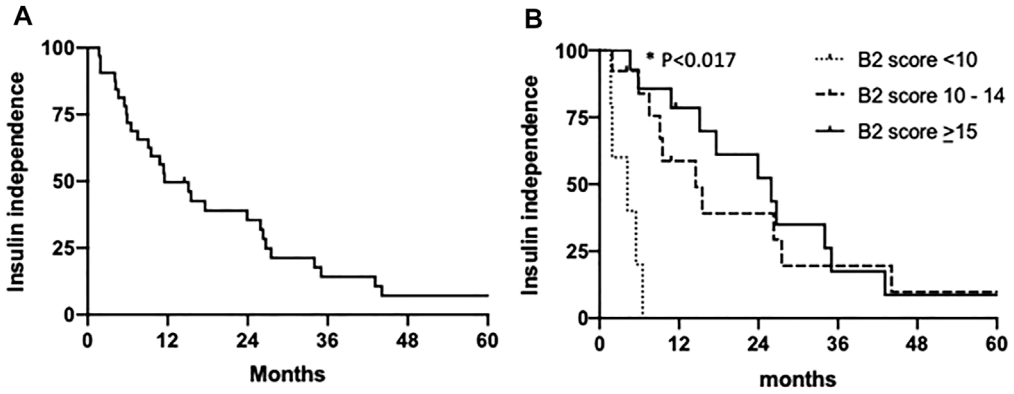


FIGURE 3 | Kaplan-Meier estimates of the proportions of patients with insulin independence **(A)** among the entire cohort and **(B)** according to 1-week post-transplant BETA-2 score <10 (n = 5), 10–14 (n = 13) or >15 (n = 14). *Durability of insulin independence was significantly lower among subjects with 1-week BETA-2 score <10 vs. 10–14 (p = 0.0002) and BETA-2 score <10 vs. > 15 (p = 0.0001) by Mantel-Cox log-rank test and Bonferroni corrected significance threshold $p < 0.017$.

recipients from group 2 received their second transplant (9476 ± 4205 IEQ/kg vs. 13,094 ± 2711 IEQ/kg, p = 0.07).

Early Graft Function

In both groups, BETA-2 score was measurable at 1-week and continued to increase before reaching a plateau 4 to 6 weeks post-transplant (**Figure 1**). BETA-2 score was significantly higher in group 1 compared to group 2 recipients as early as 1-week post-transplant (BETA-2 score 15 ± 3 vs. 9 ± 2, p = 0.001) and this difference was maintained until group 2 recipients received their second islet infusion at 4.1 ± 0.9 months (BETA-2 score 25 ± 4 vs. 17 ± 6, p = 0.07) (**Figure 1**). As expected, glycemic control as measured by HbA1c improved post-transplant in both groups (**Supplementary Figure S1**).

Early Graft Function and Transplant Outcomes

BETA-2 score at 1-week post-transplant was evaluated in an unselected cohort of recipients after their first islet transplant (n = 125) (**Table 2**). In total 26% achieved insulin independence for a median duration of 10 months (range 1.7–43 months, n = 32) while 74% remained insulin-dependent (n = 93). BETA-2 score at 1-week post-transplant was higher among those who achieved insulin independence compared to those who remained insulin-dependent (13 ± 3 vs. 9 ± 4, $p < 0.001$). BETA-2 score at 1-week also showed good discriminative ability for insulin independence (AUROC 0.83, $p < 0.001$) compared to alternative indices of graft function including the SUITO index, HOMA2-B%, CP/G and TEF (AUROC 0.55–0.77) (**Supplementary Figure S2**; **Supplementary Table S1**). Insulin independence was achieved in 8% (n = 5), 29% (n = 13), and 74% (n = 14) of recipients with BETA-2 score <10, 10–14 and ≥15, respectively ($p < 0.001$) (**Figure 2**). The odds of insulin independence increased with increasing BETA-2 score at 1 week including when adjusted for pre-transplant insulin dose, BMI, and HbA1c, as well as IE/kg transplanted (unadjusted odds ratio 1.39, 95% CI 1.21–1.59, $p < 0.001$ and adjusted odds ratio 1.44, 95% CI 1.23–1.70, $p < 0.001$). BETA-2 score at 1-week post-transplant was associated with graft survival as defined by insulin independence ($p < 0.001$, log-rank test) over a median follow-up of 12 months (range 2–119 months), with median survival of 4.2 months [IQR 1.9–5.5], 14.5 months [IQR 9.1–27.5] and 25.9 [IQR 15.1–35.0], respectively among recipients with BETA-2 score <10, 10–14 and ≥15 (BETA-2 score <10 vs. 10–14, $p < 0.002$ and vs. ≥15, $p < 0.001$) (**Figure 3**).

CONCLUSION

This study describes the evolution of islet graft function in the early period post-islet transplant using the BETA-2 score. This validated clinical score assessed weekly shows that graft function is established rapidly and increases over the first 4–6 weeks post-transplant before stabilizing. Furthermore, early engraftment estimated by the BETA-2 score as early as 1-week post-transplant is key to predicting longer-term transplant outcomes.

Vantyghem et al have shown that primary graft function as measured by the original BETA score at 1-month post-transplant is associated with prolonged graft survival (7). More recently, Witkowski et al demonstrated that the BETA-2 score on day 75 post-transplant is an early predictor of graft decline (15). In keeping with these studies, we found that it takes approximately 4–6 weeks before primary islet graft function is established and supports the association of graft function in the first 1–2 months with islet transplant outcomes.

Interestingly, our results suggest that it is possible to assess how well a graft will function even before primary graft function is fully established. We compared transplant recipients who achieved insulin independence for at least 1 year after a single transplant to those who remained insulin-dependent and found that the BETA-2 score was significantly higher at 1-week post-transplant among those who achieved insulin independence. We confirmed this in an unselected cohort of islet transplant recipients where a significantly higher BETA-2 score at 1-week was observed among those who achieved insulin independence

post-transplant. In clinical practice, this may translate into earlier identification of recipients who are unlikely to achieve insulin independence and allow for earlier intervention including repeat transplantation in recipients who are already immunosuppressed/lymphodepleted. An early endpoint such as the BETA-2 score 1-week post-transplant could serve as an intermediate outcome and allow for shorter and more efficient clinical trial testing strategies designed to improve islet engraftment.

Ourselves and others have shown previously that BETA-2 scores >13 and >15 reliably predict insulin independence (8, 9) and a BETA-2 score >17.4 on day 75 post-islet transplant has been found to be associated with durable (5 years) insulin independence (15). This is similar to our current findings: that islet transplant recipients who achieved and maintained insulin independence for at least 1 year after a single infusion had an average BETA-2 score of 15 at 1-week post-transplant, and in our unselected cohort, recipients who achieved insulin independence (minimum duration 1 month) had average BETA-2 score of 13. In both analyses, for recipients who were unable to come off insulin, the average BETA-2 score at 1-week was 9. We also found that BETA-2 score at 1-week post-transplant was associated with long-term graft survival with a longer duration of insulin independence among recipients with BETA-2 scores of 10–14 and ≥15 compared to those with BETA-2 scores <10. Taken together, it appears that a BETA-2 score cut-off of >13 at 1-week post-transplant may be useful in identifying recipients who are likely to achieve insulin independence with higher scores being associated with a longer duration of insulin independence.

A potential limitation of the current analysis is the small number of subjects being compared in groups 1 (insulin-independent for > 1 year after a single transplant) and group 2 (recipients who did not become insulin-dependent until after a second transplant 3–6 months after the first infusion which was maintained at 12 months). This was necessary to be sure that the effect of each transplant could be assessed independently by selecting groups of recipients with distinct transplant outcomes, i.e., those with optimal vs. sub-optimal graft function. Thus, patients receiving a second transplant before 3 months were not included in case they might have been able to achieve insulin independence with the first transplant. Neither were recipients of second transplants who did not remain insulin independent at 12 months since the decline in graft function might be due to other factors such as rejection, rather than engraftment estimated by BETA-2. Most recipients at our center are re-listed for a second transplant at 4 weeks and priority is given to second infusions while recipients are still lymphodepleted. Furthermore, we confirmed that early graft function (1-week post-transplant) is associated with long-term transplant outcomes in an unselected cohort of transplant recipients with BETA-2 scores consistent with previous studies showing an association between BETA-2 scores and transplant outcomes (8, 9, 15).

A limitation of using the BETA-2 score soon after islet transplant is the inclusion of 1) HbA1c which is not expected to change in the short term and 2) insulin dose which may vary depending on several factors including diet, activity, and care provider discretion. However, in our study the BETA-2 score at 1-week post-transplant had better discrimination for insulin independence compared to other simple indices of islet function (SUITO index, HOMA2-B%, TEF and CP/G) suggesting that there is merit in including these additional variables even in short term assessment of graft function. Practical considerations for calculating the BETA-2 score peri-transplant may be to measure HbA1c less frequently (i.e., bi-weekly to monthly) than fasting C-peptide and glucose and to use standardized protocols regarding insulin dose adjustments.

Our study was not designed to explore how recipient and/or donor factors relate to graft function. However, we found that higher islet equivalents were associated with insulin independence and higher 1-week BETA-2 score in keeping with previous studies demonstrating single islet transplant success in recipients who had received higher transplanted islet mass(16, 17). Lower pre-transplant BMI and insulin requirements were also associated with higher BETA-2 scores at 1-week post-transplant suggesting that transplant success appears to depend not only on the number and function of transplanted islets but also on the metabolic demand placed on them. Importantly, however, we found that the association between insulin independence and BETA-2 score at 1-week post-transplant remained relatively unchanged when adjusted for pre-transplant BMI, insulin dose, and HbA1c, as well as transplanted IE/kg.

We characterized islet function in the early period post-transplant and show that primary graft function is established over the first 4–6 weeks post-transplant and that graft function as early as 1-week post-transplant is associated with long-term graft survival. Importantly, we demonstrated that frequent and close monitoring of islet graft function soon after transplantation is possible in the clinical setting and that this may be useful in routine clinical care as well as in the development and evaluation of interventions targeted at improving islet transplant outcomes.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: This study was a retrospectives analysis of single center data of individuals newly transplanted with allogenic islets between 2009–2014. The data is not publicly available. Requests to access these datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Alberta Health Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AL and PS drafted the manuscript and analyzed and interpreted the data. AL and SI researched the data. All authors contributed to revision of the article and approved the final version of the article.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10335/full#supplementary-material

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ORIGINAL RESEARCH
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COVID-19 in Kidney Transplant Recipients With Diabetes Mellitus: A Propensity Score Matching Analysis

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Kidney transplant recipients present higher rates of pre-existing comorbidities, in particular diabetes mellitus (DM), hypertension, and cardiac disease. We aimed to verify the main risk factors related to DM that contribute to COVID-19 progression and mortality in a kidney transplant setting. From March to August 2020, we evaluated 300 kidney transplant recipients affected by COVID-19. We used propensity score matching (PSM) to estimate the impact of DM on COVID-19. After matching, all baseline characteristics were well balanced between those with and without DM (n = 100 in each group). Case fatality rate, the requirement of invasive mechanical ventilation (IMV), and acute kidney injury (AKI) were associated with previous fasting blood glucose, and C-reactive protein (CRP), and lactate dehydrogenase (LDH) levels on admission. These findings were similar in kidney transplant patients with and without DM. Glycemia on admission and estimated glomerular filtration rate (eGFR) either on admission or basal correlated to the need of IMV and development of AKI, respectively. Poor glycaemic control, eGFR, markers of inflammation (CRP) and tissue damage (LDH) were indicative of COVID-19 burden in kidney transplant recipients and may be useful tools for risk-stratifying this population, independently of the DM status, during the pandemic.

Keywords: COVID-19, diabetes mellitus, outcomes, kidney transplant, propensity score

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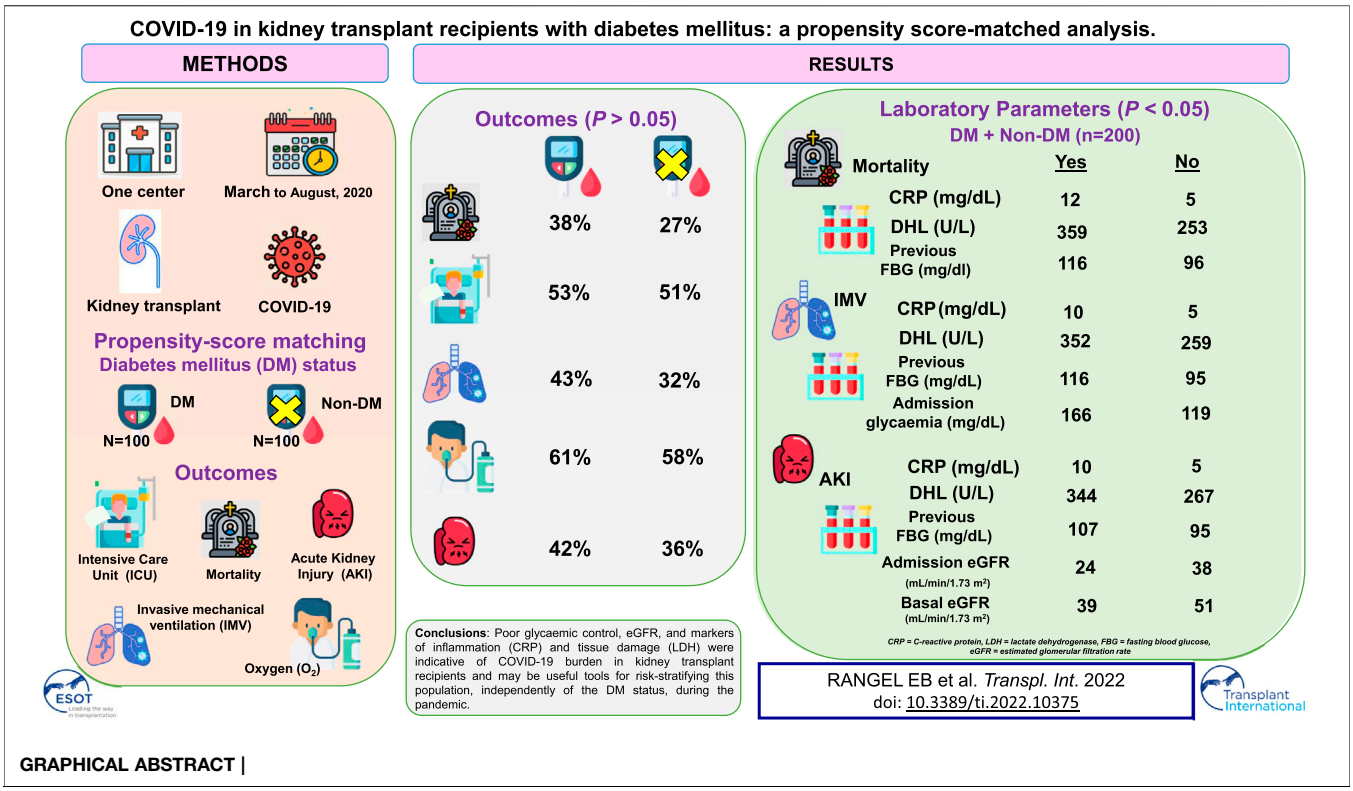
INTRODUCTION

The cardio-metabolic disease is associated with increased mortality and severity of coronavirus disease 2019 (COVID-19) pneumonia, including the transfer to intensive care unit (ICU), invasive mechanical ventilation (IMV), acute kidney injury (AKI), and death [1–4]. Cardio-metabolic disease encompasses broad pathological changes, such as insulin resistance, diabetes mellitus (DM), dyslipidemia, abdominal obesity, and hypertension, and environmental risk factors such as

Abbreviations: AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CFR, case fatality rate; CKD-EPI, chronic kidney disease epidemiology collaboration; COPD, chronic obstructive pulmonary disease; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HD, haemodialysis; ICU, intensive care unit; IMV, invasive mechanical ventilation; LDH, lactate dehydrogenase; O_2, supplemental oxygen; OR, odds ratio; PSM, propensity score matching; ROC, receiver operating characteristic; SOT, solid-organ transplantation.



GRAPHICAL ABSTRACT |

smoking, sedentary lifestyle, poor diet, and poverty. The ultimate consequences of that combination are higher rates of viral entrance, direct viral toxicity, endothelial dysfunction, thrombi-inflammation, dysregulation of the immune response, and derangement of the renin-angiotensin-aldosterone system [5].

The data describing the outcomes of solid-organ transplantation (SOT) recipients with COVID-19 has raised a debate in the literature on whether transplantation *per se* was a major risk for COVID-19 progression and mortality, or whether the presence of cardiometabolic comorbidities was the main factor responsible for the adverse outcomes [6]. Therefore, the initial reports highlighted high rates of AKI (37.8%–52.1%), transfer to ICU (33.8%–36%), respiratory failure requiring intubation (27%–29.6%), and case fatality rate (CFR; 18.7%–32%) in these population [7–9]. Importantly, a high prevalence of pre-existent comorbidities was equally documented, such as hypertension (77.4%–95.1%), DM (41.3%–52.1%), obesity/overweight (35.1%–63.8%), heart disease (21.8%–36.2%) and lung disease (10.4%–18.8%), as well as age >60–65 years-old (29.3–56.2%) and male gender (61.2–66%) in SOT setting [7–9]. When compared to non-SOT individuals, SOT individuals had increased odds of receiving IMV (2.34), developing AKI (2.41), being transferred to ICU (1.46), and mortality (1.94) [10].

Despite the growing literature focusing on the prognosis of COVID-19 in transplant recipients, data on selected high-risk clinical populations that merit special consideration, such as immunocompromised individuals with a history of DM, remain undetermined. Diabetic individuals are susceptible to a substantial burden of micro and macrovascular complications [11] and dysregulation of the immune system [12], which could predispose them to an increase in COVID-19 severity and mortality. Here, we set out to verify the clinical manifestations, outcomes, and CFR in a population of kidney transplant recipients with DM and the diagnosis of COVID-19 using the propensity-score matched analyses in a single center.

PATIENTS AND METHODS

Study Design and Setting

A cohort, cross-sectional, observational, and descriptive study was conducted at Hospital do Rim, São Paulo, SP, Brazil. The medical records of patients who were either hospitalized or non-hospitalized with the diagnosis of COVID-19 during the study period of March to August 2020 were assessed, corresponding to the first wave of COVID-19 in Brazil. We included only patients in whom SARS-CoV-2 was detected by nasopharyngeal swab RT-PCR (reverse transcriptase-polymerase chain reaction). The population at risk included 11,875 kidney transplant patients undergoing outpatient follow-up [13]. Of 590 kidney transplant recipients who became ill, 300 were included in the study. Six were excluded for being a double transplant, 4 for having lost the graft in the period before COVID-19, 4 for being a recent transplant and being in delayed graft function at the time of diagnosis of COVID-19, 1 for not using immunosuppressive drugs due to cancer treatment, 1 for being underage and 274 were excluded for missing data due to admission to other services (**Supplementary Figure S1**).

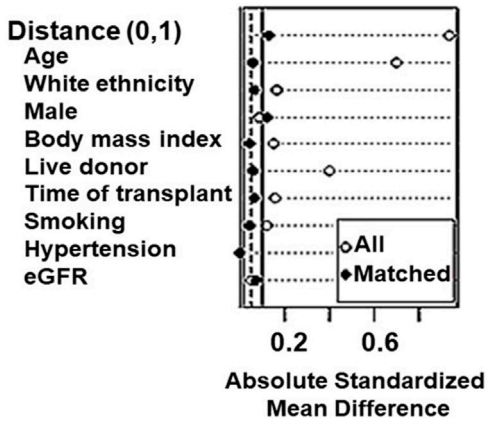


FIGURE 1 | Assessment of baseline characteristics for propensity-score matching (PSM) analyses in kidney transplant recipients.

A standardized data collection form was developed to retrospectively retrieve relevant information from medical records. Data were collected regarding patient demographics and laboratory parameters on admission with COVID-19 symptoms. The last patient was included in the study on 30th August 2020. The Ethics and Research Committee of the Federal University of São Paulo (CAEE 35311020.9.0000.8098) approved the study. Informed consent was obtained from all patients, whereas a waiver was granted for patients who died in other hospitals.

Patient demographics include age, sex, race, body mass index (BMI), type of donor, time of transplant, as well as the presence of comorbidities (smoking, hypertension, DM, chronic obstructive pulmonary disease [COPD], heart disease, liver disease, and autoimmune disease) were collected. We also evaluated the symptoms on admission.

Diabetes was defined according to the use of insulin and/or oral antidiabetics, hypertension and whether individuals were on anti-hypertensive drugs, liver disease and whether hepatitis B or C were diagnosed, and heart disease and whether heart failure and/or coronary artery disease were present.

Laboratory Testing

On admission, we evaluated in-hospital laboratory data: lymphocytes, serum creatinine, glycemia, aspartate aminotransferase (AST), alanine aminotransferase (ALT), D-dimer, lactate dehydrogenase (LDH), and C-reactive protein (CRP). As for laboratory data before admission, we collected baseline creatinine (mean the last three measurements), fasting blood glucose (FBG; last measurement within 6 months), and glycated hemoglobin (HbA_{1c}; last measurement within the 1 year).

The estimated glomerular filtration rate (eGFR) was calculated using the formula defined in the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) study: 175 × serum creatinine$^{-1.154}$ × age$^{-0.203}$ × 1.212 [if black] × 0.742 [if woman], where the glomerular filtration rate or GFR is expressed in ml/min/1.73 m^2 of the body surface [14].

Statistical Analysis

Two groups of renal receptors affected by COVID-19, e.g., diabetic or DM (+) and non-diabetic or DM (−), were separated and the outcomes were then evaluated, based on death, transfer to ICU, AKI classified in accordance do KDIGO guidelines [15], need for hemodialysis (HD) and supplemental oxygen (O_2), and IMV.

Independent samples *t*-test and Chi-square test were used to identify the association between DM and demographic and laboratory parameters, and the outcomes previously mentioned. Data were described as mean ± standard deviation or median and interquartile range, as indicated. Frequencies and percentages were reported for qualitative data.

Next, we used propensity score matching (PSM) to estimate the effect of the group accounting for confounding by the included covariates. We included in match the variables associated with COVID-19 prognosis by previous reports: age, sex, race, BMI, hypertension, time after transplantation, smoking, and eGFR. We used 1:1 nearest neighbor PSM without replacement with a caliper of 0.2, which yielded adequate balance (**Figure 1**). The propensity score was estimated using a logistic regression of the treatment (non-diabetes/diabetes) on the covariates. After matching, all standardized mean differences for the covariates were below 0.1 indicating adequate balance.

We performed a Cox regression before matching to evaluate the association between DM and covariates with 60-days death. Importantly, we included the same covariates used for PSM analysis. We did not perform Cox regression with the outcomes of ICU admission and hospitalization because these could introduce an immortal time bias. For matched cohort, we performed a Kaplan-Meier analysis of 60-days death.

Data were analyzed using IBM® SPSS (Statistical Product and Services Solutions, version 18.0, SPSS Inc., Chicago, IL, United States). A *P*-value of <0.05 was considered significant for all data analyses.

RESULTS

In our kidney transplant population of 300 patients, 57.3% were men (*n* = 172), the mean age was 52.5 ± 12.2 years, 71.6% (*n* = 215) were deceased-donor kidney transplant recipients, mean time of transplant was 94.1 ± 71.6 months (**Supplementary Table S1**). A total of 228 (76%) patients required hospitalization and the average length of stay was 23 ± 22 (median 15 days) and 89 (29.6%) deaths were registered. Immunosuppressive regimen was mainly based on Tacrolimus (TAC) and Mycophenolate (MPA) (*n* = 152; 50.6%), TAC and Azathioprine (AZA) (*n* = 49; 16.3%), TAC and mTOR inhibitor (mTORi) (*n* = 24; 8%), AZA and Cyclosporine A (CSA) (*n* = 22; 7.3%). All patients were using steroids as part of their immunosuppressive regimen.

Among the individuals included in the study, 117 (39%) were diabetic, and these individuals were older (56.9 ± 10.3 *versus* 49.6 ± 12.5 years old), had more hypertension (85.5% *versus* 67.8%), and heart disease (17.9% *versus* 6%),

TABLE 1 | Demographic variables and outcomes after applying the propensity-score matching (PSM) for kidney transplant recipients with diabetes mellitus (DM) and without DM.

Variables and outcomes	DM (–) (N = 100)	DM (+) (N = 100)	*P*
Age (median, IQR)	54 (47, 63)	56 (50, 62)	0.5
White ethnicity (n, %)	60 (60)	57 (57)	0.7
Male (n, %)	59 (59)	53 (53)	0.4
BMI (median, IQR)	27.3 (23.8, 29.7)	28.0 (24.3, 30.7)	0.6
Living donor (n,%)	23 (23%)	21 (21%)	0.7
Transplant time (months) (median, IQR)	64 (30, 143)	70 (36, 122)	>0.9
Smoking (n, %)	22 (27%)	24 (29%)	0.8
Hypertension (n, %)	83 (83%)	83 (83%)	>0.9
Basal eGFR (median, IQR)	47 (30, 60)	48 (31, 65)	0.8
Death (n, %)	27 (27%)	38 (38%)	0.10
IMV (n, %)	32 (32%)	43 (43%)	0.11
HD (n, %)	36 (36%)	42 (42%)	0.4
ICU (n, %)	51 (51%)	53 (53%)	0.8
O_2 (n, %)	58 (58%)	61 (61%)	0.7

IQR, interquartile range; BMI, body mass index; eGFR, estimated filtration glomerular rate in mL/min/1.73 m^2; IMV, invasive mechanical ventilation; HD, hemodialysis; ICU, intensive care unit; O_2, oxygen. After applying the PSM, 83 non-diabetic patients and 17 diabetic patients were excluded.

TABLE 2 | Outcomes in kidney transplant recipients with diabetes mellitus (DM) and without DM after applying the propensity-score matching (PSM).

Laboratory data	Alive (N = 135)	Not Alive (N = 65)	*P*
Previous FBG (mg/dl)	96 (86, 121)	116 (93, 194)	***<0.001***
Glycemia on admission (mg/dl)	124 (95, 217)	156 (112, 252)	0.086
Previous Hb1Ac (%)	6.20 (5.50, 7.80)	6.80 (5.60, 8.60)	0.2
CRP (mg/dl)	5 (2, 11)	12 (5, 18)	***<0.001***
LDH (U/L)	253 (217, 344)	359 (288, 483)	***<0.001***
eGFR on admission	34 (22, 50)	31 (17, 46)	0.3
Basal eGFR	47 (32, 63)	49 (27, 59)	0.5
	IMV (–) (N = 125)	**IMV (+) (N = 75)**	
Previous FBG (mg/dl)	95 (84, 114)	116 (93, 190)	***<0.001***
Glycemia on admission (mg/dl)	119 (95, 181)	166 (115, 272)	***0.009***
Previous Hb1Ac (%)	6.20 (5.50, 7.55)	6.80 (5.60, 8.70)	0.10
CRP (mg/dl)	5 (2, 11)	10 (4, 16)	***0.003***
LDH (U/L)	259 (220, 337)	352 (257, 485)	***<0.001***
eGFR on admission	34 (21, 50)	31 (18, 46)	0.2
Basal eGFR	47 (31, 64)	47 (28, 59)	0.5
	AKI (–) (N = 122)	**AKI (+) (N = 78)**	
Previous FBG (mg/dl)	95 (85, 135)	107 (92, 166)	***0.004***
Glycemia on admission (mg/dl)	137 (95, 215)	150 (108, 256)	0.2
Previous Hb1Ac (%)	6.20 (5.55, 7.85)	6.60 (5.50, 8.60)	0.5
CRP (mg/dl)	5 (2, 11)	10 (3, 15)	***0.023***
LDH (U/L)	267 (223, 342)	344 (236, 438)	***0.005***
eGFR on admission	38 (26, 52)	24 (13, 43)	***<0.001***
Basal eGFR	51 (35, 67)	39 (22, 56)	***<0.001***

All values are median and interquartile range. FBG, fasting blood glucose; Hb1Ac, glycated hemoglobin; CRP, C-reactive protein; LDH, lactate dehydrogenase; eGFR (in mL/min/1.73 m^2), estimated glomerular filtration rate; IMV, invasive mechanical ventilation; AKI, acute kidney injury. The bold-italic values mean that they are statiscally significant ($p < 0.05$).

and had more often received a kidney from deceased donors (81.2% *versus* 65.6%) (all $p < 0.05$; **Supplementary Table S1**). From a clinical perspective, we observed that anosmia was found more frequently in non-diabetics on admission (34.4% *versus* 22.2%, $p = 0.025$) (**Supplementary Table S1**).

Analyses of laboratory data disclosed poor glycaemic control and higher levels of CRP in diabetic individuals. Conversely, no differences in eGFR, LDH, lymphocytes, D-dimer, or liver tests were found between diabetic and non-diabetic kidney transplant recipients (**Table 2S**). Among the 300 kidney transplant recipients, 46.7% required ICU admission, 54.3% used supplemental O_2, 34% needed IMV, 58% developed AKI, 36.3% underwent HD, and 29.7% died (**Supplementary Table S2**). When analyzing the subgroup of diabetic kidney transplant recipients ($n = 117$), we found that the CFR was 39.3% and higher

TABLE 3 | Outcomes in kidney transplant recipients with diabetes mellitus (DM) after applying the propensity-score matching (PSM).

Laboratory data	ALIVE (N = 62)	Not ALIVE (N = 38)	P
Previous FBG (mg/dl)	114 (90, 167)	169 (119, 249)	***<0.001***
Glycemia on admission (mg/dl)	186 (109, 248)	224 (186, 327)	0.14
Previous Hb1Ac (%)	7.45 (6.20, 9.40)	8.20 (6.80, 9.40)	0.2
CRP (mg/dl)	7 (2, 13)	11 (5, 20)	0.062
LDH (U/L)	250 (214, 352)	352 (292, 492)	***0.001***
eGFR on admission	34 (21, 48)	34 (19, 46)	0.7
Basal eGFR	46 (32, 62)	51 (25, 69)	0.8
	IMV (–) (N = 57)	**IMV (+) (N = 43)**	
Previous FBG (mg/dl)	113 (90, 166)	168 (119, 247)	***<0.001***
Glycemia on admission (mg/dl)	164 (100, 238)	236 (190, 333)	***0.017***
Previous Hb1Ac (%)	7.40 (6.20, 9.30)	8.35 (6.80, 9.78)	0.10
CRP (mg/dl)	7 (2, 13)	10 (3, 18)	0.15
LDH (U/L)	265 (211, 350)	344 (256, 490)	***0.005***
eGFR on admission	34 (20, 49)	34 (20, 46)	0.6
Basal eGFR	47 (32, 64)	51 (27, 64)	>0.9
	AKI (–) (N = 58)	**AKI (+) (N = 42)**	
Previous FBG (mg/dl)	120 (91, 169)	160 (114, 249)	***0.008***
Glycemia on admission (mg/dl)	204 (148, 244)	224 (140, 333)	0.3
Previous Hb1Ac (%)	7.50 (6.30, 9.20)	8.05 (6.65, 9.85)	0.4
CRP (mg/dl)	8 (2, 13)	9 (3, 18)	0.4
LDH (U/L)	279 (222, 354)	340 (232, 427)	0.093
eGFR on admission	36 (27, 50)	28 (12, 46)	***0.017***
Basal eGFR	50 (34, 68)	47 (22, 56)	0.092

All values are median and interquartile range. Hb1Ac, glycated hemoglobin; CRP, C-reactive protein; LDH, lactate dehydrogenase; eGFR (in mL/min/1.73 m^2), estimated glomerular filtration rate; IMV, invasive mechanical ventilation; AKI, acute kidney injury. The bold-italic values mean that they are statiscally significant (p < 0.05).

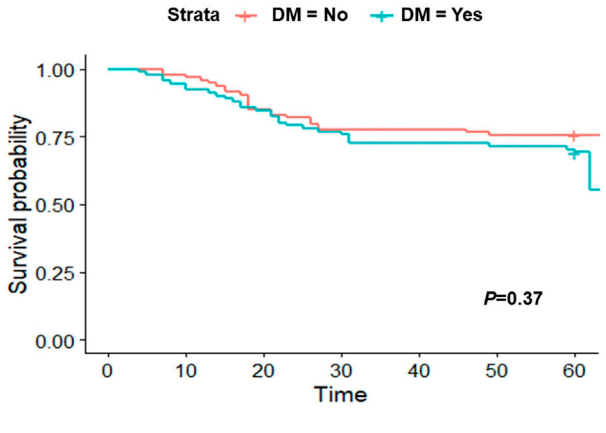


FIGURE 2 | Kaplan-Meier curve after applying the propensity-score matching (PSM) at 60 days.

rates of COVID-19 progression were noticed, including ICU admission (54.7%), the requirement of supplemental O_2 (61.5%), and IMV (44.4%), development of AKI stage 3 (47%) and the need for HD (43.6%) (all $p < 0.05$; **Supplementary Table S2**).

Next, we applied the PSM and paired 1:1 (diabetic and non-diabetic) and balanced all baseline characteristics (**Table 1**). After matching, we obtained a total of 200 patients (n = 100 diabetics and n = 100 non-diabetics). In this matched population, CFR, the requirement for IMV or O_2, development of AKI, and the need for HD were similar between diabetic and non-diabetic kidney transplant recipients (**Table 1**). Overall, CFR was 32.5%.

Evaluation of the laboratory data indicated that FBG previous to admission, CRP, and LDH levels on admission were related to an increased risk of death, the requirement of IMV, and the development of AKI in the kidney transplanted population (**Table 2**).

In addition to the variables aforementioned, glycemia on admission was associated with the requirement of IMV, which was observed in 37.5% of the kidney transplanted patients (**Table 2**). Likewise, basal and admission eGFR was associated with AKI development in 39% of both diabetic and non-diabetic patients (**Table 2**).

In transplanted patients with DM (N = 100), 38% died. Previous FBG to admission and LDH on admission were associated with CFR (**Table 3**). In addition, 43% of diabetic patients required IMV. Not only previous FBG to admission but also higher levels of glycemia and LDH levels on admission were associated with the need for IMV (**Table 3**).

When evaluating AKI outcomes in kidney transplant recipients with DM, we found that 42% of these individuals developed any stage of kidney dysfunction. Previous FBG to admission and eGFR on admission were related to AKI occurrence (**Table 3**).

To note, Cox regression analysis performed pre-PSM showed no association between DM and 60-days death (**Supplementary Table S3**), indicating similar results to those observed post-PSM. The analysis was performed as recommended after matching using weights.

For matched cohort, a Kaplan-Meier analysis showed no association between DM and 60-days death ($p = 0.37$; **Figure 2**).

Importantly, the burden of immunosuppression was not different between DM and non-DM patients after PSM, including trough levels and doses (**Supplementary Table S4**). In both groups, no patients used thymoglobulin or steroid pulse in the past 3 months. In fact, the majority of the patients were in a stable maintenance phase. Likewise, modification of the immunosuppressive regimen was not different between groups and was performed in almost two-thirds of the patients (**Supplementary Table S4**).

DISCUSSION

Here, to gain insight into the impact on outcomes when potentially severe conditions are combined, we have outlined the analysis of the subgroup of kidney transplant recipients with cardio-metabolic disease, in particular DM, and the potential of this combination to worsen COVID-19 progression and increase CFR. We found an overall CFR of 32.5%, which is in accordance with previous studies with immunocompromised individuals in transplant settings (27%–32%) [7,8,16]. The diabetic population, in particular, presented an increased CFR (38%), although not significantly different from kidney transplant recipients without DM after applying the PSM.

In our study, kidney transplant recipients with DM were older, and exhibited higher rates of hypertension and heart disease, which put them at higher risk of COVID-19 progression and mortality, as described in non-transplanted individuals with long-term DM and newly diagnosed DM [17–19].

After matching, we observed that for both groups of kidney transplant recipients, diabetic and non-diabetic, previous glycemic control and glycemia on admission, the inflammatory marker CRP and tissue damage marker LDH, as well as function were indicative of severity. Therefore, markers of coagulation and liver tests were not useful tools to stratify the risk of kidney transplant recipients diagnosed with COVID-19, in contrast to non-transplanted individuals [20].

Age, a non-modifiable variable, is associated with increased mortality from COVID-19 in the general population [21] and transplanted populations [6–8,16], as we also observed in our study. Likewise, age is related to COVID-19 progression, in particular AKI development [6,7,16]. In the general population, AKI developed in one-third of hospitalized patients with COVID-19 and the independent risk factors for its development included advanced age, black race, hypertension, DM, cardiovascular disease, use of vasopressor, and need for ventilation [22]. Furthermore, elevated values of creatinine and blood urea nitrogen, any stage of AKI-KDIGO, proteinuria, and haematuria were independent risk factors for in-hospital mortality, even after adjusting for demographic and laboratory variables [23,24].

In our population, AKI occurred in 39% of the kidney transplant recipients after applying the PSM. AKI was reported in 52% of kidney transplant recipients in TANGO International Transplant Consortium, whereas mechanical ventilation was required in 29% [7]. In this study, a high incidence of comorbidities was also present, including hypertension (95%), DM (52%), obesity (49%), and cardiac disease (28%). To note, age was greater than observed in our study. Furthermore, higher rates of mortality were associated not only with age but also with lymphocyte count, GFR, LDH, procalcitonin, and IL-6 levels [7].

In elderly individuals, AKI, IL-6 levels, and myocardial injury were equally associated with mortality, indicating the burden of COVID-19 with aging [25]. AKI occurs not only through direct damage to podocytes and tubular epithelial cells by SARS-CoV-2, but also through the inflammatory milieu, in particular the cytokine storm, and other causes, including rhabdomyolysis, cardio-renal syndrome, and secondary infections [26]. Post-mortem kidney analyses disclosed acute tubular injury in almost all cases and less frequently thrombi and collapsing segmental and focal glomerulosclerosis associated with the APOL1 variant [27]. These findings were in agreement with the histological features of kidney biopsies performed in COVID-19 patients with AKI or proteinuria and obtained from transplanted and non-transplanted individuals [28]. Importantly, recovery of kidney allograft function due to COVID-19 occurs in only 40% of the kidney transplant recipients and is associated with GFR and proteinuria on admission, previous rejection, higher SOFA score, hypotension, and KDIGO stage 3 [29].

To note, AKI is primarily seen in COVID-19 patients with respiratory failure, with almost 90% of patients on IMV developing AKI of any stage of KDIGO compared to less than 25% of non-ventilated patients, indicating a temporal relation between AKI and respiratory failure [22], as we also observed in our population. Thus, the clinical-laboratory score for risk stratification of patients showed that DM, PaO_2/FiO_2 ratio, and the inflammatory and endothelial dysfunction markers CRP and LDH are predictive for IMV requirement [30].

In our study, diabetic individuals were older and had greater cardio-metabolic comorbidity burden, in particular hypertension and cardiac disease, as reported elsewhere [19]. Not only long-term DM and newly diagnosed DM [19] but also hyperglycemia are associated with ICU admission, the need for IMV, and death [31-33]. In diabetic individuals, including newly-diagnosed DM, admittance glucose levels correlated to clinical markers, including respiratory (higher respiratory rate and lower $SatO_2$ and PaO_2/FIO_2 ratio) and hemodynamic (higher levels of systolic blood pressure) parameters [19,33] and inflammatory (CRP, IL-6, and procalcitonin), hematologic (leucocytosis, lymphopenia, anemia, and thrombocytopenia), and tissue damage

(D-dimer, ALT, troponin, and lactate) markers[19]. Admission hyperglycemia may result from an enhanced response of counter-regulatory hormones and cytokine storm exacerbating insulin resistance [34], which adversely impact the immune response. Thus, diabetic individuals present more frequently lymphopenia and higher levels of cytokines IL-2R, IL-6, IL-8, IL-10, CRP, procalcitonin, and TNF-α, as well as the distinctly reduced Th1/Th2 cytokines ratios and reduced peripheral numbers of $CD8^+$ T lymphocytes and NK cells when compared to non-diabetic individuals [35,36], which may lead to longer hospitalization time and SARS-CoV-2 shedding [37]. Therefore, exacerbated inflammatory responses within 24 h of admission correlate with COVID-19 severity in diabetic individuals, in particular IL-6 and LDH, whose longitudinal analyses hold an association with worse outcomes [38]. Additionally, FBG ≥126 mg/dl on admission in patients with COVID-19 without a previous diagnosis of DM is associated with an elevated risk of ICU admission, IMV, and death [19,32,39].

In our study, the median values of glycemia on admission in renal transplant recipients, independently of the DM status, were associated with IMV requirement (166 mg/dl *versus* 119 mg/dl). In diabetic patients, higher values of glycemia on admission were equally associated with a worse respiratory outcome (236 mg/dl *versus* 164 mg/dl). Furthermore, higher levels of the previous FBG were associated with COVID-19 severity, including, the development of AKI, the need for IMV, and CFR in both diabetic and non-diabetic kidney transplant recipients. Elevated glucose levels may regulate SARS-COV-2 replication and cytokine production, trigger mitochondrial reactive oxygen species production, and promote glycolysis in monocytes [40]. These cells are the most enriched immune cell types in the lungs of COVID-19 patients and play an important role in the pathogenicity of the disease. Monocyte-derived cytokines drive T lymphocyte dysfunction and, ultimately, may lead to cell death from diverse organs. Importantly, even after glucose control in diabetics, the macrophage is dysfunctional in these patients, exhibiting M1 pro-inflammatory phenotype and elevated levels of inflammatory chemokines CXCL1, CXCL5, and RANTES[12]. Therefore, adequate long-term glycaemic control and early identification of post-transplant DM is of paramount importance to decrease the inflammatory milieu and, ultimately, the severity of COVID-19.

In addition, pre-existing cardio-metabolic comorbidities found in kidney transplant recipients, such as DM, hypertension, and cardiac disease, are associated with chronic endothelial dysfunction. SARS-CoV-2 can directly infect endothelial cells via the angiotensin-converting enzyme 2 (ACE2) pathway and aggravate endothelial dysfunction due to endothelitis, apoptosis, and lymphocytic and mononuclear infiltrating cells [41]. Endothelial cell injury and/or activation may lead to an imbalance of the coagulation system and thromboembolic complications associated with ischemic organ damage and consequently to high morbidity and mortality [42]. Increased ACE2 expression in bronchial epithelium and alveolar cells from diabetic patients increases SARS-CoV-2 infection [43]. ACE2 and transmembrane protease serine 2 (TMPRSS2) expression in islet cells may also promote SARS-CoV-2-mediated metabolic dysregulation due to cell death by necroptosis and immune cell infiltration [44] and reduced number of insulin-secretory granules in ß-cells and impaired glucose-stimulated insulin secretion [45], yet others did not find ACE2 expression in endocrine cells within the pancreas [46]. However, ACE2 expression in other tissues may contribute to insulin resistance, such as adipose tissue, where there is a positive correlation of ACE2 expression in subcutaneous and visceral fat and body mass index and, therefore, obesity [47], and in skeletal muscle cells, where ACE2 expression is associated with direct and indirect effects of SARS-CoV-2 [48].

Unexpectedly, diabetic individuals presented a lower frequency of anosmia (**Supplementary Table S1**). Surveillance analyses documented anosmia as a symptom not associated with the risk of hospitalization, indicating a lower severity of COVID-19 [49]. Although SARS-CoV-2 enters olfactory neuroepithelium via ACE2 receptor and TMPRSS2 [50] and causes anosmia, we can speculate that chronic hyperglycemia might have caused damage to nerve fibers and olfactory network and contributed ultimately to reducing the occurrence of this symptom. However, further studies are warranted to address anosmia frequency and evolution in diabetic individuals.

Our study has some limitations, including the number of patients, retrospective analyses, and the lack of other laboratory parameters that are correlated to COVID-19 outcomes, either at admission or longitudinally. In addition, our cohort has not received COVID-19 vaccination and the potential limitations (or not) of generalizability of the study findings to a vaccinated population warrant further investigation.

CONCLUSION

Collectively, our data highlight the importance of early evaluation and identification of risk factors of COVID-19 progression and CFR for appropriately risk-stratifying kidney transplant recipients with DM, which may be extended to non-diabetics, during the pandemic. Encouraging healthy practices and strict glucose control in diabetic kidney transplant recipients and early identification of individuals at potential risk for COVID-19 progression and mortality are of paramount importance to mitigate adverse outcomes in this population during the pandemic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics and Research Committee of Federal

University of São Paulo (CAEE 35311020.9.0000.8098). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ÉR and DdL participated in the research design; DdL, IA-B, AV-A, and MC performed the research; DdL, IA-B, LdA, and ÉR participated in data analysis; ÉR and DdL participated in writing the paper; HT-S, JM-P, and ÉR gave the final approval.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10375/full#supplementary-material

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ORIGINAL RESEARCH
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COVID-19 Vaccination and Remdesivir are Associated With Protection From New or Increased Levels of Donor-Specific Antibodies Among Kidney Transplant Recipients Hospitalized With COVID-19

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Alloimmune responses in kidney transplant (KT) patients previously hospitalized with COVID-19 are understudied. We analyzed a cohort of 112 kidney transplant recipients who were hospitalized following a positive SARS-CoV-2 test result during the first 20 months of the COVID-19 pandemic. We found a cumulative incidence of 17% for the development of new donor-specific antibodies (DSA) or increased levels of pre-existing DSA in hospitalized SARS-CoV-2-infected KT patients. This risk extended 8 months post-infection. These changes in DSA status were associated with late allograft dysfunction. Risk factors for new or increased DSA responses in this KT patient cohort included the presence of circulating DSA pre-COVID-19 diagnosis and time post-transplantation. COVID-19 vaccination prior to infection and remdesivir administration during infection were each associated with decreased likelihood of developing a new or increased DSA response. These data show that new or enhanced DSA responses frequently occur among KT patients requiring admission with COVID-19 and suggest that surveillance, vaccination, and antiviral therapies may be important tools to prevent alloimmunity in these individuals.

Keywords: COVID-19, kidney transplantation, alloimmunity, donor-specific antibodies, vaccination

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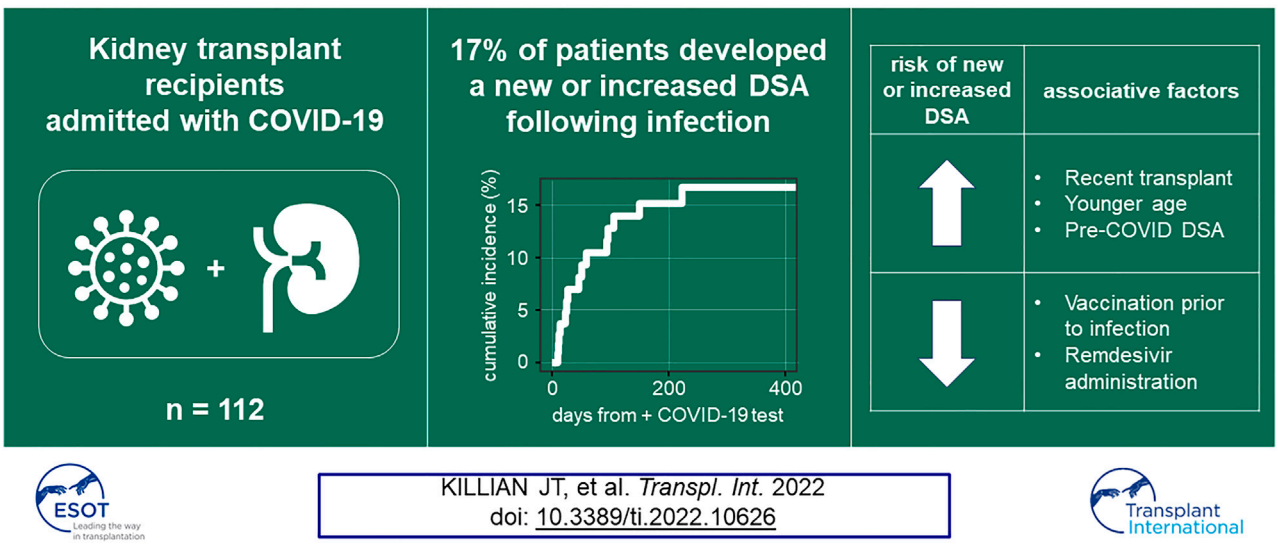
Abbreviations: KT, kidney transplant; KTR, kidney transplant recipient; nonSOT, non-solid organ transplant; DSA, donor-specific antibody; UAB, University of Alabama at Birmingham; HLA-Ab, anti-HLA antibody; MFI, mean fluorescence intensity; %SA, percentage single antigen beads; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; AKI, Acute kidney injury; KDIGO, Kidney Disease Improving Global Outcomes; sCr, serum creatinine; WHO, World Health Organization; IQR, interquartile range; KM, Kaplan-Meier; AMR, antibody-mediated rejection; EUA, emergency use authorization.



GRAPHICAL ABSTRACT |

INTRODUCTION

SARS-CoV-2 infection, which elicits acute COVID-19 clinical disease, has been devastating for kidney transplant (KT) recipients (1, 2). Compared to non-solid organ transplant (nonSOT) patients, KT recipients (KTRs) are more likely to be hospitalized and experience greater COVID-19-related morbidity and mortality (3). Given the estimated 17,000 KTRs that have been admitted with COVID-19 in the United States (4), it is critically important to characterize acute and chronic effects of COVID-19.

A recent report showed that 8% of KTRs hospitalized with COVID-19 developed a new donor-specific antibody (DSA) response or exhibited increased levels of a known pre-existing DSA within a median follow-up of 45 days post-infection (5). Given inpatient reductions in immunosuppressive therapy (5), the protracted immune response to COVID-19 (6), the impaired ability to achieve SARS-CoV-2 viral clearance among immunosuppressed individuals (7), and evidence of late allograft dysfunction following COVID-19 (8, 9), we hypothesized that KTRs may suffer alloimmune consequences from COVID-19 that extend well beyond the acute phase of infection.

To test this hypothesis, we evaluated alloimmune responses in KTRs hospitalized following SARS-CoV-2 infection. In addition, we identified risk factors associated with alloimmunity and allograft dysfunction. We found that the risk of alloimmunity in KTRs extended at least 8 months past admission for COVID-19 and observed that new or increased DSA responses were associated with decreased late allograft function. Vaccination and antiviral therapies were each associated with a reduced risk of a new or increased DSA response, suggesting that alloimmune responses in KTRs may be regulated directly or indirectly by SARS-CoV-2 infection.

MATERIALS AND METHODS

Cohort and Design

We performed a single-center prospective observational cohort study of KTRs hospitalized with COVID-19. Patients admitted to the University of Alabama at Birmingham (UAB) Hospital between 1 March 2020 and 1 November 2021 with a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction or antigen test that occurred within 14 days prior to or 7 days after the day of admission were approached for enrollment. For patients with multiple COVID-19-associated admissions, only the first hospitalization was considered. Participants or, in cases of incapacitation, legally authorized representatives provided consent. The UAB Institutional Review Board approved the study protocol (IRB-300005127).

Data Collection

We obtained data on demographics, comorbidities, medications, transplant surgical history, inpatient treatment, and outcomes. Data were extracted from the electronic medical record and transformed. We obtained all anti-HLA antibody (HLA-Ab) and renal function studies extending from 14 months prior to

COVID-19 diagnosis through 1 February 2022. HLA-Ab testing was supplemented with available research biospecimens as described below.

Outcome Assessments and Definitions

The primary outcome analyzed was the development of new DSA or increased levels of pre-existing DSA at least 10 days after COVID-19 diagnosis. DSA not previously present that crossed the 1500 mean fluorescence intensity (MFI) threshold was classified as new DSA. Although these new DSAs were also by definition *de novo* (10), we use the term *new* to indicate the appearance of the anti-HLA specificity post-COVID-19, as opposed to other patients who had *stable de novo* DSAs present both pre-COVID-19 and post-COVID-19. Increased DSA was defined as DSA that rose >1000 MFI and represented a >25% increase over baseline MFI. For patients with a history of multiple transplants, only DSA targeting HLA expressed by the functioning kidney allograft was considered. To adjudicate DSA responses, all patients required an HLA-Ab measurement within 14 months prior to the measurement with increased MFI. For most samples, HLA-Ab testing was performed for-cause and not by a prescribed protocol. In addition to HLA-Ab testing ordered by a clinician, we analyzed research serum samples collected in accordance with study protocols. We did this to establish baseline HLA-Ab measurements for patients lacking pre-COVID-19 samples and to evaluate DSA responses ≥10 days after COVID-19 diagnosis in patients without clinician-ordered HLA-Ab testing. For both HLA class I and class II, the antibody specificity with the maximum MFI across all timepoints was classified as immunodominant.

The secondary outcome analyzed was a 30% decline from the baseline estimated glomerular filtration rate (eGFR) ≥90 days from COVID-19 diagnosis (11). Two consecutive eGFR measurements below 70% of the baseline eGFR, with no subsequent eGFR recovery, defined the outcome. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate eGFR (12). Baseline eGFR was defined as the median eGFR among measurements from 365 days (or if transplanted in the year prior to COVID-19 diagnosis, from the date of post-transplant eGFR stabilization) to 7 days before COVID-19 diagnosis.

Additional outcomes analyzed included the following: Acute kidney injury (AKI) was classified using Kidney Disease Improving Global Outcomes (KDIGO) stages (13). Serum creatinine (sCr) values, used for eGFR calculations, were winsorized at the 95th percentile (14), which was 5.9 mg/dl for this study. If a patient experienced allograft failure, subsequent sCr measures were censored, and sCr at time of allograft failure was set equal to 5.9 mg/dl. Patients were defined as being vaccinated if they had received at least one dose of a COVID-19 vaccine prior to admission. World Health Organization (WHO)-defined COVID-19 disease severity was scored from 3 (admission without supplemental oxygen requirement) to 8 (death) (15).

Inpatient reduction of immunosuppression was calculated as the percentage reduction of each outpatient immunosuppressive dose. This reduction was calculated by dividing the total inpatient administered dose of each medication by the total expected dose based upon the outpatient maintenance regimen documented by the admitting physician, excluding inpatient medications administered on the day of admission or discharge. Corticosteroid doses were transformed into prednisone equivalent doses based upon relative potencies (16). Remdesivir was administered per institutional protocols for patients requiring supplemental oxygen and with eGFR >30 mL/min per 1.73 m^2 and serum alanine transferase <260 units/L.

Statistical Analyses

Categorical variables were presented as count and percentage. Continuous variables were presented with median and interquartile range (IQR). Pearson's Chi-squared test was used to compare percentages between categorical variables when all expected cell counts were ≥5, and Fisher's exact test was used when any expected cell count was <5. Group mean ranks were compared with Mann-Whitney tests. Differences in matched samples were compared using the Wilcoxon matched-pairs signed rank test. Multiple comparisons of group mean ranks were performed using the Friedman test adjusted with Dunn's multiple comparisons. An alpha of 0.05 was used as the cutoff for statistical significance. All tests were two-tailed.

The two main outcomes analyzed, development of new or increased levels of DSA and the loss of 30% of baseline eGFR, were estimated using the Kaplan-Meier (KM) method. eGFR loss was estimated using a landmark analysis, starting at a landmark of 90 days after COVID-19 diagnosis (17). Time-to-event analyses were censored by death and the end of the study follow-up period. The logrank test was used to evaluate for statistical differences in the probability of the outcome of interest at any time point. All statistical analyses were performed using R version 4.0.2 and GraphPad Prism version 9.3.1.

RESULTS

Cohort Characteristics

We prospectively enrolled KTRs hospitalized with a COVID-19 diagnosis at UAB between March 1, 2020 and November 1, 2021 (**Figure 1**). Of 115 consented KTRs with functioning allografts, 3 patients for whom DSA adjudication was impossible were excluded, yielding 112 KTRs for analysis (**Table 1**). 64 (57%) patients had at least one HLA-Ab test ≥10 days after their COVID-19 diagnosis (subcohort A). Baseline HLA-Ab testing was available at a median of 66 days prior to COVID-19 diagnosis (IQR 15–110 days). For a single patient, the baseline HLA-Ab measure was conducted over 365 days prior to COVID-19 diagnosis (at 414 days before COVID-19), and this patient had been followed regularly with clinic visits and stable allograft function for the year prior to COVID-19. These 64 patients had a total of 197 HLA-Ab tests (on average, 3.1 tests per patient). 175 of these tests were ordered by the attending

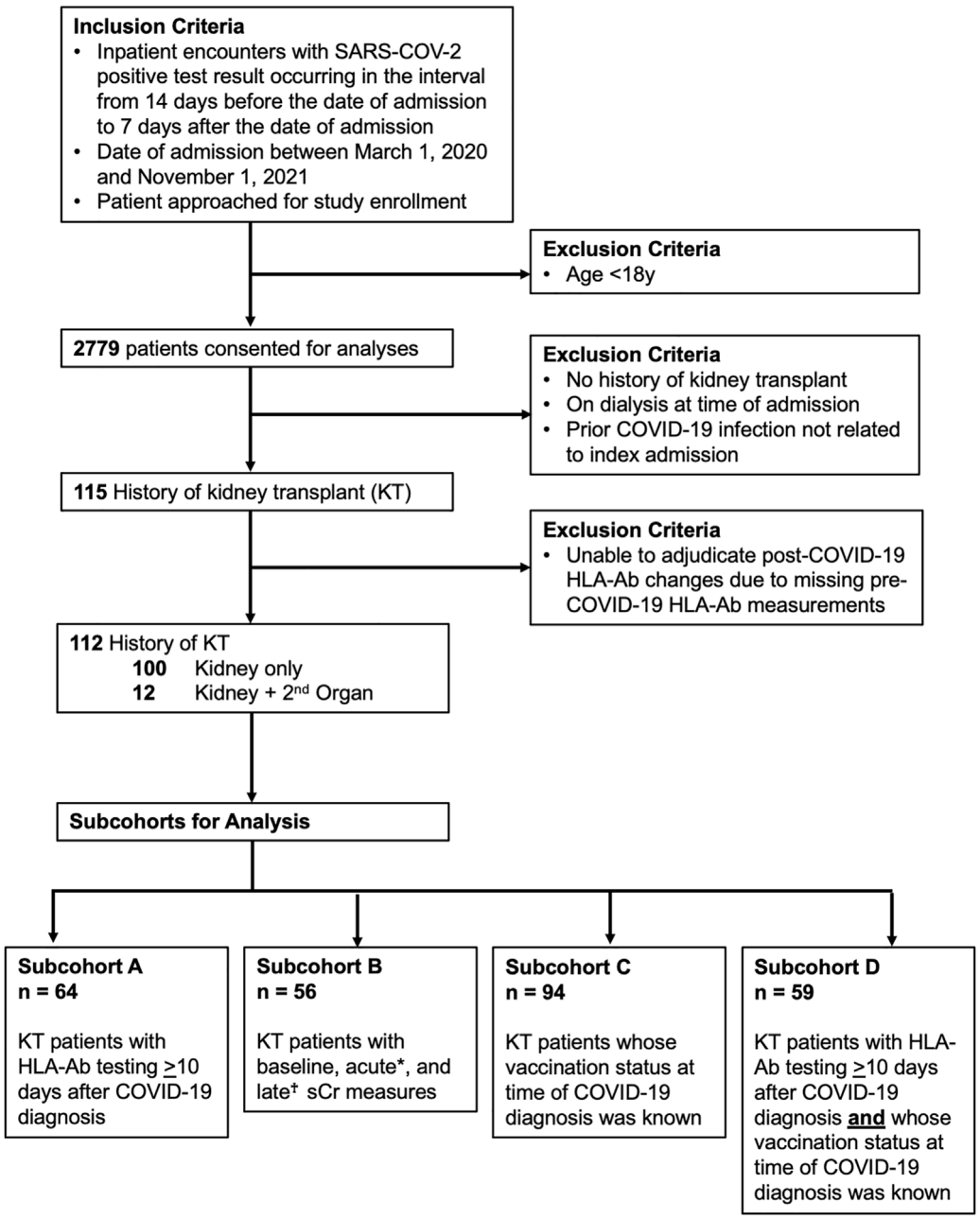


FIGURE 1 | Cohort inclusion criteria and data availability. Flowchart for included participants and four subcohorts used for analyses. sCr indicates serum creatinine. KT indicates kidney transplant. HLA-Ab indicates anti-HLA antibody. *Acute sCr refers to measures occurring both 1) during the index admission and 2) within 30 days of the COVID-19 diagnosis. †Late sCr refers to measures occurring at least 90 days after the COVID-19 diagnosis.

transplant nephrologist, and 22 were research serum specimens that were subsequently tested in the HLA laboratory (**Supplementary Figure S1**). 56 (50%) patients (subcohort B) had baseline, acute, and late (≥90 days from COVID-19 diagnosis) sCr measurements. 94 (84%) patients had a known COVID-19 vaccination status at the time of admission (subcohort C), and 59 (53%) patients had both HLA-Ab testing ≥10 days after COVID-19 diagnosis and known COVID-19 vaccination status (subcohort D).

New or Increased Donor-Specific Antibody Responses Were Frequently Observed in Kidney Transplant Patients Following Admission for COVID-19

Fifteen patients developed new or increased DSA responses at ≥10 days following COVID-19 diagnosis (**Figure 2A**). Ten of fifteen (67%) patients developed a new DSA specificity not present pre-COVID-19 (defined as increasing above a 1500

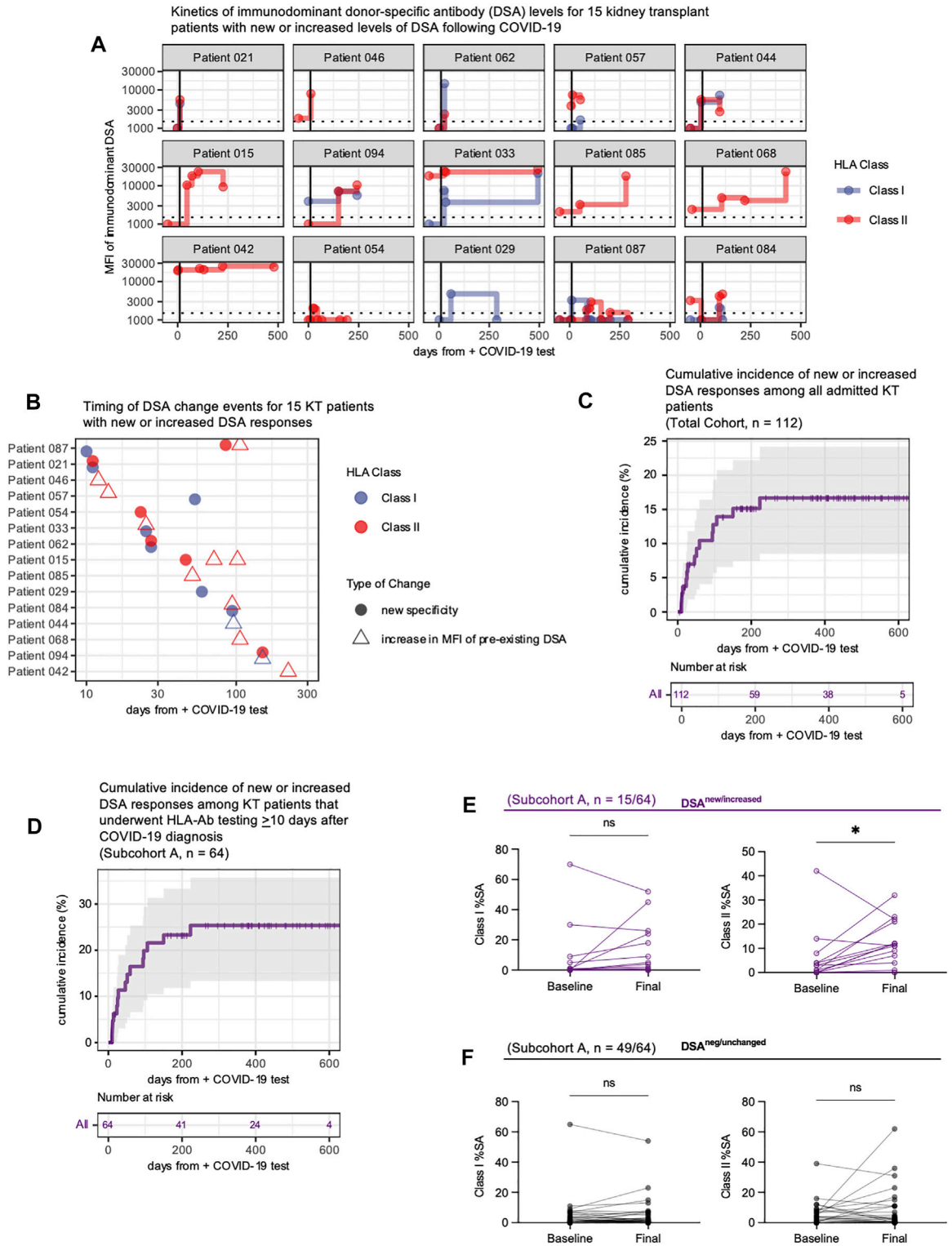


FIGURE 2 | COVID-19 infection is associated with development of new or increased DSA responses for up to 8 months. **(A)** Longitudinal assessment of the immunodominant class I and/or class II DSA levels for 15 patients who developed new or increased DSA responses following diagnosis of COVID-19. Each facet plot shows a single patient. DSA responses are shown as the mean fluorescence intensity (MFI) of binding by serum antibodies to the HLA bead array. The horizontal dashed line indicates the laboratory threshold for a positive DSA (MFI = 1500). Results below this MFI threshold are plotted at MFI = 1000. The vertical black line indicates 10 days after COVID-19 diagnosis. Any baseline measurement that occurred greater than 50 days before the COVID-19 diagnosis is plotted at x = −50 days. **(B)** The

(*Continued*)

FIGURE 2 | timing of the DSA changes shown in **A**, plotted based on DSA reactivity to individual HLA class I (blue symbols) or class II (red symbols) antigens, and whether the DSA was reactive to a new specificity (filled circle) or represented an increase in a preexisting DSA response (open triangle). **(C,D)** Cumulative incidence of new or increased DSA for the entire hospitalized COVID-19 KT patient cohort [n = 112, panel **(C)**] or those individuals with HLA-Ab testing ≥10 days after COVID-19 diagnosis [subcohort A, n = 64, panel **(D)**]. Incidence defined as the development of a new DSA specificity or an increase in a DSA specificity (MFI change >1000 and an overall increase of >25%). Patients are censored at time of death or at the end of the study follow-up period. **(E,F)** Analysis of breadth of anti-HLA reactivity in patient serum collected pre-COVID-19 (Baseline) and at the final measurement post-COVID-19 (Final). Samples were analyzed against a full panel of microbeads coated with single HLA class I or class II antigens. The percentage of positive (defined as an MFI ≥4 standard deviations above the background MFI) single antigen beads (%SA) for each sample was determined. Data shown are for patients in subcohort A with %SA measurements at both timepoints and include patients (n = 15) with new or increased DSA levels **(E)** and patients (n = 49) whose DSA responses were negative or unchanged **(F)**. Data were analyzed using the Kaplan-Meier method **(C,D)** or the Wilcoxon matched-pairs signed rank test **(E,F)**. ns, $p > 0.05$; *$p \leq 0.05$, **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

TABLE 1 | Overview of cohort demographics, transplant history, and disease severity.

	N = 112
Demographics	
Age	56 (49, 64)
Gender	
Female	45 (40%)
Male	67 (60%)
Race	
Black	57 (51%)
Other	3 (2.7%)
White	52 (46%)
Ethnicity	
Hispanic	3 (2.7%)
Non-Hispanic	109 (97%)
Transplant-related history	
Type of transplant	
Kidney	100 (89%)
Kidney, Heart	1 (0.9%)
Kidney, Liver	4 (3.6%)
Kidney, Lung	1 (0.9%)
Kidney, Pancreas	6 (5.4%)
Time from kidney transplant <1 year	31 (28%)
Presence of DSA pre-COVID-19	12 (19%)
Unknown	48
Disease Severity	
Highest WHO COVID-19 disease severity scale	
3 (no supplemental oxygen)	17 (15%)
4 (supplemental oxygen via nasal cannula)	38 (34%)
5 (supplemental oxygen via high-flow nasal cannula, BiPap, or CPAP)	19 (17%)
6 (endotracheal intubation and mechanical ventilation)	5 (4.5%)
7 (endotracheal intubation and mechanical ventilation + vasopressor support or ECMO)	3 (2.7%)
8 (death)	30 (27%)

Cell values presented as median (IQR) for continuous variables and n (%) for categorical variables. DSA, donor-specific antibody; WHO, World Health Organization; ECMO, extracorporeal membrane oxygenation.

MFI threshold), while five had increases (defined as an increase of both >1000 MFI and >25% from baseline MFI) in pre-existing DSA levels (**Figure 2A**, **Supplementary Table S1**). Four patients showed subsequent resolution of at least one DSA specificity within 1 year of COVID-19 diagnosis, but for all other patients, DSA responses persisted (**Figure 2A**). Development of new or increased DSA occurred within 6 months of COVID-19 diagnosis for 14/15 (93%) patients (**Figure 2B**). Within the entire cohort of 112 KTRs, which included those with and without post-COVID HLA-Ab testing, the cumulative incidence of new or increased DSA responses was 17% at 230 days post-COVID-19 (**Figure 2C**). When we restricted our analysis to the subcohort of patients with post-COVID-19 HLA-Ab testing (subcohort A, n = 64), we calculated the cumulative incidence to be 25% at 230 days post-COVID-19 (**Figure 2D**). Thus, we concluded that a minimum of 17% of admitted KTRs in our study developed a new or increased DSA response within 8 months following admission with COVID-19.

We then asked whether this DSA response developed in the context of a broad anti-HLA response. To assess the breadth of anti-HLA reactivity we measured reactivity of serum samples against an array of HLA class I and class II single antigen (SA) beads prior to COVID-19 diagnosis and at the final observation post-COVID-19. At each timepoint, we determined the percentage of HLA class I and class II antigens (%SA) that were bound by donor serum (defined as MFI ≥4 standard

TABLE 2 | Comparison of demographics, comorbidities, and transplant-related history based upon the development of new or increased DSA responses following COVID-19.

	DSA negative/unchanged/unknown, N = 97	DSA new/increased, N = 15	*p*-value
Demographics			
Age	57 (50, 64)	48 (40, 55)	0.010
Gender			0.3
Female	41 (42%)	4 (27%)	
Male	56 (58%)	11 (73%)	
Race			0.067
Black	45 (46%)	12 (80%)	
Other	3 (3.1%)	0 (0%)	
White	49 (51%)	3 (20%)	
Ethnicity			>0.9
Hispanic	3 (3.1%)	0 (0%)	
Non-Hispanic	94 (97%)	15 (100%)	
Comorbidities			
Diabetes mellitus	67 (69%)	12 (80%)	0.5
COPD	13 (13%)	3 (20%)	0.4
Hypertension	94 (97%)	14 (93%)	0.4
Coronary artery disease	38 (39%)	5 (33%)	0.7
Congestive heart failure	33 (34%)	6 (40%)	0.7
Never smoker	27 (28%)	1 (6.7%)	0.11
Obesity			0.5
Not obese	54 (56%)	7 (47%)	
Obese	43 (44%)	8 (53%)	
Transplant-related history			
Type of transplant			0.3
Kidney	87 (90%)	13 (87%)	
Kidney, Heart	1 (1.0%)	0 (0%)	
Kidney, Liver	4 (4.1%)	0 (0%)	
Kidney, Lung	0 (0%)	1 (6.7%)	
Kidney, Pancreas	5 (5.2%)	1 (6.7%)	
Time from kidney transplant (days)	1,496 (475, 2,958)	145 (100, 2,324)	0.068
Time from kidney transplant <1 year	23 (24%)	8 (53%)	0.028
Presence of DSA pre-COVID-19	5 (10%)	7 (47%)	0.004
Unknown	48	0	

Cell values presented as median (IQR) for continuous variables and n (%) for categorical variables. Pearson's Chi-squared test was used to compare percentages between categorical variables when all expected cell counts were ≥5, and Fisher's exact test was used when any expected cell count was <5. Group mean ranks were compared with Mann-Whitney tests. DSA, donor-specific antibody. COPD, chronic obstructive pulmonary disease.

deviations above background MFI). Patients with new or increased DSA responses showed an increase in the breadth of anti-class II responses (**Figure 2E**). However no other changes in the breadth of anti-HLA reactivity were statistically significant, and patients with negative or unchanged DSA responses had no significant increase in the breadth of anti-HLA reactivity (**Figure 2F**). Thus, the alloimmune response that develops following COVID-19 appears to be donor-specific and not merely the result of COVID-19 eliciting a broadly reactive anti-HLA response.

Kidney Transplant Patients With New or Increased Donor-Specific Antibody Responses Had Distinguishing Clinical Features

Our data showed new or increased DSA responses in a substantial fraction of KTRs hospitalized with COVID-19. To assess whether this alloimmune response was associated with specific clinical features, we assessed demographics, comorbidities and transplant history using an unadjusted bivariate analysis. In **Table 2**, we show that the 15 patients with new or increased DSA levels were younger, more likely to have been recently transplanted (53% vs. 24%, $p = 0.028$), and more likely to have pre-COVID-19 DSA (47% vs. 10%, $p = 0.004$). By contrast, other baseline demographics and comorbidities were similar between groups. Thus, younger age, pre-COVID-19 DSA and recency of transplant were associated with the development of new or increased DSA levels.

Next, we asked whether disease severity was associated with new or increased DSA responses. In **Table 3**, we show that length of stay, level of care, mortality, and the WHO COVID-19 disease severity score were all similar between the 15 KTRs who experienced DSA changes post-COVID-19 diagnosis and those without DSA changes. Overall, baseline and acute allograft function were similar between groups. Although rates and severity of AKI were similar between groups, patients with new or increased DSA levels showed significantly greater decreases in eGFR at late timepoints (**Table 3**). Sensitivity analyses of KTRs with baseline, acute and late serum creatine

TABLE 3 | Comparison of disease severity and renal function based upon the development of new or increased DSA responses following COVID-19.

	DSA negative/unchanged/ unknown, N = 97	DSA new/increased, N = 15	*p*-value
Measures of disease severity			
Length of stay (days)	10 (4, 19)	11 (4, 17)	>0.9
ICU requirement	40 (41%)	7 (47%)	0.7
Highest WHO COVID-19 disease severity scale			0.13
3 (no supplemental oxygen)	14 (14%)	3 (20%)	
4 (supplemental oxygen via nasal cannula)	34 (35%)	4 (27%)	
5 (supplemental oxygen via high-flow nasal cannula, BiPap, or CPAP)	17 (18%)	2 (13%)	
6 (endotracheal intubation and mechanical ventilation)	2 (2.1%)	3 (20%)	
7 (endotracheal intubation and mechanical ventilation + vasopressor support or ECMO)	3 (3.1%)	0 (0%)	
8 (death)	27 (28%)	3 (20%)	
Discharge disposition			0.7
Expired	27 (28%)	3 (20%)	
Home	59 (61%)	11 (73%)	
Long-term care facility	11 (11%)	1 (6.7%)	
Renal function			
AKI Grade (KDIGO)			0.3
0	20 (24%)	3 (23%)	
1	34 (41%)	4 (31%)	
2	5 (6.0%)	3 (23%)	
3	24 (29%)	3 (23%)	
Unknown	14	2	
CRRT/HD while inpatient	22 (23%)	3 (20%)	>0.9
Baseline eGFR (mL/min/1.73 m^2)	43 (33, 61)	56 (41, 63)	0.3
Unknown	21	4	
Absolute change in eGFR on 90+ day follow-up	1 (–8, 7)	–14 (–17, –5)	0.013
Unknown	51	5	
Percentage change in eGFR on 90+ day follow-up	4 (–19, 17)	–29 (–56, –9)	0.005
Unknown	51	5	
At least 30% loss in eGFR on 90+ day follow-up			0.027
<30%	42 (91%)	6 (60%)	
≥30%	4 (8.7%)	4 (40%)	
Unknown	51	5	

Cell values presented as median (IQR) for continuous variables and n (%) for categorical variables. Pearson's Chi-squared test was used to compare percentages between categorical variables when all expected cell counts were ≥5, and Fisher's exact test was used when any expected cell count was <5. Group mean ranks were compared with Mann-Whitney tests. DSA, donor-specific antibody. WHO, World Health Organization· ECMO, extracorporeal membrane oxygenation. AKI, acute kidney injury. KDIGO, Kidney Disease Improving Global Outcomes. CRRT, continuous renal replacement therapy. HD, hemodialysis. eGFR, estimated glomerular filtration rate.

(sCr) measurements (subcohort B, **Supplementary Table S2**) and subcohort B patients who also had post-COVID-19 HLA-Ab testing (**Supplementary Table S3**) confirmed those findings. Thus, while acute clinical severity was similar between groups, patients with new or increased DSA levels exhibited greater loss of eGFR at ≥90 days post-COVID-19.

New or Increased Donor-Specific Antibody Was Associated With Late Loss of Allograft Function

To better understand the progression of renal dysfunction post-COVID-19, we analyzed the change in eGFR over time, comparing patients with new or increased DSA levels to patients whose DSA levels were negative/unchanged/unknown (subcohort B, $n = 56$). Among the subset of patients with baseline, early inpatient, and late eGFR measurements, there was a significant decline in eGFR during admission (**Figures 3A,B**), with subsequent late recovery for many patients (**Figures 3A,B**). We then measured the absolute (**Figure 3C**) and relative (**Figure 3D**) changes in eGFR and found that patients with new or increased DSA levels had significantly greater late loss of eGFR. To estimate the incidence of a clinically meaningful (11, 18) late 30% loss of baseline eGFR, we performed a time-to-event analysis. This confirmed that patients with new or increased DSA levels were more likely to experience a 30% loss of baseline eGFR (**Figure 3E**, $p = 0.046$). Thus, patients who developed new or increased DSA levels following COVID-19 diagnosis experienced greater late loss of renal function.

Kidney Transplant Patients Who Developed New or Increased Levels of Donor-Specific Antibodies Received Distinct Medical Management

Our data showed a substantial proportion of KTRs hospitalized with COVID-19 went on to develop new or increased DSA levels that were associated with late allograft dysfunction. Given the temporal association between infection and DSA responses, we hypothesized that antiviral and immunosuppressive therapies would be associated with DSA events following infection. To test this hypothesis, we assessed inpatient medications,

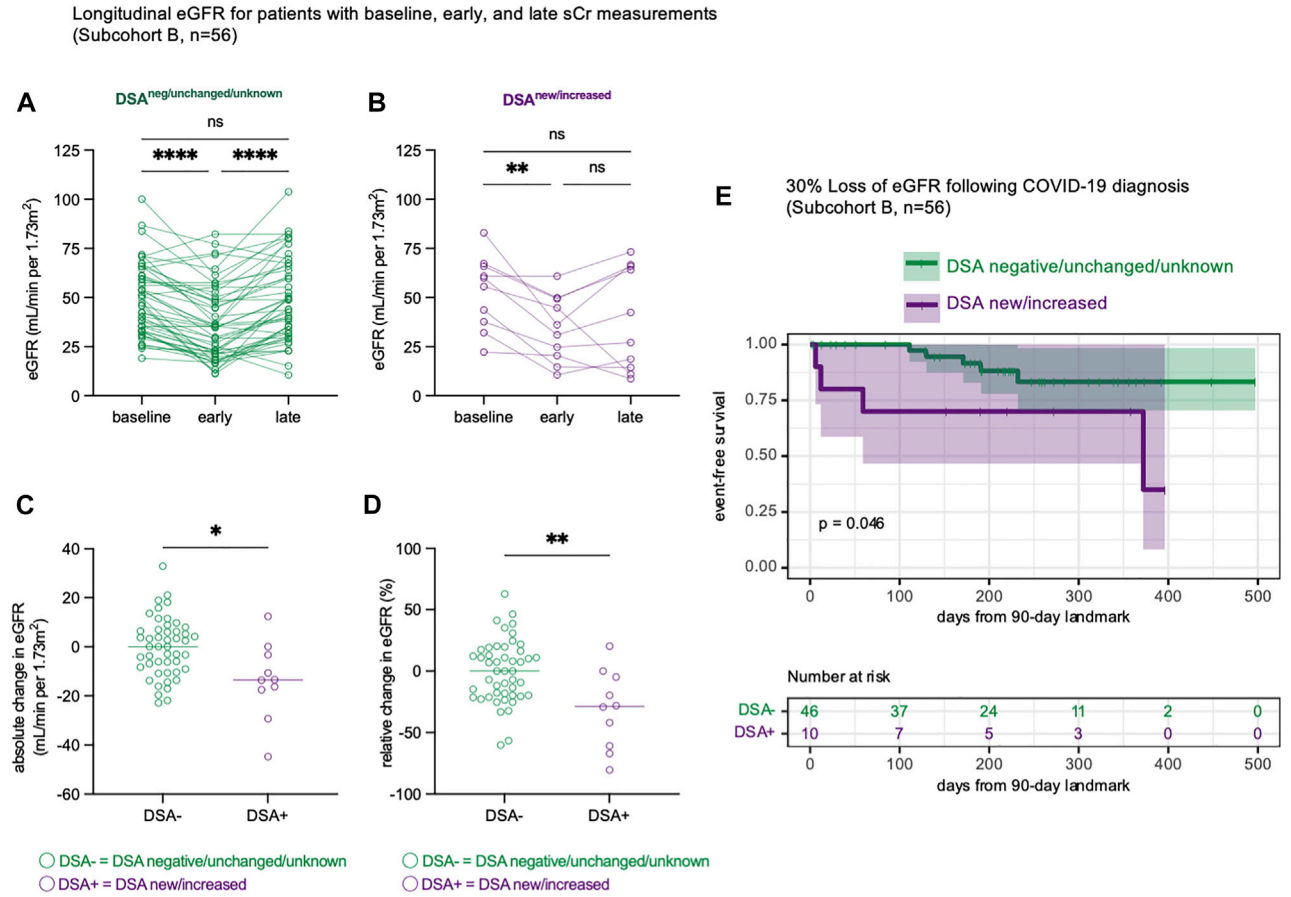


FIGURE 3 | Late renal function is impaired in KT patients with new or increased DSA responses. **(A,B)** Estimated glomerular filtration rate (eGFR) trajectories for n = 56 patients (subcohort B) with baseline, early and late eGFR measurements. The baseline measurement represents the median eGFR in the year prior to COVID-19 infection. The early measurement represents the nadir eGFR during the hospital admission for COVID-19. The late measurement represents the final eGFR measurement that occurred at least 90 days following COVID-19 diagnosis. Data shown as eGFR measurements in patients whose DSA responses were negative, unchanged, or unknown [**(A)**, n = 46] and in patients with new or increased DSA responses [**(B)**, n = 10]. **(C)** The absolute change in eGFR from each patient's baseline eGFR to their final eGFR, at least 90 days after COVID-19 diagnosis. **(D)** The relative change in eGFR from each patient's baseline eGFR to their final eGFR, calculated as a percentage change of the baseline eGFR. **(E)** A time-to-event analysis modeling the late loss of at least 30% of baseline eGFR, starting at 90 days after COVID-19 infection. This is performed as a landmark analysis, where x = 0 is landmarked to 90 days after COVID-19 diagnosis. Statistical analyses were performed using Friedman's test with the application of Dunn's multiple comparisons test **(A,B)**, Mann-Whitney Test **(C,D)** or the logrank test **(E)**. ns, $p > 0.05$; *$p \leq 0.05$, **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

vaccination history, and immunosuppressive regimens for the entire cohort (n = 112, **Table 4**). We found that patients who developed new or increased DSA levels were less likely to receive remdesivir (33% vs. 65%, p = 0.02, **Table 4**), which was notable considering rates of AKI were similar between groups. Patients with new or increased DSA levels were less likely to have received at least one dose of a COVID-19 vaccination prior to infection (0% vs. 28%, p = 0.018, **Table 4**) and were admitted earlier in the pandemic. Among the 22 KTRs that had been vaccinated prior to infection, the median time from the first vaccine dose to infection was 137 days (**Supplementary Table S4**). 16 (72%) received a second vaccine dose ≥3 weeks prior to infection. Patients with new or increased DSA levels had higher pre-admission immunosuppressant doses and presented with lower absolute lymphocyte counts, consistent with more recent transplantation and more intensive immunosuppression. However, inpatient percentage reduction in immunosuppression was not significantly different across any immunosuppressant between groups. In addition, there was no significant difference in the receipt of convalescent plasma between groups. Thus, patients who did not develop new or increased levels of DSA were more likely to (1) have received remdesivir, (2) have received at least one dose of a COVID-19 vaccine prior to infection, or (3) have lower doses of maintenance immunosuppression at the time of admission.

Vaccination Was Associated With Protection From New or Increased Donor-Specific Antibodies

Given that vaccination status and development of new or increased DSA responses were inversely correlated, we analyzed the temporal relationship between COVID-19 admission date and whether an individual developed a new or

TABLE 4 | Comparison of medical management based upon the development of new or increased DSA responses following COVID-19.

	DSA negative/unchanged/unknown, N = 97	DSA new/increased, N = 15	*p*-value
Antimicrobial therapies			
Convalescent plasma	3 (3.1%)	1 (6.7%)	0.4
Remdesivir	63 (65%)	5 (33%)	0.020
Any antibiotics while inpatient	68 (70%)	9 (60%)	0.6
Vaccine-related			
Time from EUA to COVID-19 diagnosis (days)	38 (–37, 234)	–57 (–132, 4)	0.001
COVID-19 diagnosis occurred after EUA	64 (66%)	6 (40%)	0.053
At least one vaccine dose prior to COVID-19 infection	22 (28%)	0 (0%)	0.018
Home immunosuppressive regimen at time of admission			
Home immunosuppression drug regimen			0.2
Other	14 (14%)	0 (0%)	
Tac + MMF (Steroid Free)	6 (6.2%)	0 (0%)	
Tac + MMF + Prednisone	77 (79%)	15 (100%)	
Home prednisone dose (mg)	10.00 (7.50, 10.00)	10.00 (10.00, 10.00)	0.006
Home tacrolimus dose (mg)	4.0 (3.0, 7.0)	10.0 (4.0, 11.5)	0.043
Home MMF dose (mg)	1,000 (750, 1,500)	1,500 (1,260, 2,000)	0.009
Inpatient immunosuppression doses			
Average daily inpatient steroid dose (prednisone equivalent mg)	28 (11, 40)	20 (11, 35)	0.4
Average daily inpatient tacrolimus dose (mg)	1.31 (0.38, 2.85)	2.50 (0.22, 4.00)	0.4
Median inpatient tacrolimus level (ng/ml)	5.03 (3.15, 7.17)	6.70 (4.70, 7.60)	0.3
Average daily inpatient MMF dose (mg)	524 (344, 1,000)	646 (34, 1,034)	0.8
Inpatient immunosuppression as a percentage of home regimen			
Percentage of home prednisone dose (%)	358 (123, 497)	150 (106, 303)	0.10
Percentage of home tacrolimus dose (%)	33 (13, 55)	37 (5, 57)	>0.9
Percentage of home MMF dose (%)	52 (38, 70)	44 (2, 65)	0.2
Immune parameters			
Admission C-reactive protein (mg/L)	88 (36, 149)	87 (48, 111)	>0.9
Admission absolute lymphocytes (103 cells/uL)	0.56 (0.28, 0.81)	0.27 (0.18, 0.45)	0.083

Cell values presented as median (IQR) for continuous variables and n (%) for categorical variables. Pearson's Chi-squared test was used to compare percentages between categorical variables when all expected cell counts were ≥5, and Fisher's exact test was used when any expected cell count was <5. Group mean ranks were compared with Mann-Whitney tests. DSA, donor-specific antibody. EUA, emergency-use authorization for BNT-162b2 (Pfizer/BioNTech) on 11 December 2020. Tac, tacrolimus. MMF, mycophenolate mofetil.

increased DSA response. All patients who developed new or increased DSA responses were admitted before 1 March 2021, and none had been vaccinated prior to COVID-19 infection (**Figure 4A**). The proportion of KTRs who developed new or increased DSA declined as the pandemic progressed (**Figure 4B**). We found these same relationships for patients with HLA-Ab testing ≥10 days after COVID-19 (subcohort A, **Figures 4C,D**). Next, using a time-to-event analysis for the entire cohort, we found that patients who had received at least one vaccine dose were significantly less likely to develop a new or increased DSA response (**Figure 4E**, $p = 0.047$). As a sensitivity analysis, we performed the same comparison for those individuals for whom HLA-Ab testing post-COVID-19 was available (subcohort D). Importantly, we observed a similar inverse correlation between vaccination status and likelihood of developing a new or increased DSA response (**Figure 4F**, $p = 0.074$). Of note, although patients that were vaccinated and subsequently admitted had shorter follow-up time given their more recent admissions, these patients had still had a median follow-up time of 151 days, and our data (**Figure 2B**) suggests that the majority of post-COVID-19 DSA events occur within this follow-up interval. Thus, KTRs vaccinated prior to infection or admitted later in the course of the pandemic were less likely to develop new or increased DSA post-COVID-19.

Secular Trends in COVID-19 Severity and Management

Our data showed that in addition to transplant-specific risk factors, vaccination and remdesivir use were associated with protection from new or increased DSA responses. As disease management, variant prevalence, and outcomes changed during the pandemic, we asked whether other secular trends might be associated with the declining risk of DSA events in this cohort. We observed a trend towards slightly greater disease severity as the pandemic progressed, as measured by WHO clinical severity and the proportion of patients experiencing a grade 3 AKI (**Figures 5A,B**). We also found that remdesivir use increased during the fall of 2020 and stayed stable following that time (**Figure 5C**), and that vaccination increased in 2021 (**Figure 5D**). These findings support the notion that the declining incidence of DSA events is not explained by declining disease severity, and instead may be associated with trends in management.

DISCUSSION

We showed that new or increased DSA responses in KTRs following admission with COVID-19 was relatively common, with a cumulative incidence of 17% at 8 months following

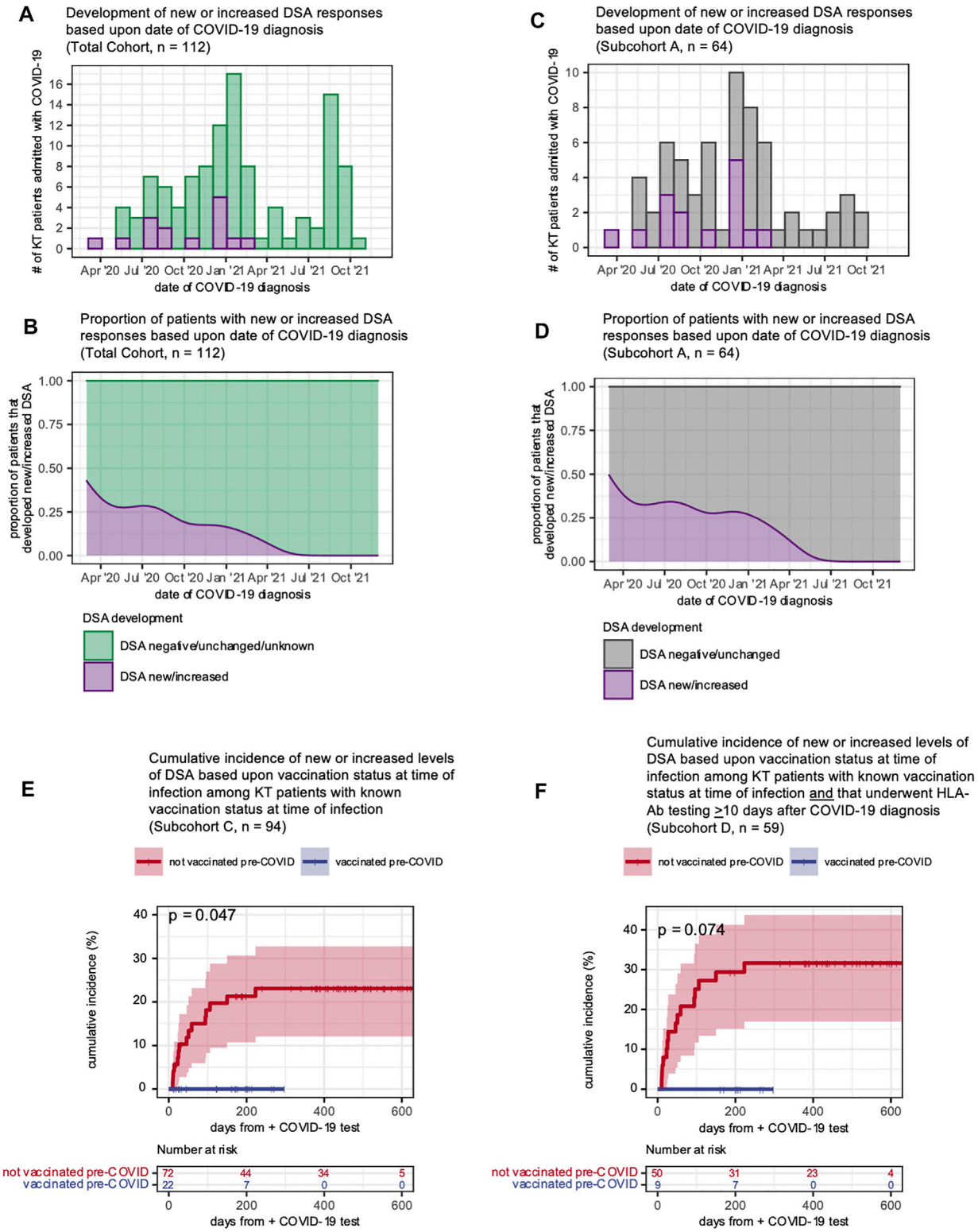


FIGURE 4 | Vaccinated KT patients were less likely to develop new or increased DSA responses following COVID-19 infection. **(A–D)** Development of new or increased DSA responses shown as histograms **(A,C)** displaying the distribution of patients over the study period and smoothed kernel density estimates **(B,D)** that reflect the proportion of patients admitted over time who subsequently developed a new or increased DSA response. Panels **(A,B)** show all KT patients (*n* = 112) hospitalized with a COVID-19 diagnosis over the study period and include patients who developed a new or increased DSA response after COVID-19 (purple) and

(*Continued*)

FIGURE 4 | patients whose DSA status was negative, unchanged or unknown (green). Panels **(C,D)** show subcohort A (patients with HLA-Ab testing ≥10 days after COVID-19 diagnosis) with a new or increased DSA response after COVID-19 (purple) and patients whose DSA status was negative or unchanged (gray). **(E)** The cumulative of incidence of new or increased DSA levels based upon the patient vaccination status at the time of COVID-19 diagnosis. Data shown for $n = 94$ patients (subcohort C) for whom vaccination status was known at the time of admission. **(F)** The cumulative of incidence of new or increased DSA levels based upon the patient vaccination status at the time of COVID-19 diagnosis. Data shown for $n = 59$ patients (subcohort D) for whom vaccination status was known at the time of admission and that had post-COVID-19 HLA-Ab testing. Curves in **(E,F)** were estimated using the Kaplan-Meier method and analyzed with the logrank test.

infection. This DSA response was associated with impaired allograft function. We further found that the risk of developing a new or increased DSA response was significantly higher in recently transplanted patients. By contrast, COVID-19 vaccination prior to infection as well as the administration of remdesivir during infection were associated with protection from the development of a new or increased DSA response.

Our study is not the first to examine DSA changes in KTRs following COVID-19 infection (5). Masset *et al.* showed that 8% of KTRs hospitalized with COVID-19 developed new or increased levels of DSA within a median follow-up of 45 days (5). Consistent with the Masset et al. study (5), we observed that recent transplant, younger age, and pre-COVID-19 DSA were each associated with an increased risk for a change in DSA levels. By extending the follow-up time, we found the cumulative incidence of a new or increased DSA response was 17% within 8 months of COVID-19 diagnosis. Importantly, these changes in DSA responses occurred throughout those 8 months. Interestingly, of the 15 individuals who experienced a change in their DSA response following infection, 10 (66%) developed a new DSA specificity, considerably higher than the expected incidence of *de novo* DSA in a general KT population (19). Our data also show that these DSA responses do not only occur immediately following the acute infection. We speculate that these delayed alloimmune events may be related to ongoing inflammation or tissue injury. Given that SARS-CoV-2 may persist in the immunocompetent human host for at least 4 months (20) and that immunosuppressed patients may have relatively prolonged viral shedding (21), extended infection may promote alloimmunity, either through chronic immune activation (22) or direct allograft injury (23–25).

Consistent with data showing SOT patient outcomes improved as clinical practices were refined during the pandemic (26), our temporal analysis revealed that patients admitted in the post-EUA era were significantly less likely to develop new or increased DSA responses. This reduction in alloimmunity was associated with COVID-19 vaccination pre-infection as well as with administration of remdesivir during acute infection. Given the lack of association between DSA responses and acute disease severity or acute kidney injury, these data suggest that interventions like vaccination or remdesivir treatment that decrease viral replication (27, 28) may be associated with a reduced risk of alloimmunity.

Our data suggest three potential clinical considerations for KTRs in the ongoing COVID-19 pandemic. First, for KTRs hospitalized with COVID-19, new or increased DSA formation was relatively common, particularly among non-vaccinated patients. Given the association between DSA changes and impaired late allograft function, it may be prudent to consider more frequent DSA surveillance in this population, particularly among patients with increased alloimmune risk (a recent transplant or pre-COVID-19 DSA). Second, given the vulnerability of recently transplanted patients, the impaired efficacy of vaccination in the immunosuppressed patient (29), and concerns surrounding vaccination policies (30, 31), our findings suggest that pre-transplant vaccination in this cohort has the potential to be beneficial in reducing the incidence of alloimmune responses post-COVID-19. Importantly, our data provide no evidence that vaccines promote alloimmunity. Third, we observed that administration of remdesivir was associated with a reduced risk of developing a new or increased DSA response. It will be important to assess the efficacy of new antiviral therapies in this vulnerable KT patient population—both for reducing disease severity as well as for any association with abrogating alloimmune responses.

Our study was conducted at a single center and was limited to KTRs hospitalized with COVID-19. Although we did not genotype SARS-CoV-2 strains, we think it is unlikely that changing variants could explain our findings as all admissions predated Omicron's emergence (32), and pre-Omicron variants have shown similar disease severity (33) and similar tropism for the kidney (34). Our analysis of other secular trends indicate that while management features changed, disease severity largely did not. Although we were able to document vaccine administration, we did not have pre-disease antiviral serologic data as a surrogate for vaccine efficacy. Since most testing and sample collection in our study group was guided by clinical care rather than research protocols, HLA-Ab testing was likely biased towards patients with increased immune risk or evidence of allograft dysfunction. As we did not have equivalent samples across the entire cohort, we may have underestimated the incidence of DSA events in untested patients. Finally, although we found an association between DSA events and loss of eGFR, we lacked sufficient allograft biopsy data to correlate DSA events with histopathological lesions. Importantly, the conclusions that were reached for the whole cohort were well-supported by the subcohort-based sensitivity analyses. Finally, the relatively small number of events for our outcomes of interest limited our ability to perform multivariate analysis, and thus our findings should be interpreted as correlational.

Despite the limitations associated with our single center study, we demonstrated that KTRs hospitalized with COVID-19 often developed new or increased DSA responses. These changes in DSA status were associated with impaired late allograft function. Vaccination and administration of remdesivir were each associated with protection from the development of a new or enhanced DSA response. Recent transplant and the presence of pre-COVID-19 DSA were positively associated with new or increased DSA responses. These data, which identify patients

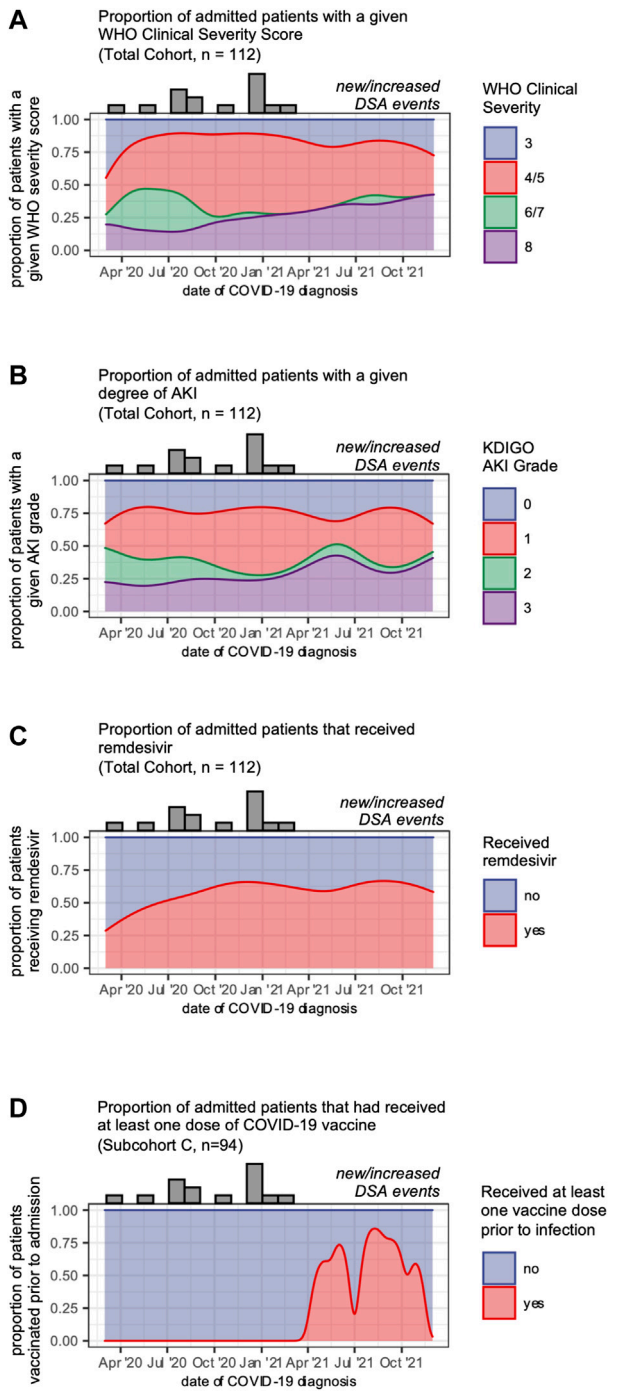


FIGURE 5 | Secular trends in COVID-19 disease severity and management. **(A)** Disease severity of patients admitted over the study period shown as smoothed kernel density estimates that reflect the proportion of patients admitted over time who reached a given WHO clinical severity score. **(B)** Degree of acute kidney injury for patients admitted over the study period shown as smoothed kernel density estimates that reflect the proportion of patients admitted over time who reached a given grade of AKI. **(C)** Remdesivir use for patients admitted over the study period shown as smoothed kernel density estimates that reflect the proportion of patients admitted over time who received remdesivir. **(D)** Vaccination status for patients admitted over the study period shown as smoothed kernel density estimates that reflect the proportion of patients admitted over time who had received at least one dose of a COVID-19 vaccine prior to admission. Along the top of each graph are counts of the number of admitted patients who developed a new or increased DSA response in each month.

(*Continued*)

at higher risk of developing humoral alloimmune responses, suggest a role for more intensive HLA-Ab surveillance and provide indirect evidence of the possible benefits of preventing alloimmunity in SARS-CoV-2-infected KTRs through vaccination or antiviral therapies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the UAB Institutional Review Board (IRB-300005127). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK, JH, AK, SO, VH-D, PP, EL, VK, and FL contributed to conception and design of the study. JK, JH, GB, SR, AN, GA, CK, BO, JL, PP, EL, VK, and FL contributed to clinical data extraction, transformation, and analysis. NE, PG, SL, and EZ contributed to sample acquisition and patient enrollment. JK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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AUTHOR DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CONFLICT OF INTEREST

EL receives research support from Amgen and consults for Novartis. AN serves on the scientific advisory board of

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10626/full#supplementary-material

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BRIEF RESEARCH REPORT
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An Analysis by the European Committee on Organ Transplantation of the Council of Europe Outlining the International Landscape of Donors and Recipients Sex in Solid Organ Transplantation

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Discrepancies in donation and transplantation by sex and gender have previously been reported. However, whether such differences are invariably the inevitable, unintended outcome of a legitimate process has yet to be determined. The European Committee on Organ Transplantation of the Council of Europe (CD-P-TO) is the committee that actively promotes the development of ethical, quality and safety standards in the field of transplantation in Europe. Whilst the ultimate objective is to shed light on the processes underlying potential gender inequities in transplantation, our initial goal was to represent the distribution by sex among organ donors and recipients in the CD-P-TO Member States and observer countries. Our survey confirms previous evidence that, in most countries, men represent the prevalent source of deceased donors (63.3% in 64 countries: 60.7% and 71.9% for donation after brain and circulatory death, respectively). In contrast, women represent the leading source of organs recovered from living kidney and liver donors (61.1% and 51.2% in 55 and 32 countries, respectively). Across countries, most recovered organs are transplanted into men (65% in 57 countries). These observations may be explained, at least in part, by the higher burden of certain diseases in men, childbearing related immune sensitization in women, and donor-recipient size mismatch. Future research should establish whether gender-related socially-constructed roles and socioeconomic status may play a detrimental role reducing the access of women to transplantation.

Keywords: donors, sex, inequalities, recipients, Council of Europe

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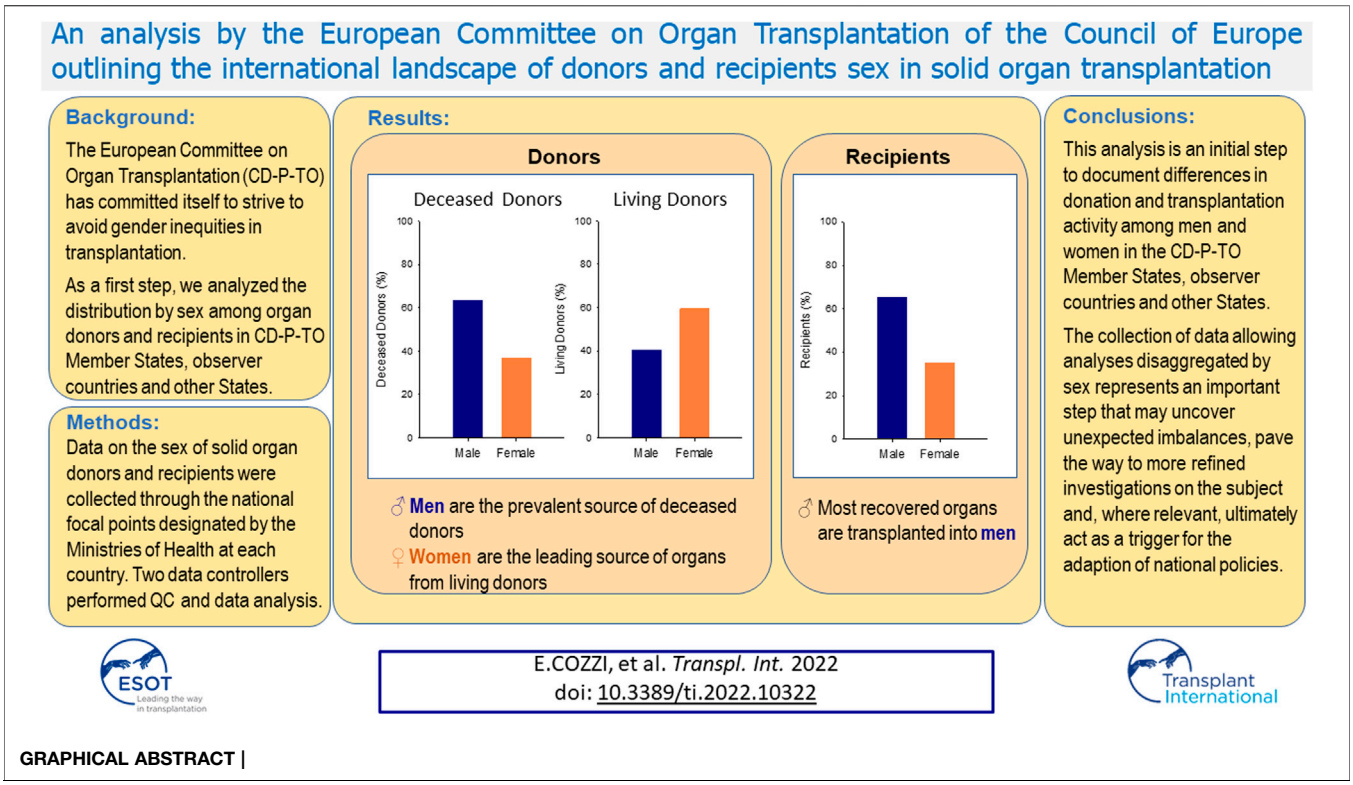
Abbreviations: CD-P-TO, European Committee on Organ Transplantation of the Council of Europe; DBD, donor/donation after brain death; DD, deceased donor; DCDD, donor/donation after circulatory determination of death; LD, living donor; LKD, living kidney donation; LLD, living liver donation; ONT, Organización Nacional de Trasplantes; PMP, per million population.



GRAPHICAL ABSTRACT |

INTRODUCTION

Sex and gender represent two fundamental variables that must be taken into due consideration to ensure health policies are efficient and adapted to the current needs and circumstances of the global population (1). Accordingly, the European Committee on Organ Transplantation of the Council of Europe (CD-P-TO)[1] has committed itself to take into account the impact of gender and sex in the performance of its tasks and to strive to avoid inequities in each of its policy areas.

To date, the terms gender and sex have often been used interchangeably. However, gender and sex have very specific meanings and must be applied in well-defined and distinct circumstances. Whilst sex exclusively refers to biological traits, gender regards non-biological attributes that are socially constructed and are the ultimate result of an individual's roles, culture, and conventions (2–3).

Gender inequities in access to transplantation were previously reported (4–8). However, to the best of our knowledge, the sex of donors and recipients of solid organ transplants across the countries represented in the Council of Europe has not been investigated to date. Appreciating the importance of studying determinants of potential gender inequities in transplantation at the international level, the CD-P-TO decided, as an initial step, to collect data on the sex of solid organ donors and recipients in its annual data collection on donation and transplantation activities. These data are made available through "Newsletter Transplant", the official annual publication of the Committee. Here we report the main findings of analyses conducted using the data provided for the year 2019 by Member States of the Council of Europe, Observer Countries, and other States. Indeed, the figures regarding the year 2019 represent the latest set of data that were not impacted by the Covid-19 pandemic.

[1]The CD-P-TO is the steering committee in charge of organ, tissue and cell donation and transplantation activities at the European Directorate for the Quality of Medicines and HealthCare of the Council of Europe. It actively promotes the non-commercialization of organ, tissue and cell donation, the fight against organ trafficking and the development of ethical, quality and safety standards in the field of organ, tissue and cell transplantation. Its activities include the collection of international data and monitoring of practices in Europe, the transfer of knowledge and expertise between organisations and experts through training and networking and the elaboration of reports, surveys, and recommendations. As of November 2021, the CD-P-TO was composed of 39 members (Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Republic of Moldova, Turkey, Ukraine, and United Kingdom) and 22 observers (Armenia, Belarus, Canada, Georgia, Israel, Russian Federation, United States, Council of Europe Committee on Bioethics, DTI Foundation, European Association of Tissue and Cell Banks, European Commission, European Eye Bank Association, European Society for Blood and Marrow Transplantation, European Society for Organ Transplantation, European Society of Human Reproduction and Embryology, Eurotransplant, Scandiatransplant, South-Europe Alliance for Transplants (SAT), The Transplantation Society, United Network for Organ Sharing (UNOS), World Health Organization (WHO), and World Marrow Donors Association).

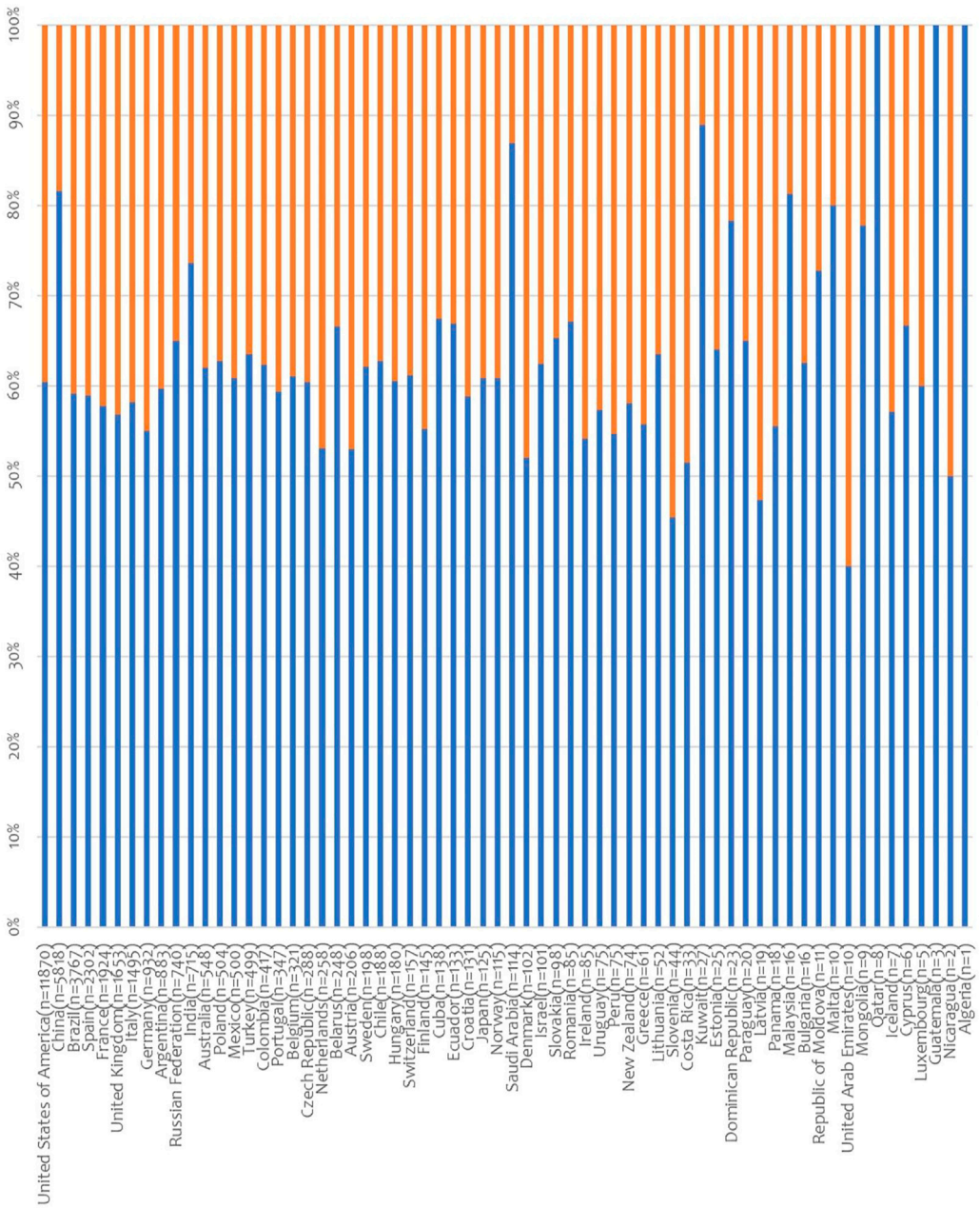


FIGURE 1 | Distribution of deceased organ donors (DBD and DCDD) by sex. Data on donor sex was provided by 64 countries (in brackets: number of donors; blue lines: % male donors; orange lines: % female donors).

METHODS

To investigate inequities in organ transplantation, questions on the sex of living and deceased organ donors and recipients were incorporated into the questionnaire that the Organización Nacional de Trasplantes (ONT) submits yearly to countries participating in the Newsletter Transplant (available at www.edqm.eu/freepub).

As far as deceased organ donors are considered, countries were first invited to provide national figures (absolute numbers). Subsequently, countries were asked to stratify the data by deceased donor type into donors after brain death (DBD) and donors after circulatory determination of death (DCDD). Countries were then requested to further provide the distribution of donors by sex. To examine the situation relating to living donation, countries were likewise invited to

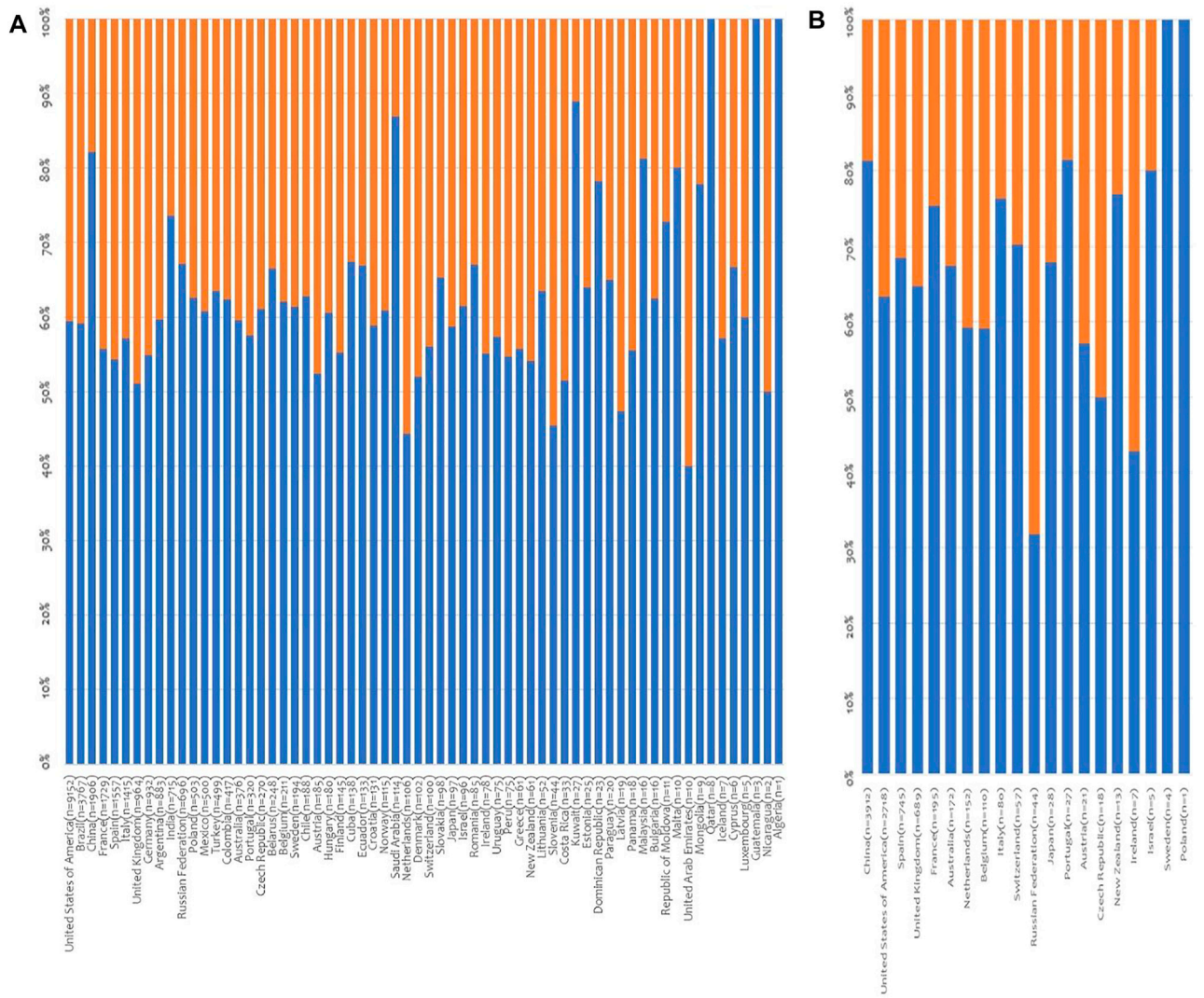


FIGURE 2 | Distribution of deceased organ donors by sex and donor type. **(A)** Distribution of DBD by donor sex. Data on donor sex was provided by 64 countries; **(B)** Distribution of DCDD by donor sex. Data on donor sex was provided by 20 countries (in brackets: number of donors; blue lines: % male donors; orange lines: % female donors).

provide national figures relating to the sex of living kidney donors (LKD) and living liver donors (LLD). Finally, countries were also asked to provide data on the sex of recipients of solid organ transplants originating from both deceased and living donors.

The questionnaire was completed by national focal points designated by the Ministries of Health at each country. ONT then compiled the information collected by the questionnaires, performed the corresponding quality control of the data reported, and the analysis. Quality control of the data involved the review of each questionnaire by two data controllers. In the presence of inconsistencies, the ONT contacted the designated focal point in each country for a final data check. Analyses were carried out using SPSS v.25.0 and Excel. To calculate rates per million population (PMP), the country population was obtained from the United Nations Population Fund (UNFPA) report (www.unfpa.org).

RESULTS

Participating Countries

A total of 69 countries responded to this initiative and provided thorough information on the sex of donors and recipients. In particular, the countries involved in the study include 36 Council of Europe Members States (Armenia, Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of Moldova, Republic of North Macedonia, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom), 3 Observer Countries (Mexico, Israel, and United States), 15 countries of Iberoamerican Network/Council of Donation and Transplantation- RCIDT

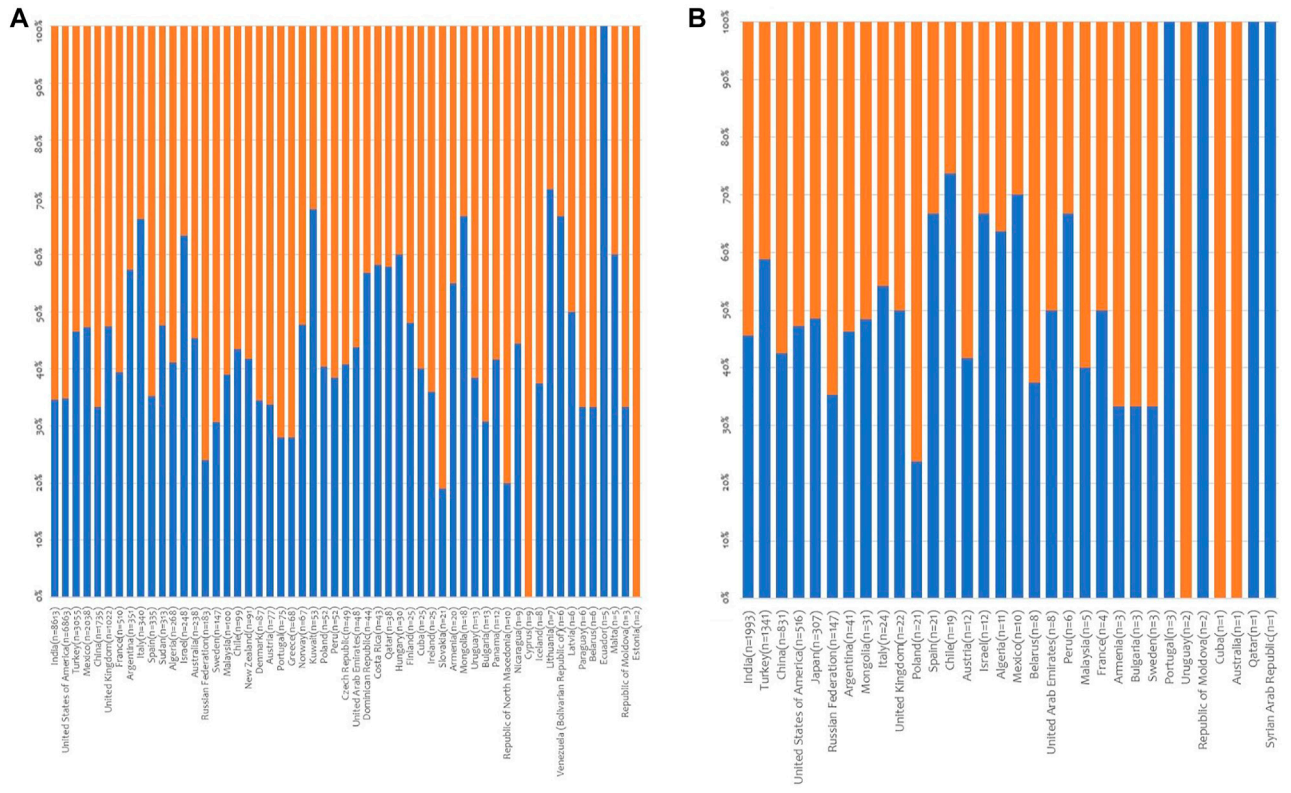


FIGURE 3 | Distribution of living donors by sex. **(A)** Distribution of living kidney donors by sex. Data on donor sex was provided by 55 countries; **(B)** Distribution of living liver donors by sex. Data on donor sex was provided by 32 countries (in brackets: number of donors; blue lines: % male donors; orange lines: % female donors).

(Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Guatemala, Nicaragua, Panama, Paraguay, Peru, Uruguay, Venezuela) and 15 additional countries from 4 continents (Algeria, Australia, Belarus, China, India, Japan, Kuwait, Malaysia, Mongolia, New Zealand, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, United Arab Emirates).

Sex of Deceased Organ Donors

Globally, in 2019 there were 38,983 deceased organ donors recorded in the 69 participating countries. In the latter, DBD and DCDD activity was reported in 65 and 20 countries, respectively. Deceased donors PMP ranged from 0 to 49.6 (**Supplementary Figure S1**). Information about sex was available for 38,980 deceased donors (99.9%) in 64 countries, and men added up to 63.3% of these (**Figure 1**, **Supplementary Table S1**). When deceased donors were divided into DBD and DCDD donors, once again the percentage of male donors was prevalent (60.7% and 71.9% for DBD and DCDD, respectively). Except for 4 countries (United Arab Emirates, Slovenia, Latvia, and Nicaragua), the majority of deceased donors were invariably represented by men (range: from 40% to 100%). In all countries but 5 (United Arab Emirates, Slovenia, Latvia, Netherland, and Nicaragua), the percentage of female DBD never exceeded that of males (**Figure 2A**). Similarly, in all countries but 3 (Russian Federation, Ireland, and Czech Republic), the percentage of female DCDD never exceeded that of males (**Figure 2B**). Interestingly, in the case of deceased donors, an average of 2,67 organs could be retrieved from each donor.

Sex of Living Donors

Internationally, there were 39,090 living donors (33,116 LKD and 5,974 LLD) recorded in the period considered. Information about sex was available for 32996 living donors (84.4%), and women added up to 59.5% of these. As far as LKD, a therapeutic approach that takes place in 67 of the participating countries, information about sex was available for 27586 donors (83.3%). Women accounted for 61.1% of the LKD ranging from 0 (Ecuador) to 100% (Estonia and Cyprus) (**Figure 3A**, **Supplementary Table S2**). Except for Ecuador, Lithuania, Kuwait, Venezuela, Mongolia, Italy, Israel, Malta, Hungary, Costa Rica, Qatar, Argentina, Dominican Republic, Armenia and Latvia, in reporting countries women accounted for the majority of LKD.

Similarly, as far as LLD, a therapeutic approach available in 40 of the participating countries, information about sex was available for 5,410 donors (90.6%). Female donors accounted for 51.2% of the livers transplanted, ranging from 0 (Portugal, Moldova, Syria and Qatar) to 100% (Uruguay, Australia and Cuba) (**Figure 3B**, **Supplementary Table S2**) Except for Portugal, Moldova, Syria, Qatar, Chile, Mexico, Peru, Israel, Spain, Algeria, Turkey, Italy, France, UAE and UK, in reporting countries women accounted for the majority of living liver donors.

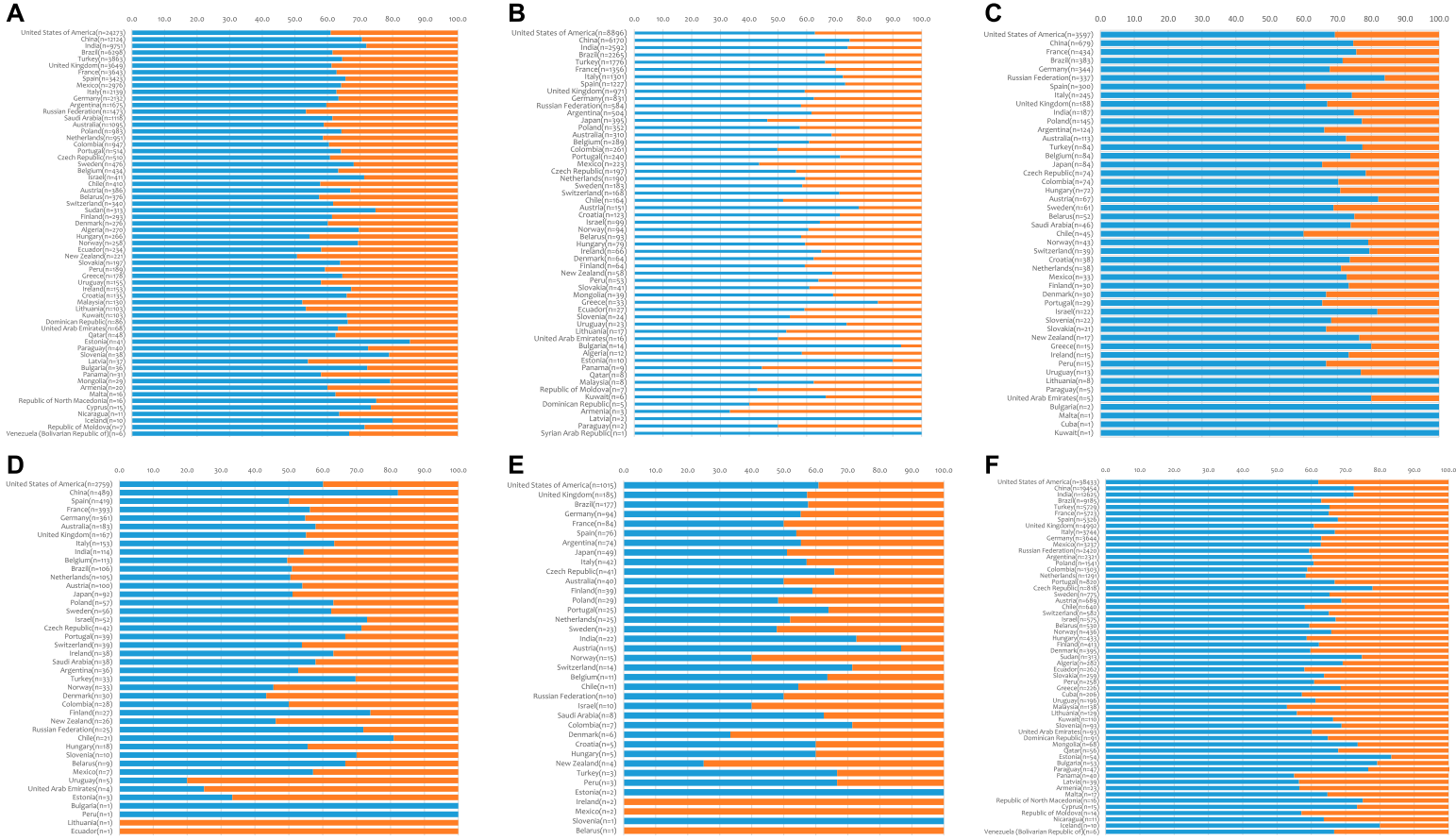


FIGURE 4 | Distribution of solid organ transplant recipients by sex. **(A)** kidney transplant recipients (62 countries); **(B)** liver transplant recipients (56 countries); **(C)** heart transplant recipients (47 countries); **(D)** lung transplant recipients (42 countries); **(E)** pancreas transplant recipients (37 countries); **(F)** all transplant recipients (57 countries).

Altogether, it is of interest that, in contrast to DD, for both kidney and liver the percentage of women amongst living donors exceeded that of men.

Sex of the Patients Transplanted

Finally, our studies have been extended to determine the sex of the recipients of the organs allocated in 2019 (N = 139,230) in the participating countries for which information about sex was available (N = 133,694, 96%), irrespective of the source of the organ implanted (deceased versus living donation) (**Figure 4**, **Supplementary Table S3**).

Men consistently received the vast majority of the organs transplanted in 2019 (65% of the total). In particular, men received 65% of the kidneys, 67% of the livers, 71% of the hearts, 60% of the lungs and 58% of the pancreases available. At a national level, men received the majority of the kidneys, livers, hearts, lungs and pancreases in 100%, 84%, 100%, 76%, and 68% of the surveyed countries, respectively.

DISCUSSION

Transplantation represents the ideal treatment option for patients with terminal organ failure. In the case of end-stage renal disease, transplantation is associated with improved quality of life and increased life expectancy compared to any other form of kidney replacement therapy (9–11). Likewise, transplantation represents the only form of treatment for terminal heart, lung, or liver failure. Unfortunately, due to the limited availability of organs, transplantation is precluded in many patients who could benefit from such a treatment (11). In this light, to avoid inequities it is fundamental that access to transplantation is carefully regulated and is the legitimate outcome of a fair and transparent process. Sex and gender differences in access to transplantation have been observed previously for kidney, liver and heart transplantation (4–8). However, it is yet to be established whether these differences are invariably the inevitable, fortuitous outcome of a legitimate process.

In an effort to shed some light on potential inequities in access to transplantation, we commenced by collecting and analysing data on donation and transplantation activity by organ donors' and recipients' sex in the CD-P-TO Member States, observer countries, and other States. As previously reported (12), our analysis of data collected prior to the COVID-19 pandemic in 69 countries and 6 continents, confirms that in most but not all countries, men are the prevalent source of both DBD and DCDD deceased donors. In this regard, it is of interest that, coherently, individuals who meet the biological criteria and who may eventually become deceased donors are more frequently men hospitalized in intensive care units as a consequence of severe and unrecoverable acute brain injury (due trauma or stroke) (13).

In contrast, our study clearly demonstrates that women are the leading source of kidneys recovered from living donors. In a context where donor voluntariness is an important determinant (14, 15), this observation may be explained by the more generous and altruistic nature of women in comparison to men (16–19). Yet, it is also important to recall that certain situational, group specific, or individual factors might reduce the degree of voluntariness. For example, as a consequence of their social role, women may perceive it as their maternal or spousal duty to become living donors and help their child or partner (20). Additionally, women may feel more pressured to donate and may be made to feel less autonomous because of societal and socioeconomic pressures (15) as men are often the prevailing source of family income (17, 21). Interestingly, a study involving men and women who served as living kidney donors, did not demonstrate differences in psychosocial profiles or greater vulnerability to family pressure between them (22). Future analyses should re-evaluate differences in living kidney donation among men and women as the contribution of women to family earnings increases. In the context of living liver donation, on the other hand, the number of organs provided by women only marginally exceeded the number of livers provided by men.

In all cases, the majority of the organs recovered are transplanted into men. Several reasons may account for such an observation that is valid collectively but also for each of the organs considered separately. First, certain diseases more frequently affect men resulting in a larger number of men being waitlisted for transplantation. For instance, chronic liver diseases are more frequently observed in men. Likewise, men more often develop kidney diseases (23) and, in most countries, men represent the larger proportion of patients on dialysis due to end-stage renal disease. Second, women are not infrequently penalized in accessing transplantation due to their immunological profile. In particular, women listed for a transplant may present greater immune sensitization (measured by pre-transplant panel reactive antibodies (PRA)) as a consequence of previous pregnancies (24). Third, women may not be selected for transplantation due to donor-recipient size mismatch (25). However, other gender related factors may also be at play. For example, the interplay between psychosocial and cultural pressures on women, and subtle differences in perception of women as transplant candidates, limit the full use of transplant treatment options for women (26). A recent North American study, for example, showed that, whilst in men only a BMI $\geq$40 kg/m^2 was associated with lower likelihood of transplantation from any donor source, in the case of women, BMI $\geq$25 kg/m^2 was associated with a lower access to transplantation from both deceased and living donors (27). In the case of paediatric candidates, in addition to physician attitudes, patient and caretaker motivation toward transplantation may also contribute to gender inequity in girls' access to pre-emptive transplants (28). In certain countries, limited education and health literacy (29) as well as socioeconomic dependence may affect some women. Future studies should shed light on patient, health care provider, and system-related factors that may contribute to reduced access to transplantation among women compared to men. Similarly, currently available data prevents us from verifying whether, at least in some countries, gender-related issues or socioeconomic variables may play a detrimental role, possibly reducing the access of women to the transplant waiting lists and, ultimately, to transplantation.

We would like to acknowledge several limitations of the current study. Information on sex was not available for all donors and recipients involved in the transplantation activity of the year

considered in all the participating countries. Furthermore, the data collection undertaken did not enable an analysis of the findings according to the four possible donor-recipient sex combinations (M-M; M-F; F-F; F-M). Additionally, we did not have access to additional pertinent donor and recipient variables, including age, socioeconomic status, the relationship between donor-recipient pairs, and national statistics on organ failure and waiting lists among men and women (e.g., cause for end-stage disease, waiting time, death whilst listed for transplantation). Because of the cross-sectional nature of this study, we cannot rule out that our observations on the sex of donors and recipients in organ transplantation may have differed in preceding years or may change further as a consequence of the ongoing covid-19 pandemic. Finally, while our findings preclude a thorough assessment of the processes underlying potential inequities in access to transplantation by patients' sex and gender, they represent an initial step in documenting the current state of affairs on their distribution among transplant donors and recipients at an international level.

In summary, this brief report is an initial step to document differences in donation and transplantation activity among men and women in the CD-P-TO Member States, observer countries and other States (69 countries in 6 continents). We are convinced that the collection of data allowing analyses disaggregated by sex represents an important step that may uncover unexpected imbalances, pave the way to more refined investigations on the subject and, where relevant, ultimately act as a trigger for the adaption of national policies. Accordingly, the CD-P-TO has decided to invest further resources into this research topic in the years to come. A follow up and more detailed questionnaire is expected to be submitted to the Health Authorities of the Council of Europe Member States in the second trimester of the year 2022.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: EC, ML-F, JF, MasC, and BD-G. Supervision: EC, ML-F, JF, MasC, and BD-G. Formal analysis: all authors. Methodology: EC, ML-F, JF, MasC, and BD-G. Validation: MA, MarC, and BM. Data Curation: MA, MarC, and BM. Writing—original draft preparation: EC, ML-F, JF, MasC, BD-G, and ML. Writing—review and editing: all authors.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10322/full#supplementary-material

Supplementary Figure S1 | Deceased donor rates per million population (PMP). Data from 69 participating countries (in brackets: absolute number).

Supplementary Table S1 | Distribution of deceased organ donors (DBD and DCDD) by sex. Data on donor sex was provided by 64 countries.

Supplementary Table S2 | Distribution of living kidney and liver donors by sex. Data on donor sex was provided by 55 and 32 countries for kidney and liver donors, respectively.

Supplementary Table S3 | Distribution of solid organ transplant recipients by sex. Data on recipient sex for kidney, liver, heart, lung, pancreas transplants was provided by 62, 56 countries, 47, 42 and 37 countries, respectively.

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LETTER TO THE EDITOR
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Use of Perfluorohexyloctane for Preservation of Rat Liver After Circulatory Death and a Prolonged Cold Preservation Model for Hepatocyte Transplantation

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Keywords: donation after circulatory death, preservation solution, perfluorohexyloctane, hepatocyte transplantation, prolonged cold preservation model

Dear Editors,

Liver transplantation has been established as the standard therapy for end-stage liver disease, and hepatocyte transplantation (HTx) has been gaining acceptance as an alternative for the treatment of patients with inherited metabolic liver diseases and acute hepatic failure. However, due to donor shortage, the main tissue sources for HTx are marginal-quality livers (i.e., livers donated after circulatory death, those exposed to prolonged cold storage, etc.). In the field of pancreatic islet transplantation, which is a cell therapy similar to HTx, pancreas oxygenation using perfluorohexyloctane (F6H8) has been shown to effectively prevent ischemically induced damage incurred during cold preservation [1–3]. F6H8 has a high lipophilic character compared to conventionally used perfluorodecaline, subsequently resulting in a high oxidizing capacity for the graft. Therefore, the present study assessed whether or not hepatocyte isolation using livers from donation after circulatory death and prolonged cold ischemic time could be improved using F6H8.

Male F344/NSLc rats were anesthetized and systemically heparinized, and then warm ischemia was induced by incising the diaphragm. The heart stopped beating within approximately 6 min of starting the procedure. The period of warm ischemic time was 15 min, and then the portal vein was cannulated. University of Wisconsin (UW) solution was flushed *via* the portal vein. Grafts were assigned to 2 groups: those preserved in UW solution (UW group) or in the oxygenated F6H8 (F6H8 group) at 4°C for 72 h. Therefore, cold ischemic time in this experiment was 72 h. F6H8 was oxygenated with gaseous oxygen for 10 min before use. After preservation, in both groups, the hepatocytes were isolated and purified using a modified two-step collagenase perfusion technique as previously described [4]. Hepatocyte viability was evaluated by trypan blue exclusion (TBE) and the ADP/ATP ratio [5, 6]. Ten million hepatocytes were then directly injected into the portal vein of analbuminemic rats [7], and the serum albumin levels were quantified on days 0, 14, and 28. All animals were handled according to the Guide for the Care and Use of Laboratory Animals, and the guidelines for animal experiments at Tohoku University (protocol ID: 2014 NICHe-Animal-001).

The TBE viability of the UW group was significantly higher than that of the F6H8 group (76.50 ± 3.77% and 70.53 ± 5.59%, n = 8, p = 0.025). In terms of the ADP/ATP ratio, no significant difference was observed between groups (UW: 0.175 ± 0.057, F6H8: 0.149 ± 0.046,

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TABLE 1 | The results of Cell yield, TBE viability, ADP/ATP ratio, serum albumin levels on days 0, on days 14 and on days 28 in UW group and F6H8 group.

	Cell Yield (10^8 cells)	TBE viability (%)	ADP/ATP Ratio	Serum albumin levels on days 0 (μg/ml)	Serum albumin levels on days 14 (μg/ml)	Serum albumin levels on days 28 (μg/ml)
UW group	4.90 ± 1.10	76.50 ± 3.77	0.175 ± 0.057	6.13 ± 1.24	6.33 ± 2.90	6.16 ± 1.31
F6H8 group	4.49 ± 0.77	70.53 ± 5.59	0.149 ± 0.046	5.56 ± 1.33	10.34 ± 10.06	5.64 ± 0.69

TBE, trypan blue exclusion; UW, University of Wisconsin, F6H8 = perfluorohexyloctane.

$n = 8$, $p = 0.329$). In terms of the serum albumin levels, no significant difference was observed between the UW (day 0: 6.13 ± 1.24 μg/ml, day 14: 6.33 ± 2.90 μg/ml, day 28: 6.16 ± 1.31 μg/ml, $n = 10$) and F6H8 (day 0: 5.56 ± 1.33 μg/ml, day 14: 10.34 ± 10.06 μg/ml, day 28: 5.64 ± 0.69 μg/ml, $n = 10$) ($p = 0.19$) groups (**Table 1**). The TBE and ADP/ATP ratio were analyzed by Student's t-test, and the serum albumin levels were analyzed by a two-way analysis of variance.

Unexpectedly, the strategy for preventing ischemically induced damage in marginal graft model using oxygenated F6H8 compared to conventional storage of UW does not seem to improve the outcomes of HTx. We found TBE viability of the UW group was significantly higher than that of the F6H8 group. But in terms of the ADP/ATP ratio and serum albumin levels, we could not find any statistically significant differences between both groups, suggesting that only a limited increase of necrotic (but not apoptotic) hepatocytes might be observed in the F6H8 group for unknown reasons, but this difference was too small to be reflected in the transplant outcomes. In this experiment, the warm ischemic time and cold ischemic time were longer than those in real clinical settings. We performed a preliminary experiment with a shorter cold ischemic time, but no significant differences were observed.

In conclusion, UW solution, in comparison to the F6H8, may have better cytoprotective effect on preserving liver tissues from marginal-quality donors. However, this effect was not enough to facilitate the engraftment of hepatocytes. More robust approaches to improve the quality of liver grafts are needed for HTx using marginal-quality donors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Guide for the Care and Use of Laboratory Animals, and the guidelines for animal experiments at Tohoku University (protocol ID: 2014 NICHe-Animal-001).

AUTHOR CONTRIBUTIONS

MM participated in the research design, the performance of the research and the writing of the paper. TI, HO, and SM participated in the performance of the research. AI participated in the performance of the research and the writing of the paper. KO, MU, and TK participated in the writing of the paper. MG participated in the research design, the performance of the research and the writing of the paper.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LETTER TO THE EDITOR
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A Payer's Perspective: A Comparison and Simulation of the Costs of Hemodialysis Versus Living Donor Kidney Transplant for Patients With End-Stage Renal Disease in Nigeria

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Keywords: dialysis, transplantation, policies, cost, access to transplantation, cost-comparison

Dear Editors,

Chronic kidney disease (CKD) carries substantial disease and economic burden in Nigeria. The prevalence of CKD in Nigeria has been estimated to range from 11.4% to 26% (1). Due to factors such as limited education on the early asymptomatic stages of CKD, poor screening practices, and limited nephrology care, many cases of CKD result in progression to end-stage renal disease (ESRD) (2). Living Donor Kidney Transplant (LDKT) is considered the gold standard treatment for patients with ESRD or stage five chronic kidney disease (CKD) (3). LDKT offers ESRD and CKD patients a significantly better quality of life and life expectancy than those receiving hemodialysis (HD) or other renal replacement therapies (4, 5). The feasibility of LDKT compared to other forms of RRT may differ on a country-by-country basis due to factors such as availability/accessibility of transplantation and HD centers, health insurance coverage for ESRD and CKD care, robustness of the donor organ procurement network, and government support (6).

In 2016, there were approximately 80 hemodialysis centers and five transplant centers in Nigeria (7). The outlook for patients with CKD and ESRD is poor, as there is no national insurance or health aid scheme (e.g., Medicare in the United States) to financially support these patients, leaving the financial burden of RTT fully upon patients and their families (8). Furthermore, there is no structured organ procurement and donation network in place in Nigeria, meaning that most kidney donors in Nigeria are usually genetically or emotionally related to the donor (6).

To date, data regarding the cost of LDKT and HD in Nigeria are limited. Through our experience with the Clarion Call transplant program across Western Nigeria since 2014, we aimed to address this gap. The aim of this study was to primarily quantify, compare, and conduct a simulation of costs of LDKT and HD in Nigeria from a payer's perspective and secondarily inform future cost-effectiveness studies to guide health care decision-making of kidney disease management in the country.

Our analysis takes on the perspective of the ESKD patient payer in Nigeria. Data from the Clarion Call Transplant Program in Nigeria from 53 patients who underwent hemodialysis at centers across Nigeria from July 2014 to June 2020 and 20 patients who received a LDKT between June 2017 and May 2020 was used in this study. Cost estimates were determined through direct supplier pricing and patient utilization data, and confirmed by expert consultation *via* two transplant nephrologists, two transplant surgeons, and one transplant coordinator in Nigeria. All costs were reported in both 2020 USD and 2020 Nigerian Naira

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TABLE 1 | Costs of model inputs for hemodialysis, living donor kidney transplant, and acute rejection.

Treatment modality	Input	Description	Yearly cost (₦)	Yearly cost (USD)
Hemodialysis	Dialysis treatment (three sessions per week)	30,000 N per session	₦ 4,680,000	$12,168.00
	Dialysis treatment (two sessions per week)	30,000 N per session	₦ 3,120,000	$8,112.00
	Labs (pre dialysis)	Once per session	₦ 800,800	$2,082.08
	Labs (post dialysis)	Once per session	₦ 572,000	$1,487.20
	CVC placement	One time	₦ 150,000	$390.00
	AV fistula creation	One time	₦ 120,000	$312.00
	Epogen injection	Per protocol	₦ 1,560,000	$4,056.00
	Iron injection	Per protocol	₦ 312,000	$811.20
	Vitamin D	Per protocol	₦ 48,000	$124.80
	Phosphorus binders	Per protocol	₦ 80,000	$208.00
	B-Complex vitamin and folic acid	Per protocol	₦ 10,400	$27.04
	Nephrologist consultation	Per protocol	₦ 300,000	$780.00
	Nutritionist consultation	Once per year	₦ 250,000	$650.00
	First year total (three sessions per week)		₦ 8,883,200	$23,096.32
	Recurring costs (three sessions per week)		₦ 8,363,200	$22,394.32
	3 Year total (three sessions per week)		₦ 26,109,600	$67,884.96
	First year total (two sessions per week)		₦ 7,323,200	$19,040.32
	Recurring costs (two sessions per week)		₦ 7,053,200	$18,338.32
	3 Year total (two sessions per week)		₦ 21,429,600	$55,716.96
Living donor kidney transplant	Work up	One time	₦ 1,500,000	$3,900.00
	Transplant surgery	One time	₦ 10,000,000	$26,000.00
	Follow-up labs	One time	₦ 528,000	$1,372.80
	Tacrolimus (1 mg ×100 caps)	45,000 per unit	₦ 1,296,000	$3,369.60
	Cellcept (500 mg ×100 caps)	50,000 per unit	₦ 720,000	$1,872.00
	Prednisone (5 mg ×100 tabs)	10,000 per unit	₦ 36,500	$94.90
	Anti-viral	Valcyte	₦ 180,000	$468.00
	Anti-fungal	Fluconazole	₦ 36,500	$94.90
	Anti-bacterial	Bactrim	₦ 38,000	$98.80
	First year total		₦ 14,335,000	$37,271.00
	Recurring costs		₦ 2,580,500	$6,709.30
	3 Year total		₦ 19,496,000	$50,689.60
Acute rejection	Kidney biopsy	Per protocol	₦ 200,000	$520.00
	Anti-thymocyte globulin	Per protocol (cell-mediated)	₦ 250,000	$650.00
	IV immunoglobulin	Per protocol (antibody-mediated)	₦ 250,000	$650.00
	Plasmapheresis	Per protocol (antibody-mediated)	₦ 480,000	$1,248.00
	Total (cell-mediated)		₦ 450,000	$1,170.00
	Total (antibody-mediated)		₦ 930,000	$2,418.00

Note: 3 year totals are not discounted.

using the conversion rate in 2020. One year and yearly recurring costs of LDKT and HD were calculated to compare RRT modalities. HD costs were projected for both three sessions per week and two sessions per week to simulate a more feasible alternative, although three sessions per week is the standard of care in most resource-rich countries.

Costs and model inputs are shown in **Table 1**. We estimated one-year costs of US $23,096.32 for HD (three sessions per week) and $37,271.00 for LDKT. Yearly recurring costs were $22,394.32 for HD and $6,709.30 for LDKT. Costs of acute rejection for LDKT were $2,418.00 for antibody-mediated rejection and $1,170.00 for cell-mediated rejection. One-time costs dominated the one-year cost of LDKT at 82.0%, while alternatively 97.0% of one-year costs of HD were recurring costs. A discounted simulation (6% discount rate, as is recommended in resource-limited countries) of three-year costs when survival was assumed yielded a cost-savings for LDKT in comparison to HD of US $12,421.23 for three sessions per week, and $1,655.86 for two sessions per week.

Costs of LDKT in Nigeria are higher than that of HD in the first year but are markedly decreased in subsequent years. The cost of HD and LDKT is primarily an out-of-pocket expense paid by patients with kidney failure in Nigeria. The maintenance cost of HD is three times more than the maintenance cost of immunosuppression post kidney transplantation. Our data demonstrates favorable long-term cost profile of LDKT vs. HD in Nigeria when cost is borne directly by patients. These potential cost-savings are in line with cost comparisons of the two RRT modalities in many settings globally and demonstrate the benefit of LDKT from a cost perspective.

This study considers no treatment as a non-viable option going forward. One study of a Nigeria HD center found median duration of treatment for those receiving HD in Nigeria to be as low as 1 week, with only 30% with continued dialysis after 3 months. Median survival for those on HD is abysmal, with

median survival for females as low as 5 weeks and males 20 weeks (8). A larger, 6 years study of 1,167 patients from five HD centers across Northwestern Nigeria found rates of sustained dialysis past 90 days to be as low as 15.1% at one center, with only 41.7% of patients receiving more than three sessions in total, and only one patient referred for kidney transplant over the period from 2011 to 2017 (9). This study is limited by lack of data regarding access to RRT modalities or survival rates of either HD or LDKT, as well as the lack of data regarding complications including hospital visits, rejection rates, etc., and merits further study.

We found a favorable long-term cost of LDKT versus HD in patients with ESRD at our transplant program in Nigeria. Despite high up-front costs of LDKT, maintenance costs were demonstrated lower than that of HD. Cost data from this study can be used for further study of the comparative cost-effectiveness of these RRT modalities using survival data and outcomes to assess cost-effectiveness and help inform local policymakers with aim of increasing access to LDKT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: JL, CL, IJ, DR-A, CO, and OE; Methodology: JL and OE; Software: JL; Validation: JL, CL, IJ, DR-A, CO, and OE; Formal Analysis: JL and OE; Investigation: JL, CL, IJ, DR-A, CO, and OE; Resources: JL, CL, IJ, DR-A, CO, and OE; Data curation: JL, CL, IJ, DR-A, CO, and OE; Writing-original draft: JL, CL, and OE; Writing—reviewing and editing: JL, CL, IJ, DR-A, CO, and OE; Visualization: JL and OE; Supervision: OE; Project administration: JL, CL, IJ, DR-A, CO, and OE.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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