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## Breathing new life into sensitized patients

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Transplant International

# Breathing new life into sensitized patients



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POINT OF VIEW
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# Promoting and Supporting Positive Conversations and Knowledge Mobilisation About Organ Donation in NHS Staff: a Hashtag "#" Series of Projects

*Natalie L. Clark[1]*, Dorothy Coe[2], Hannah Gillespie[2], Marcus Diamond[3], Michael O'Malley[3], David Reaich[1] and Caroline Wroe[2]*

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Implementation of the "soft" opt-out legislation in England has not had the desired impact in increasing the number of deceased donations and consent. The need for organs continues to be greater than the number of organs available, consent rates have fallen and organ donor registrations have stagnated. Introducing the legislation during the pandemic has had a profound effect with public awareness campaigns withheld, leaving a significant proportion of the population unaware of the change. Strategies to increase the public's awareness and understanding of organ donation and the opt-out legislation are needed, as well as to encourage decision-making and sharing this with their families. We outline several "#" projects (#conversations, #options, #speak) with NHS staff to demonstrate how we can successfully utilise this specific population as trusted individuals and advocates to promote positive communications about organ donation and the opt-out legislation. NHS England is one of the biggest employers and most ethnically diverse across Europe. We know that NHS staff are more supportive, more aware and are more likely to have made an organ donation decision and had conversations with their families than the public. This places them in a unique and valuable position to lead positive conversations about organ donation.

**Keywords: organ donation, opt-out legislation, national health service (NHS), United Kingdom, England**

Point of View

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## INTRODUCTION

Within the United Kingdom (UK), the number actively waiting for a transplant exceeds the number of organs available for transplantation, resulting in patients being removed from the list due to deteriorating health or from dying while waiting. In May 2020, the organ donation legislation in England changed from opt-in to "soft" opt-out [1]. This means consent to be an organ donor is

**Abbreviations:** BLS, Basic Life Support; ICU, Intensive Care Unit; IRODT, International Registry in Organ Donation and Transplantation; NHS, National Health Service; NHSBT, National Health Service Blood and Transplant; ODR, organ donor register; PMP, per million population; SNOD, Specialist Nurses-Organ Donation; UK, United Kingdom.

assumed unless an individual explicitly registers their decision on the organ donor register (ODR) that they opt-out or they meet exclusion criteria whereby assumed consent cannot apply. With the "soft" opt-out system, a family member will still be consulted and can override the donor's wishes in comparison to a "hard" opt-out system whereby a donor's decision is the primary factor [2]. By June 2023, this legislation had been implemented across the UK, with Wales first implementing this in December 2015 [1].

The change was introduced with the intention to improve the number of available deceased donors, as has proven successful in countries like Spain [3, 4]. However, this change in legislation coincided with the COVID-19 pandemic which impacted adversely on organ donation internationally and overshadowed the planned public awareness campaigns, with the "Pass it on" slogan removed [5]. The impact of implementing the opt-out legislation within the UK is likely to have been overestimated whilst additional factors such as economic implications, the role of family members, public health initiatives, engagement with stakeholders, and training of healthcare professionals, have been underestimated [6–8]. Subsequently, a significant number of the population are unaware of the change and vulnerable to misinformation.

By 2023, consent rates across England, Scotland and Wales failed to recover beyond pre-pandemic levels, from 68.3%, 63.0% and 63.6% in April-June 2019 to 63.2%, 60.5% and 56.3% in April-June 2023, respectively [3]. The latest statistics from National Health Service Blood and Transplant (NHSBT) showed those registering an opt-in decision on the ODR stagnated from 27.7million registered in 2022 to 28.4million in 2025 [9]. The list of patients actively waiting for an organ transplant had started to decrease up until the COVID-19 pandemic, however the 2024/25 NHSBT activity report states there are 8,096 patients waiting [9], this figure being the highest seen in 15-year where approximately 8,000 were on the waiting list in 2009/10 [10].

## The Spanish Model of Organ Donation

Spain is recognised as the world leader for organ donation and transplantation, operating an opt-out legislation since 1979 [11]. It is estimated to have been approximately 10 years before the effects were seen [12]. However, the legislative change in isolation did not contribute to the country's success and normalisation of organ donation within their society. The Spanish Model of Organ Donation consists of three components, a solid legislative framework, strong clinical leadership and a highly organised logistics framework [11, 13–15]. Factors constituting the model include simpler consent processes, access to more intensive care unit (ICU) beds, better resources, ensuring organ donation is embedded within the overall healthcare system, providing legal protection for organ donors, and greater education and training for healthcare professionals. Spain's Model should be viewed as an international exemplar for deceased donation and could be replicated in other countries, like the UK, to improve deceased donation rates alongside the legislative changes.

To understand the trends of deceased donation in the UK from 2018 to 2023 (inclusive), we used open access data from the International Registry in Organ Donation and Transplantation (IRODT) [16] and compared the rates of deceased donation against four countries of a similar population size (Spain, Germany, France, Italy) (**Figure 1**). Pre-pandemic (2018–2019), deceased donation per million population (PMP) rates were increasing across the UK, Spain and France. In 2020, UK, Spain, France and Italy all experienced a decline in deceased donation PMP rates, though they began to recover the following year.

However, by 2023 and compared to 2019, the UK (24.88 vs. 22.35 PMP) and France (33.25 vs. 27.63 PMP) had not returned to pre-pandemic deceased donation PMP rates, respectively. Germany deceased donation PMP rates stayed consistent throughout 2018–2023 at around 11 PMP. Whereas, both Spain and Italy had improved beyond their pre-pandemic rates. Italy similarly implemented an eight-step organ donation and transplantation programme [12, 17], heavily influenced by the Spanish Model, evidencing successful replicability. Recent figures from 2024 [16] suggest deceased donation PMP rates in the UK have fallen (20.37) whilst Spain remains the highest globally.

The "soft" opt-out legislation in England means an organ donor's family are still consulted, as is the case for Spain. The NHSBT 2024/25 statistics highlight that the family are more likely to be supportive when a donor has expressed a decision to opt-in, with consent/authorisation at 87% versus when a decision was unknown at 48% [9]. Ethnicity is a known influencing factor on consent rates, with much lower family consent/authorisation rates (33%) from ethnic minority groups [1, 9]. In Spain, the number of refusals by family has progressively declined, one explanation being improved communication amongst families regarding sharing individual wishes in conjunction with an overall positive attitude towards donation within society [11, 18]. A priority within the UK is to encourage conversations with families and sharing organ donation decisions which can then increase consent to organ donation [11, 18, 19].

## The Unique and Valuable Position of NHS Staff

The National Health Service (NHS) is one of the biggest employers and most ethnically diverse across Europe, with over 1.5 million employees in NHS England as of January 2025 [20, 21]. Our objective is to demonstrate how we can successfully utilise this specific group of individuals to become organ donation advocates and lead positive conversations and foster constructive dialogue about organ donation, extending into our communities. We define a positive conversation about organ donation as one that is supportive, engages empathetic listening, and is respectful of one's wishes [22].

In 2017, we conducted a pilot survey over 5-week in partnership with "ExtraLife" (**Table 1**). Our survey aimed to explore the views about and barriers to organ donation in the local workforce, including NHS staff based within two Trusts (one acute medical, one mental health). The questions were derived from the NHSBT Optimisa Survey of the General Population, 2013 [19]. We found NHS staff to be largely supportive of organ donation (96%) with the majority willing to donate their organs after death (90%). Approximately 74% of respondents were already registered to the ODR, 71% had shared their decision with someone else and 97% understood the

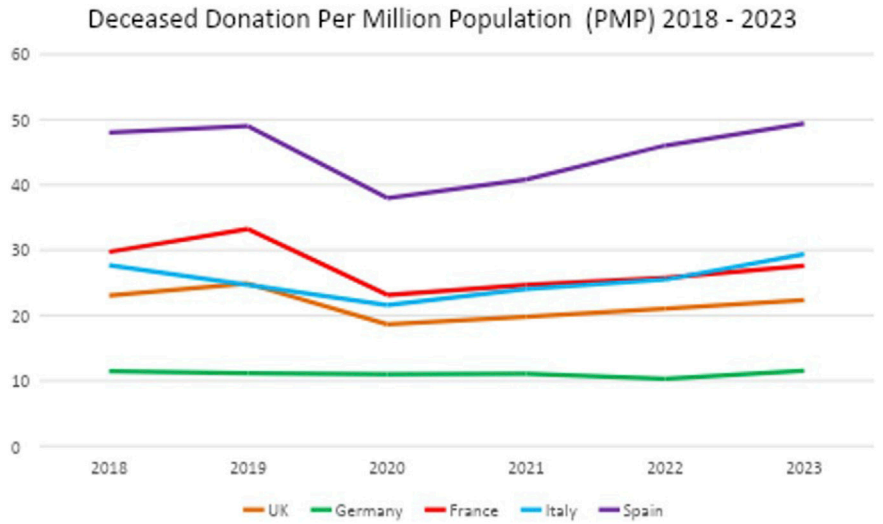


**FIGURE 1 |** Deceased donation per million population (PMP) from 2018 to 2023 in the UK, Germany, France, Italy and Spain.

importance of making their family members aware of their decision (**Table 2**; **Supplementary Material S1**)

Following this, we conducted a larger survey between July-December 2020 called #options [23], with approximately 6,000 NHS staff across NHS organisations (primary care, secondary care, mental health, ambulance, community services) in the North-East and North Cumbria and North Thames (**Table 1**). We aimed to explore NHS staff views of organ donation and the opt-out legislation, including awareness, support and action taken around the ODR and conversations with family and friends. We compared our findings to those of the public, evidencing NHS staff were much more likely to be aware and supportive of the legislative change. They were also three times more likely to have registered a decision on the ODR, whilst 75% of NHS staff had reported already having had conversations with their family, compared to 12% of the public. More importantly, we highlighted greater awareness and support in ethnic minority groups within NHS staff, though this group were more likely to request more information to improve their knowledge of organ donation and the opt-out legislation [24].

Through both surveys, we have established higher levels of support for and awareness of organ donation amongst NHS staff, irrespective of whether they are directly/indirectly involved in organ donation and transplantation. Notably, the #options survey [23] was the largest survey of NHS staff to evaluate awareness and support for organ donation, including staff working across a variety of healthcare settings.

Due to the size of the NHS workforce, it is likely most families have a member, or know someone, who works for the NHS [13]. This places those individuals in a unique and valuable position as a trusted individual and advocate, to lead positive conversations about organ donation. These conversations have the scope to extend beyond healthcare settings into wider communities. Interventions, specifically for NHS staff, aiming to improve communication about organ donation are needed to support the sharing of accurate information (e.g., processes, reasons behind the change, family involvement [24]) and challenge any myths and misconceptions.

## Educational Resources for NHS Staff

We know from the #options survey that NHS staff, in clinical and non-clinical roles and those without direct involvement in organ donation, have expressed a desire for more information about organ donation and the opt-out legislation [24].

Educational interventions have previously proved effective in improving organ donation knowledge amongst healthcare professionals [25]. These interventions also aimed to increase organ donor numbers, improve identification of potential organ donors, improve referral processes, increase education, and provide extra support to families. However, the interventions were mostly delivered to and tailored for ICU staff or Specialist Nurses in Organ Donation (SNODs) who, due to the nature of their roles, we would already anticipate being organ donation advocates. Providing training and educating beyond these roles is of paramount importance to promote a collaborative approach, improve communication between healthcare professionals, patients and their family, and provide adequate support to families. This will further encourage all staff within an NHS setting to become more knowledgeable and aware.

In May 2024, we conducted the #speak project using two focus groups with 14 NHS staff from the North-East and North Cumbria, to explore gaps in educational resources for the wider NHS staff workforce, what they felt was missing and how this should be delivered (**Tables 1**, **3**). There were several suggestions regarding the content that should be included. Examples being, providing clarity around the new assumed consent, overcoming barriers, clarifying eligibility criteria and the family involvement. As the target audience for these educational resources would be NHS staff, the focus group felt they could include more facts and statistics compared to if this was aimed at the public.

**TABLE 1 |** Summary of the ExtraLife survey and the three # projects (#conversations, #options, #speak).

| Project | Target population | Objectives | Results | Impact |
|---|---|---|---|---|
| ExtraLife Survey<br>*2017*<br>Funded by the local organisations involved | Employees (n = 549) from Teesside working in the NHS (one mental health Trust and one acute medical Trust), and the local College, Football Club, Council and University | To explore views about and uncover barriers to organ donation in the local Teesside workforce<br>To use the findings to develop workplace-based education to support organ donation | NHS staff were<br>• The most supportive of organ donation (96%)<br>• Most likely to be registered on the Organ Donor Register (ODR) (74%)<br>• Most likely to agree on the importance of making their family aware of their decision (97%)<br>However<br>• 30% did not know how to put their name on the ODR<br>• 25% did not have enough information to decide | NHS staff were identified as a large potential advocacy resource for organ donation<br>Results were shared with transplant patient group and reviewed by Graphics Design students and lecturers at Teesside University to develop novel educational tools to support organ donation conversations (the #conversations short film) |
| #conversations short film<br>*January to February 2019*<br>Funded by ExtraLife, Principle Sounds and Northern Counties Kidney Research Fund | NHS staff working in one Acute Medical Trust in Teesside (n = 338) attending mandatory Basic Life Support (BLS) training | To create a short educational film that can be delivered to NHS staff during BLS training to promote positive conversations about organ donation<br>To evaluate the effectiveness of the #conversations short film as an educational tool for NHS staff | Before watching the short film<br>• 99% supported organ donation<br>• 72% discussed organ donation with friends/family<br>After watching the short film<br>• Understanding of the need for organ donation (95%) and impact on family (94%) increased<br>• 43% had talked to their colleagues about organ donation<br>• 51% had talked to their family about organ donation<br>• 42% were more supportive of organ donation | The #conversations short film promoted impactful and thought-provoking conversations about organ donation and was effectively delivered within BLS training with minimal additional resources |
| #options survey<br>*July to December 2020*<br>Funded by the Northern Counties Kidney Research Fund | NHS staff working in primary and secondary care, mental health, ambulance and community services in the North East and North Cumbria (n = 4986), and North Thames(n = 803) | To investigate the levels of awareness, support and action taken towards the new organ donation legislation in England, in NHS staff<br>To understand what influences opinions (e.g., demographics, geographical location)<br>To use the findings to support the development of educational resources around organ donation and the change in legislation for NHS staff | NHS staff were<br>• More aware than the public (68% vs. 60%)<br>• More supportive (83%)<br>• 6x more likely to have had a conversations about organ donation<br>• 3x more likely to already be on the ODR.<br>The survey demonstrated higher levels of awareness and support across ethnic minority groups<br>NHS staff also requested more information about<br>• The process of organ donation<br>• How relatives are informed<br>• How to opt-out on behalf of loved ones<br>• Storage of data and decisions<br>• Understanding the rationale behind the legislative change<br>• What has been communicated to the public and patients | Notably, NHS staff, compared to the general public, have taken more positive action in response to the change in legislation and were more likely to have had conversations with their family and friends<br>There was a desire from NHS staff to find out more information<br>Based on the findings, there is a unique opportunity to support NHS staff to be advocates and ambassadors for organ donation and the change in legislation |
| #speak focus groups<br>*May 2024*<br>Funded by the Northern Counties Kidney Research Fund | Participants of the #options survey who consented and provided their contact details to take part in future focus group work and attended focus group sessions (n = 14) | To conduct focus group discussions with NHS staff to review two educational resources, #conversations short film and an NHSBT resource<br>To determine any educational gaps | NHS staff liked it when the videos<br>• Had accessible language<br>• Used real people to make it personal<br>• Graphics were not too overwhelming | The focus groups helped to understand how the #conversations short film can be adapted, upscaled and implemented more widely across other NHS Trusts delivering BLS training or in other similar settings |

(Continued on following page)

**TABLE 1 |** (*Continued*) Summary of the ExtraLife survey and the three # projects (#conversations, #options, #speak).

| Project | Target population | Objectives | Results | Impact |
|---|---|---|---|---|
| | | | NHS staff felt the videos<br>• Missed emphasis improving quality of life<br>• Should ensure statements were phrased appropriately<br>• Should include how you can opt-in<br>NHS staff felt a new resource could<br>• Include a tangible message<br>• Be a part of annual event<br>• Include about how to overcome barriers<br>• Empower families | A greater understanding of the specific information NHS staff (clinical and non-clinical) want to know about organ donation, following on from the #options survey<br>Using these findings, we can develop an educational resource to further support the wider NHS workforce to be more knowledgeable about organ donation, and support conversations with their family, friends, colleagues and the wider communities |

**TABLE 2 |** Pilot survey findings of NHS staff based within one acute medical and one mental health NHS Trust in the North-East and North Cumbria to understand current attitudes and barriers towards organ donation.

| Question | Yes (%) | No (%) | Unsure (%) |
|---|---|---|---|
| Do you support the principle of organ donation? | 343 (96) | 3 (1) | 10 (3) |
| Would you be willing to consider giving your organs after death? | 320 (90) | 11 (3) | 25 (7) |
| Have you put your name on the NHS ODR? | 260 (74) | 78 (22) | 14 (4) |
| Have you told anyone that you have put your name on the NHS ODR? | 246 (71) | 69 (20) | 30 (9) |
| | **Very/quite important** | **Neither important/ unimportant** | **Quite/very unimportant** |
| How important do you think it is to tell those closest to you of your wishes about donating your organs after death? | 344 (98) | 4 (1) | 2 (1) |

**TABLE 3 |** Focus group summary of gaps in educational resources for the wider NHS staff workforce, what they felt was missing and how this should be delivered.

| Theme | Recommendations |
|---|---|
| Format | • Technological poverty, provide information in other ways<br>• Range of different delivery methods because it's not a one size fits all<br>• Use different approaches for different target audiences<br>• More general education<br>• If you're tailoring it towards NHS staff you can be a bit sort of more numbery and statistics<br>• Trying to be positive in what can be quite an awful time<br>• Any big existing myths exist that would make someone not want to donate - a Q&A document "busting" these myths<br>• It's about managing expectations |
| Delivery | • Avoid making this another mandatory e-Learning course<br>• Mandatory training can be seen as a tick box exercise and we definitely don't want this to be that<br>• Screensavers on the Intranet<br>• As a member of the NHS staff you are in a unique position of understanding that the work that we do impacts the work you do now but it could also impact the future and showing the importance the uniqueness and the value of that position to encourage people to engage with it which I think is a totally different offer than a mandatory training |
| Process (including consent, eligibility etc.) | • Personally confused around presumed consent then we're still getting consent<br>• What happens in terms of getting the body back for funeral arrangements<br>• Process is what actually happens to somebody's body<br>• Who owns your body, that dark side of is it the state or do you own your body up until that point of your last breath<br>• Making sure that everybody is aware of what that eligibility criteria is and not just the basics |
| Family | • Does your family know your views, whether it's opt-in or opt-out<br>• Empowering families to ask if organ donation is possible for your family member - I know my family members wishes<br>• Knowing your bit won't be affected knowing you can still be with your loved one<br>• Nothing being disruptive in the most disruptive time |

**TABLE 4 |** Focus groups summary of the short film (#conversations) and suggestions for improvement.

| | #conversations |
|---|---|
| Positives | • Real people would make it more personal<br>• Great hearing real life stories<br>• Addition of the mum at the end was a really nice touch<br>• Led me on a journey to why it was a good idea and seeing some good results<br>• Appealed to my compassionate side<br>• Lends to your emotional side<br>• Definitely hits the spot, it's very emotive, that's what's needed<br>• Having the lady talking through her story with her son and the picture of her son was really impactful<br>• The son being so young it was saying that everybody could have the conversation it's not just for an older audience<br>• What kind of impact that has and how everyday things that you do take for granted are special |
| Suggested improvements and recommendations | • Music slightly too loud<br>• I felt like it was too much, I couldn't focus<br>• Having everything all in one go is a bit too much for me<br>• I felt quite immediately sort of moved then I don't know whether that makes it a bit harder to tune into the rest of it your arousal level is quite high<br>• Need to be left with a tangible message – those are the conversations that people will lastingly have with their family<br>• If you think more people can do it or everybody can do it there's less personal onus on you to do, you're in a very unique and privileged position<br>• Ongoing thing rather than a thing that's done and then that's it<br>• World awareness day - awareness stand, pens and posters, advertisements, people can go to the stand, walking past and having a look, having it annually pops up and you can create a buzz around it |

## A Short Film to Promote Conversations

Basic Life Support (BLS) training is mandated in the UK by the Resuscitation Council that all NHS clinical staff must undergo BLS training, with annual refresher sessions to maintain knowledge and skills [26]. Non-clinical staff within the NHS are expected to have the same BLS skillset as a lay person.

In a previous project (#conversations) [27], we explored how a short film could be embedded within in-person BLS training at one NHS Trust, using existing resources, and delivered to staff to promote conversations about organ donation (**Table 1**). We developed the film in collaboration with families from the North-East and North Cumbria who had direct experience of organ donation, placing emphasis on the personal narrative, and the ease and normality of having conversations around organ donation in everyday life [28–32]. Sharing personal stories of organ donation have proven to significantly impact registrations in the UK [1]. A bespoke soundtrack was developed by Principle Sounds©, supporting the message through emotional engagement [33]. The film was reviewed by families, a staff focus group and BLS trainers for feedback.

Over a 6-week period, the film was delivered to 228 NHS staff. We collected a baseline questionnaire of opinions towards organ donation [19] and followed-up NHS staff 5 days after attending BLS training and watching the film to assess impact.

Like our previously reported #options survey findings [23], the NHS staff demonstrated high levels of support for organ donation (99%) and were more likely to have shared their views towards organ donation with their family or friends (72%). Comparative data from NHSBT's attitudinal survey in June 2020 showed only 49% of the public have had a conversation about organ donation, with even fewer saying they have shared their decision. Those completing the follow-up questionnaire felt that the film increased their understanding of the need for organ donation (95%) and the impact it has on families (94%). A further 43% of staff had talked to their colleagues and 51% had talked to their family about organ donation. Support for organ donation did not decrease after watching the film, with 42% even more supportive. Despite the widespread support at the time of the study, 25% were not comfortable with the proposed legislative change, 16% were unsure and 9% were unsupportive.

During the recent #speak project we utilised the focus groups to gather feedback about the #conversations film. With consent, we recorded group discussions, anonymised the recordings and analysed the data. We aimed to generate suggestions for improvement (**Table 4**). NHS staff particularly liked that the film led them "*on a journey*" to "*seeing some good results*" using real-life people and "*hearing real life stories*" to "*make it more personal*" and "*really impactful*". Suggestions of improvements included, amending the volume of the music as they felt it was "*slightly too loud*" and at times, the way in which the graphics was used was sometimes perceived as being "*too much*" and this made it difficult to focus on the voiceover. Though #conversations was intended to be emotionally thought-provoking, some felt this made it a "*bit harder to tune into the rest of it because your arousal level is quite high*". However, it was reiterated, "*that's what's needed*".

NHS staff summarised that any new film, it should finish with a "*tangible message*", and it is this message "*that people will lastingly have with their family*". They also suggested that the content should aim to empower families, doing so will put them in position to "*ask if organ donation is possible for [their] family member*" as they will "*know [their] family members wishes*". Ultimately, they agreed that "*there's never a good time to talk about it so the conversations that we have earlier on the better*". One NHS staff member suggested that this needed to be "*a regular ongoing thing*", rather than something that is done once and then forgotten but not overdone "*to the point where people get desensitised*", such as delivering any resources around significant awareness events (e.g., Organ Donation Week).

## SUMMARY

Implementation of the opt-out legislation in England has not had the desired impact on increasing the number of potential eligible donors, consent rates and deceased donation. Introducing the legislation during the COVID-19 pandemic has undoubtedly had a profound effect and it is difficult to disentangle the true response to the change from the lasting effects of the pandemic. However, it is apparent that an opt-out legislation in isolation will not be effective in increasing the number of the opt-in registrations and consent. Instead, the UK would benefit from supporting an organ donation and transplantation programme that is embedded within society to improve public trust and confidence in deceased organ donation systems [3], as we have successfully seen in Spain and Italy [11–14, 17]. Currently, the public's awareness and understanding of organ donation and the opt-out legislation is low, as is the number of individuals who have made a decision and shared this with their families. Increasing public awareness, understanding and support would be paramount within the programme.

We have outlined several projects with NHS staff from a variety of NHS organisations, with the film (#conversations) being delivered to and focus group (#speak) work specifically being with NHS staff from the North-East and North Cumbria. These findings are promising, demonstrating how we could work with this specific population to promote positive communications about organ donation and the opt-out legislation. There is a recognised need to involve NHS staff outside of those with direct involvement in organ donation and transplantation to maximise opportunities for deceased donation [34]. To effectively do so, we need to ensure that the wider NHS workforce is appropriately educated using tailored resources which will help them initiate conversations and provide support.

We must develop effective strategies to empower individuals to make their decision in life and share this with their families to reduce the number of family refusals. Doing so will begin to improve organ donation rates, and both save and improve the quality of lives of individuals actively waiting for a transplant. This could be done through revising and relaunching public health campaigns as some evidence suggests [34].

To further demonstrate the effectiveness of such resources, a consistent and systematic approach is needed, including standardising implementation across settings, clear outcome measures and appropriate data collection methods to evaluate impact reliably. We propose updating the film first used in #conversations by incorporating the feedback from the #speak focus groups and literature post-legislative change. The aim being to disseminate as part of a public health campaign that can be evaluated within a regional pilot study and funded by a partnership between the Department of Health and Social Care, NHSBT and local Integrated Care Boards. This will be evaluated within a regional pilot study. To evaluate impact, we will gather pre-/post-test regional data from NHSBT on ODR registrations, deceased donation consent rates, and transplant activity, further evaluating how impact varies across demographics (e.g., age, ethnicity) and geographical location, these factors being known to influence organ donation consent rates [9].

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable request to the corresponding author.

## ETHICS STATEMENT

Ethical approval was obtained for the short film #conversations (IRAS Project ID: 233611) and the #options survey with NHS staff in the North-East and North Cumbria and North Thames (IRAS Project ID: 275992). Further ethical review was not required for #speak focus groups as these included participants from #options who had provided consent to be contacted. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

## AUTHOR CONTRIBUTIONS

NC, DC, HG, and CW contributed to the concept and design of the study, the acquisition of data, the analysis and interpretation of the data and drafted the article. MD and MO'M contributed to the concept and design of the study, created and edited the film and contributed to the final approval. DR contributed to the concept and design of the study, the analysis and interpretation of the data and to the final approval. All authors contributed to the article and approved the submitted version.

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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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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15131/full#supplementary-material

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# Long Term Outcomes of Lung Transplantation in Sensitized Patients Following Eculizumab Use With the Desensitization Protocol

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Lung transplantation remains a life-saving option for end-stage pulmonary diseases, but sensitized patients with anti HLA antibodies carry high risk; recent desensitization advances, such as eculizumab, may permit outcomes comparable to non-sensitized recipients with tailored perioperative care. In this prospective cohort study of 399 adult lung transplant recipients, 36 sensitized patients underwent a protocol combining preoperative plasmapheresis, a defined eculizumab regimen, anti-thymocyte globulin, and IVIG. In comparison, 363 non-sensitized recipients received standard immunosuppression. We compared recipient/donor characteristics, intraoperative parameters, and postoperative outcomes, including primary graft dysfunction, infection, rejection, and overall survival. Desensitized patients were older, predominantly female, and had significantly higher panel reactive antibody levels and preformed donor-specific antibodies; intraoperatively, they required more blood transfusions and VA-ECMO support. Postoperatively, they exhibited higher rates of *de novo* donor-specific antibodies, antibody-mediated rejection, longer ICU stays, increased dialysis requirement, and more frequent CMV infections. Despite these differences, rates of acute cellular rejection, chronic lung allograft dysfunction, and one-year and overall survival were similar between groups. Our findings suggest that lung transplantation in sensitized patients managed with a desensitization protocol, including eculizumab, is feasible and safe, achieving outcomes comparable to those of non-sensitized recipients.

**Keywords: lung transplantation, sensitized, eculizumab, ACR, AMR**

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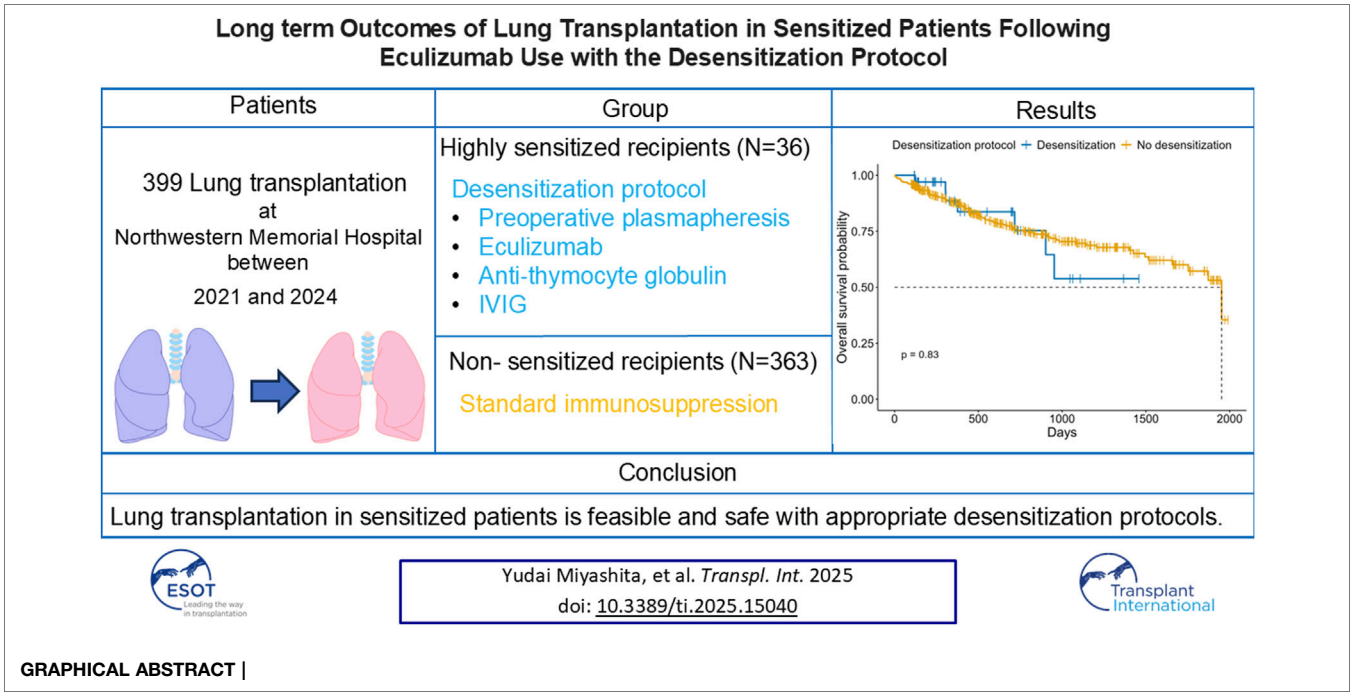


**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Lung transplantation is a life-saving procedure for patients with end-stage pulmonary diseases, offering improved survival and quality of life [1]. However, some transplant candidates are sensitized, harboring elevated levels of pre-formed anti-HLA antibodies. Historically, these patients have been considered at higher risk for complications such as hyperacute rejection and severe infections, rendering them less favorable candidates for transplantation [2, 3]. Recent advances in immunology and desensitization protocols have prompted a re-evaluation of lung transplantation in sensitized patients [4, 5]. While traditional approaches have often excluded these patients from transplant candidacy, emerging evidence suggests that perioperative desensitization strategies—such as repeated plasmapheresis, administration of high-dose IVIG, and targeted immunomodulatory agents—can mitigate the risks associated with pre-formed donor-specific antibodies (DSA) [6, 7]. Despite the theoretical risk of higher rates of rejection and infection, these interventions hold the potential to enable safe transplantation in a group previously deemed ineligible. Nevertheless, data on the clinical outcomes of lung transplantation in sensitized recipients remain limited, particularly regarding long-term survival and complication rates. While study have reported comparable survival and CLAD-free survival between sensitized and non-sensitized recipients following desensitization protocols, detailed evaluations of complications in this context are still lacking [8]. Several prior single-center experiences have demonstrated the feasibility of perioperative desensitization in lung-transplant cohorts. In 2015, Tinckam et al. described 340 first-time transplants—including 53 DSA-positive patients—managed with perioperative plasma exchange (PLEX), IVIG, antithymocyte globulin (ATG), and mycophenolate, reporting similar one-year graft survival and freedom from acute rejection compared with unsensitized controls [9]. Aversa et al. subsequently evaluated 74 virtual-crossmatch-positive/flow-crossmatch-positive recipients treated with PLEX, IVIG, and ATG and found 5-year allograft and CLAD-free survival equivalent to VXM-negative patients [10]. Parquin et al. implemented a virtual-crossmatch–based protocol in 39 high-DSA candidates at Foch Hospital—using PLEX, rituximab, and IVIG—and demonstrated comparable 3-year graft survival and CLAD-free survival versus non-sensitized recipients [11]. More recently, Heise et al. reported a 9-year, single-center experience in 62 sensitized recipients treated with IgA- and IgM-enriched IVIG (IgGAM), PLEX, and a single dose of rituximab, achieving 73% DSA clearance and long-term outcomes analogous to those of unsensitized patients [12]. Together, these studies showed the diverse of perioperative regimens—incorporating PLEX, IVIG (or IgGAM), ATG (or basiliximab), and rituximab,—can safely expand transplant access for sensitized candidates without compromising medium-term outcomes. Despite these encouraging results, Marfo et al. indicates an increased incidence of infections and episodes of antibody-mediated rejection (AMR) [13]. Still, overall survival may remain comparable to that of non-sensitized recipients if rigorous surveillance and specialized immunosuppressive regimens are in place. Recent evidence has highlighted the potential of preventative treatment with eculizumab, a terminal complement inhibitor, in mitigating the risk of AMR in sensitized patients undergoing heart and kidney

transplantation [14, 15]. Building on this, we previously demonstrated the feasibility of performing successful multiorgan transplantation in sensitized patients with positive crossmatch donors by implementing a perioperative desensitization protocol incorporating eculizumab [16]. This approach not only mitigated the heightened immunological risk but also highlighted the importance of tailored strategies in expanding transplant opportunities for this challenging patient population while maintaining acceptable long-term outcomes.

In this study, we evaluated our institution's experience with sensitized patients who underwent lung transplantation following a desensitization protocol with Eculizumab. We compared perioperative and postoperative outcomes—including rates of primary graft dysfunction (PGD), infection, rejection, and survival—between sensitized patients receiving desensitization therapy and non-sensitized patients. We aimed to determine whether lung transplantation can be performed safely and effectively in the sensitized population without compromising overall postoperative outcomes.

# PATIENTS AND METHODS

## Study Design

This is a cohort study of adult patients who underwent lung transplantation at a single institution between September 2021 and August 2024. Patient data were collected prospectively using electronic medical records. The study was approved by the Institutional Review Board of Northwestern University (STU00207250, STU00213616, and STU00217958). The need for patient consent for data collection was waived by the institutional review board due to the retrospective nature of this study. Recipient and donor characteristics, preoperative laboratory values, and intra- and postoperative outcomes were compared in lung transplant patients.

## Peri- and Post-Operative Protocol for Sensitized Patients

The protocol has been previously reported by our group [16]. Specifically, sensitized patients with PRA above 40 (details in supplemental methods about HLA testing) received plasmapheresis 4–6 h prior to lung transplant. Both sensitized and non-sensitized patients received steroid and simlect as induction therapy at the time of lung transplant. Sensitized patients received total 5 sessions of plasma exchange (Pre-operative, POD0, 1, 2, and 3), eculizumab (Pre-operative: 1200mg, POD 0: 900 mg, POD 1: 600 mg, 2: 600 mg, 3: 1200 mg), anti-thymocyte globulin (POD5-, 1 mg/kg/day, total cumulative dose 4–8 mg/kg), IVIG 300 mg/kg if plasma IgG <500 (**Supplementary Table S1**; **Supplementary Figure S1**).

## Statistical Analysis

Continuous data are shown as median (Interquartile Range; IQR), and discrete data are shown as number (%). Recipient and donor characteristics, preoperative laboratory values, and intra- and postoperative outcomes were compared between lung transplant patients. The Mann-Whitney U test was used to compare independent continuous variables between the groups. Fisher's exact test was used to compare categorical variables, which were reported as numbers and percentages. The Kaplan-Meier method was used to estimate survival, and the log-rank test was performed to compare survival between the groups. Hazard ratio (HR) was obtained using a univariate and multivariate cox proportional hazard analysis and odds ratio was obtained using a univariate and multivariate logistic regression analysis. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the JMP Pro 17.0.0 software program (SAS Institute Inc.).

# RESULT

## Patient Characteristics

399 lung transplant recipients were analyzed, comprising 36 patients who underwent desensitization protocols and 363 who did not (**Table 1**). The median age of the desensitization group was significantly higher than the non-desensitization group [63.0 years (56.0–68.0) vs. 53.5 years (48.3–67.8), $p = 0.023$]. The proportion of female recipients was significantly greater in the desensitization group (86.1% vs. 38.3%, $p < 0.0001$). Patients in the desensitization group had significantly higher Panel Reactive Antibody (PRA) levels for both Class I and Class II [Class I: 44.0% (23.3–87.0) vs. 0.0%, $p < 0.0001$; Class II: 10.5% (0.0–83.8) vs. 0.0%, $p < 0.0001$]. All patients in the desensitization group tested positive for PRA (100.0% vs. 32.8%, $p < 0.0001$), and a significantly higher proportion had positive T cell flow cytometry crossmatch (FC-XM) (63.9% vs. 0.0%, $p < 0.0001$), B cell FC-XM (72.2% vs. 0.0%, $p < 0.0001$), and both T and B cell FC-XM (61.1% vs. 0.0%, $p < 0.0001$). Preformed DSA were also markedly more frequent in the desensitization group (75.0% vs. 6.4%, $p < 0.0001$) (**Supplementary Table S2**). Regarding etiology, interstitial lung disease (ILD) was less prevalent in the desensitization group (19.4% vs. 39.9%), whereas COVID-19-related indications were more common (27.8% vs. 10.2%, $p = 0.0023$).

## Intraoperative and Postoperative Outcomes

Intraoperative outcomes are shown in **Table 2**. Patients undergoing desensitization required significantly more intraoperative blood transfusion, including packed red blood cells (pRBC) [0.0 units (0.0–4.5) vs. 0.0 units (0.0–2.0), $p = 0.0075$] and fresh frozen plasma (FFP) [0.0 units (0.0–3.8) vs. 0.0 units (0.0–0.0), $p = 0.01$]. Ischemic time was comparable [5.4 h (4.4–6.2) vs. 5.2 h (4.1–6.1), $p = 0.60$]. VA-ECMO was used significantly more often in the desensitization group (80.6% vs. 60.6%, $p = 0.019$).

Postoperative outcomes revealed that *de novo* DSA was significantly more frequent in the desensitization group (55.6% vs. 12.4%, $p < 0.0001$). PGD grade at 72 h post-transplantation showed a trend toward higher grades in the desensitization group, though this was not statistically significant ($p = 0.079$). Specifically, PGD grade 0 was less frequent in the desensitization group (27.8% vs. 46.0%), while grades 1 and

**TABLE 1 |** Characteristics of patients.

| Variable | Desensitization protocol (n = 36) | No Desensitization protocol (n = 363) | p value |
|---|---|---|---|
| Recipient factors | | | |
| Age, years | 63.0 (56.0–68.0) | 53.5 (48.3–67.8) | 0.023 |
| Female | 31 (86.1%) | 139 (38.3%) | <0.0001 |
| BMI, kg/m2* | 27.8 (24.3–29.8) | 26.4 (22.1–29.4) | 0.13 |
| BSA, m2* | 1.8 (1.6–19) | 1.9 (1.7–2.1) | 0.0079 |
| Smoking history | 13 (36.1%) | 185 (51.0%) | 0.12 |
| Hypertension | 18 (50.0%) | 204 (56.2%) | 0.49 |
| Diabetes | 10 (27.8%) | 112 (30.9%) | 0.85 |
| CKD | 1 (2.8%) | 32 (8.8%) | 0.34 |
| Bilateral | 26 (72.2%) | 222 (61.2%) | 0.21 |
| PRA | | | |
| Class I | 44.0 (23.3–87.0) | 0.0 (0.0–0.0) | <0.0001 |
| Class II | 10.5 (0.0–83.8) | 0.0 (0.0–0.0) | <0.0001 |
| any PRA | 36 (100.0%) | 119 (32.8%) | <0.0001 |
| Positive T cell FC-XM | 23 (63.9%) | 0 (0.0%) | <0.0001 |
| Positive B cell FC-XM | 26 (72.2%) | 0 (0.0%) | <0.0001 |
| Positive T and B cell FC-XM | 22 (61.1%) | 0 (0.0%) | <0.0001 |
| Any positive T and B cell FC-XM | 27 (75.0%) | 0 (0.0%) | <0.0001 |
| preformed DSA | 27 (75.0%) | 23 (6.4%) | <0.0001 |
| Etiology | | | 0.0023 |
| ILD | 7 (19.4%) | 145 (39.9%) | |
| COPD | 3 (8.3%) | 72 (19.8%) | |
| PAH | 3 (8.3%) | 22 (6.1%) | |
| COVID-19 | 10 (27.8%) | 37 (10.2%) | |
| other | 13 (36.1%) | 87 (24.0%) | |
| Laboratory | | | |
| Hemoglobin, g/dL* | 10.5 (8.8–13.1) | 11.9 (9.9–13.4) | 0.053 |
| WBC, 1,000/mm3* | 9.8 (7.8–12.6) | 8.7 (7.0–11.3) | 0.25 |
| Platelets, 1,000/mm3* | 256.0 (199.0–304.8) | 238.5 (189.0–302.8) | 0.57 |
| Sodium, mEq/L | 140.0 (138.0–141.8) | 139.0 (138.0–141.0) | 0.42 |
| BUN, mg/dL | 14.0 (12.0–17.8) | 16.0 (13.0–20.0) | 0.079 |
| Creatinine, mg/dL | 0.7 (0.6–0.8) | 0.8 (0.6–0.9) | 0.0045 |
| ALT, U/L* | 16.0 (12.0–21.0) | 17.0 (11.0–25.0) | 0.90 |
| AST, U/L* | 19.5 (16.3–34.8) | 21.0 (17.0–28.0) | 0.90 |
| Albumin, g/dL* | 4.1 (3.7–4.3) | 4.0 (3.6–4.3) | 0.16 |
| Total bilirubin, mg/dL | 0.4 (0.4–0.8) | 0.5 (0.3–0.7) | 0.84 |
| INR | 1.1 (1.0–1.1) | 1.0 (1.0–1.1) | 0.62 |
| Arterial blood gas | | | |
| pH | 7.4 (7.4–7.4) | 7.4 (7.3–7.4) | 0.39 |
| PaCO2 | 50.5 (40.5–58) | 48 (42.0–55.0) | 0.52 |
| PaO2 | 307.0 (193.3–375.5) | 281.0 (195.0–358.0) | 0.50 |
| Donor | | | |
| Age, years | 37.0 (31.3–46.8) | 33.0 (24.0–45.0) | 0.17 |
| Female | 16 (44.4%) | 111 (30.6%) | 0.094 |
| Cause of death | | | 0.29 |
| Anoxia | 19 (52.8%) | 146 (40.2%) | |
| Head trauma | 9 (25.0%) | 128 (35.3%) | |
| Stroke | 6 (16.7%) | 78 (21.5%) | |
| Other | 2 (5.6%) | 11 (3.0%) | |

*Continuous data are shown as median (interquartile range) and discrete data are shown as number (%). BMI, body mass index; BSA, body surface area; CKD, chronic kidney disease; PRA, panel reactive antibody; FC-XM, flow cytometry crossmatching; DSA, donor specific antibody; ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; PAH, pulmonary arterial hypertension; WBC, white blood cell; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio. *Unknown cases were excluded.*

2 were more common. AKI occurred at a similar rate in both groups (47.2% vs. 46.8%, p = 1.00); however, the need for dialysis was significantly higher in the desensitization group (30.6% vs. 13.5%, p = 0.012). Patients in the desensitization group had a longer median ICU stay [10.0 days (5.3–25.8) vs. 7.0 days (4.8–15.0), p = 0.050] and required longer post-transplant ventilator support [2.5 days (2.0–3.8) vs. 2.0 days (1.0–3.0), p = 0.049]. Hospital stay also tended to be longer in the desensitization group [20.0 days (13.0–44.0) vs. 17.0 days (12.0–31.0), p = 0.087], although this difference did not reach statistical significance. Despite these differences, the two groups' one-year survival rates were comparable (91.7% vs. 89.0%, p = 0.78).

**TABLE 2 |** Intraoperative and Postoperative outcomes.

| Variable | Desensitization protocol (n = 36) | No Desensitization protocol (n = 363) | p value |
|---|---|---|---|
| Intraoperative outcomes | | | |
| Operative time (hours) | 6.3 (4.8–7.5) | 5.7 (4.4–7.5) | 0.40 |
| Intra-op blood transfusion (unit) | | | |
| pRBC | 0.0 (0.0–4.5) | 0.0 (0.0–2.0) | 0.0075 |
| FFP | 0.0 (0.0–3.8) | 0.0 (0.0–0.0) | 0.01 |
| Plt | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 0.47 |
| Ischemic time (hours) | 5.4 (4.4–6.2) | 5.2 (4.1–6.1) | 0.60 |
| VA-ECMO use | 29 (80.6%) | 220 (60.6%) | 0.019 |
| VA-ECMO time (hours) | 2.7 (2.3–3.3) | 2.9 (0.4–3.5) | 0.71 |
| Postoperative outcomes | | | |
| *de novo* DSA | 20 (55.6%) | 45 (12.4%) | <0.0001 |
| PGD | | | |
| Any grade | 26 (72.2%) | 196 (54.0%) | 0.053 |
| Grade>=2 | 14 (38.9%) | 98 (27.0%) | 0.17 |
| Grade3 | 4 (11.1%) | 46 (12.7%) | 1.00 |
| AKI | 17 (47.2%) | 170 (46.8%) | 1.00 |
| Dialysis | 11 (30.6%) | 49 (13.5%) | 0.012 |
| CVA | 0 (0.0%) | 12 (3.3%) | 0.61 |
| Bowel ischemia | 1 (2.8%) | 5 (1.4%) | 0.44 |
| Digital ischemia | 1 (2.8%) | 5 (1.4%) | 0.44 |
| ICU stay (days) | 10.0 (5.3–25.8) | 7.0 (4.8–15.0) | 0.050 |
| Post transplant ventilator (days) | 2.5 (2.0–3.8) | 2.0 (1.0–3.0) | 0.049 |
| Hospital stay (days) | 20.0 (13.0–44.0) | 17.0 (12.0–31.0) | 0.087 |
| 1-year survival | 91.7% | 89.0% | 0.78 |
| Follow-up period (days) | 367.5 (228.5–729.8) | 567.0 (235.0–1077.0) | 0.061 |

*Continuous data are shown as median (interquartile range) and discrete data are shown as number (%). pRBC, packed red blood cells; FFP, fresh frozen plasma; Plt, platelets; VA ECMO, veno-arterial extracorporeal membrane oxygenation; DSA, donor specific antibody; PGD, primary graft dysfunction; AKI, acute kidney injury; CVA, cerebrovascular attack; ICU, intensive care unit.*

## Infection Outcomes

The infection outcome is shown in **Table 3** and **Figures 1A,B**. The overall incidence of infections was similar between the desensitization and non-desensitization groups (69.4% vs. 61.9%, p = 0.47). Respiratory infections occurred at similar rates between the two groups (50.0% vs. 52.2%, p = 0.86), as did recurrent respiratory infections (19.4% vs. 16.4%, p = 0.64). **Figure 1A** demonstrates that respiratory infection-free survival did not differ significantly between the two groups (p = 0.93). However, CMV infections were significantly more frequent in the desensitization group compared to the non-desensitization group (36.1% vs. 13.1%, p = 0.0009). Baseline donor/recipient CMV serostatus also differed between cohorts (p = 0.003): in the desensitization group, none were donor–recipient seronegative (−/−), ten (76.9%) were donor-negative/recipient-positive (−/+), none were donor-positive/recipient-negative (+/−), and three (23.1%) were donor–recipient seropositive (+/+), whereas in the non-desensitization group five (34.0%) were −/−, fourteen (29.8%) were −/+, eight (17.0%) were +/−, and nine (19.1%) were +/+. This difference is illustrated in **Figure 1B**, where CMV infection-free survival was significantly worse in the desensitization group (p < 0.0001). The desensitization group experienced a higher and earlier incidence of CMV infections following transplantation. Positive aspergillus galactomannan antigen tests tended to be less frequent in the desensitization group, though the difference did not reach statistical significance (8.3% vs. 21.9%, p = 0.055). Additionally, blood culture positivity rates for bacterial infections (2.8% vs. 6.9%, p = 0.49) and fungal infections (5.6% vs. 3.3%, p = 0.37) were comparable between the two groups.

## CMV Infection Incidence and Risk Within 1 Year

**Supplementary Table S2** shows that, over a uniform one-year follow-up, CMV infection occurred in 9 of 36 patients (25.0%) who received perioperative desensitization versus 33 of 363 (9.1%) who did not (p = 0.007). Within the desensitized cohort, none of the nine CMV-infected patients were donor-negative/recipient-negative or donor-negative/recipient-positive; three (33.3%) were donor-positive/recipient-negative and six (66.7%) were donor-positive/recipient-positive. By contrast, among the 33 infected patients in the non-desensitized cohort, five (15.2%) were −/−, ten (30.3%) were −/+, seven (21.2%) were +/−, and 11 (33.3%) were +/+ (p = 0.003). In logistic regression—including mismatch status, perioperative desensitization protocol, and their interaction—the mismatch effect was not significant (OR 0.69, 95% CI 0.29–1.47; p = 0.36), whereas desensitization independently increased CMV risk more than fourfold (OR 4.23, 95% CI 1.90–9.20; p < 0.001). The interaction term yielded an OR effectively 0.00 (95% CI not estimable; p = 0.98), indicating no synergistic effect between mismatch and desensitization on CMV incidence (**Supplementary Table S3**).

**TABLE 3 |** Infection and rejection outcomes.

| Variable | Desensitization protocol (n = 36) | No Desensitization protocol (n = 363) | p value |
|---|---|---|---|
| Any infection | 25 (69.4%) | 223 (61.9%) | 0.47 |
| Respiratory infection | 18 (50.0%) | 188 (52.2%) | 0.86 |
| Recurrence respiratory infection | 7 (19.4%) | 59 (16.4%) | 0.64 |
| CMV infection | 13 (36.1%) | 47 (13.1%) | 0.0009 |
| Donor/Recipient CMV status | | | 0.003 |
| −/− | - | 16 (34.0%) | |
| −/+ | 10 (76.9%) | 14 (29.8%) | |
| +/− | - | 8 (17.0%) | |
| +/+ | 3 (23.1%) | 9 (19.1%) | |
| Positive aspergillus galactomannan antigen | 3 (8.3%) | 79 (21.9%) | 0.055 |
| Blood culture positive | | | |
| bacterial | 1 (2.8%) | 25 (6.9%) | 0.49 |
| fungal | 2 (5.6%) | 12 (3.3%) | 0.37 |
| ACR | 6 (16.7%) | 95 (26.4%) | 0.23 |
| number of ACR episodes | 1.0 (1.0–2.0) | 1.0 (1.0–2.0) | 0.74 |
| AMR | 8 (22.2%) | 12 (3.3%) | 0.0001 |
| number of AMR episodes | 1.0 (1.0–2.5) | 1.0 (1.0–1.0) | 0.51 |
| CLAD | 5 (13.9%) | 50 (13.8%) | 1.00 |
| BOS | 2 (5.6%) | 39 (10.7%) | 0.56 |
| RAS/mixed | 3 (8.3%) | 11 (3.0%) | 0.12 |
| Endpoint | Group | Median Event-Free Days (95% CI) | Log-rank p |
| ACR-free survival | Desensitized | NA (NA–NA) | |
| | No Desensitization | NA (NA–NA) | 0.20 |
| AMR-free survival | Desensitized | NA (NA–NA) | |
| | No Desensitization | NA (NA–NA) | <0.0001 |
| Respiratory infection-free survival | Desensitized | 184 (91–NA) | |
| | No Desensitization | 274 (189–468) | 0.93 |
| CMV-free survival | Desensitized | NA (417–NA) | |
| | No Desensitization | NA (NA–NA) | <0.0001 |

*Data are shown as number (%). CMV, Cytomegalovirus. *over 3 infections per year lasting over 4 weeks. ACR, acute cellular rejection; AMR, Antibody-mediated rejection; CLAD, chronic lung allograft dysfunction;BOS, bronchiolitis obliterans syndrome; RAS, restrictive allograft syndrome. Unknown date were excluded. NA, indicates that fewer than 50% of patients in that group experienced the event during follow-up, so the median event-free time is not reached.*

## Rejection Outcomes

Rejection outcomes demonstrated no significant difference in the incidence of ACR between the desensitization and non-desensitization groups (16.7% vs. 26.4%, p = 0.23) (**Table 3**). The median number of ACR episodes was also similar between the groups [1.0 (1.0–2.0) vs. 1.0 (1.0–2.0), p = 0.74]. **Figure 2A** further illustrates ACR-free survival, showing no significant difference in survival rates between the two groups (p = 0.20). In contrast, AMR was significantly more frequent in the desensitization group compared to the non-desensitization group (22.2% vs. 3.3%, p = 0.0001). Although the median number of AMR episodes was comparable between the groups [1.0 (1.0–2.5) vs. 1.0 (1.0–1.0), p = 0.51], **Figure 2B** reveals a significantly worse AMR-free survival in the desensitization group (p < 0.0001). The desensitization group showed a higher and earlier incidence of AMR events following lung transplantation, no case of AMR persisted after corticosteroid pulse therapy and repeat TBLB confirmed histologic resolution. No difference was observed in CLAD between desensitization and non-desensitization groups.

## Predictors of PGD

**Table 4** shows the risk factors associated with PGD of grade 2 or higher. Univariate logistic regression analysis identified several variables significantly associated with PGD. Among recipient factors, higher body mass index (BMI) was associated with an increased risk of PGD [odds ratio (OR) 1.05, 95% confidence interval (CI) 1.00–1.11, p = 0.037]. Bilateral lung transplantation (OR 1.67, 95% CI 1.05–2.70, p = 0.032) and higher PRA levels (OR 1.71, 95% CI 1.10–5.67, p = 0.017) were also significant predictors. Regarding etiology, pulmonary arterial hypertension (PAH) (OR 2.53, 95% CI 1.10–5.76, p = 0.029) and COVID-19-related indications (OR 2.32, 95% CI 1.23–4.31, p = 0.0094) were associated with increased PGD risk. Laboratory results showed that lower hemoglobin levels (OR 0.91, 95% CI 0.84–0.99, p = 0.037) and albumin levels (OR 0.60, 95% CI 0.40–0.91, p = 0.016) were significant predictors. Additionally, arterial oxygen pressure (PaO2) (OR 1.00, p = 0.028) and intraoperative factors, including operative time (OR 1.20, 95% CI 1.07–1.33, p = 0.0010), pRBC transfusion (OR 1.07, 95% CI 1.03–1.15, p = 0.0012),FFP transfusion (OR 1.11, 95% CI 1.02–1.21, p = 0.014), and VA-ECMO use (OR 1.85, 95% CI 1.16–3.01, p = 0.0096), were significant.

In the multivariate analysis, higher BMI (OR 1.08, 95% CI 1.02–1.14, p = 0.0072) and PRA (OR 1.64, 95% CI 1.02–2.65, p = 0.042) remained significant independent predictors of PGD. Lower albumin levels were also independently associated with PGD (OR 0.61, 95% CI 0.38–0.98, p = 0.043).

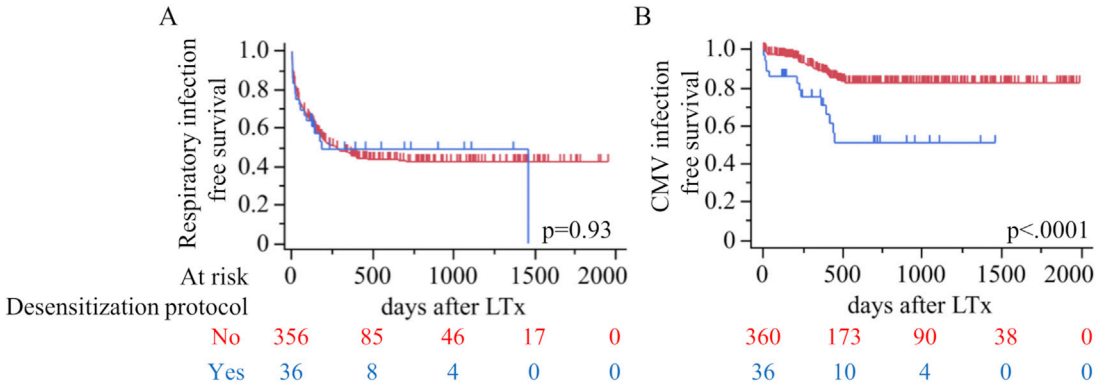


**FIGURE 1 |** Kaplan–Meier curves comparing outcomes after lung transplantation by use of a perioperative desensitization protocol (red, No; blue, Yes). **(A)** Respiratory infection–free survival (log-rank p=0.93). **(B)** Cytomegalovirus (CMV) infection–free survival (p<0.0001). LTx, lung transplantation.

## Predictors of CLAD

**Table 5** presents both univariate and multivariate Cox proportional-hazards analyses for CLAD. In the univariate models, only two variables emerged as significant predictors: each 1 kg/m$^2$ increase in BMI conferred an 8% higher hazard of CLAD (HR 1.08, 95% CI 1.02–1.15; p = 0.01), and each 1 g/dL rise in pre-transplant hemoglobin was associated with a 14% increase in risk (HR 1.14, 95% CI 1.02–1.27; p = 0.02). In contrast, variables such as PGD ≥2, CMV infection, and acute antibody-mediated rejection showed no significant univariate associations. In the fully adjusted multivariate model—including desensitization protocol, PGD ≥2, CMV, AMR, and recipient age—none of these factors remained independently significant. The desensitization protocol itself carried an adjusted hazard ratio of 1.75 (95% CI 0.67–4.57; p = 0.26), indicating that, after controlling for established risk factors, desensitization did not independently influence CLAD development.

## Overall Survival

**Figure 3** illustrates overall survival following lung transplantation for patients in the desensitization and non-desensitization groups. The Kaplan-Meier analysis revealed no significant difference in overall survival between the two groups (p = 0.83). The mean follow-up period for the cohort was 688.2 days (the sensitized group; 509.3 days, the desensitization group; 706.0 days). Additionally, **Table 6** presents a univariate and multivariate cox proportional hazard analysis identifying predictors of overall survival. Significant findings from the multivariate analysis included bilateral lung transplantation (HR 0.44, 95% CI 0.28–0.69, p = 0.0004) and serum albumin levels (HR 0.14, 95% CI 0.030–0.64, p = 0.012), which were independently associated with improved survival. Postoperative outcomes such as PGD grade ≥2 (HR 1.76, 95% CI 1.11–2.80, p = 0.017), AKI (HR 1.82, 95% CI 1.15–2.88, p = 0.011), cerebrovascular accidents (CVA) (OR 3.48, 95% CI 1.33–9.09, p = 0.011), bowel ischemia (HR 3.04, 95% CI 1.06–8.71, p = 0.039), and digital ischemia (HR 6.48, 95% CI 2.45–17.11, p = 0.0002) were significant risk factors for reduced survival.

# DISCUSSION

In this study, we examined the outcomes of sensitized patients who underwent lung transplantation following the desensitization protocol with eculizumab, comparing their perioperative and postoperative courses to those of non-sensitized patients. Several key findings emerged [1]: despite receiving intensified immunosuppressive therapy, the desensitization group had comparable one-year and overall survival rates [2]; the incidence of infections was broadly similar between the two groups, except a significantly higher rate of CMV infection in the desensitization group [3], ACR and CLAD rates did not differ significantly, yet AMR was more frequent in the desensitization group, and [4] although the desensitization group experienced longer ICU stays and required more intraoperative transfusions, their one-year graft and patient survival remained comparable to non-sensitized controls. These findings suggest that lung transplantation can be performed safely in sensitized recipients when the desensitization protocol with eculizumab is implemented alongside meticulous postoperative monitoring. One of the critical observations of this study is the increased incidence of AMR in the desensitization group. The presence of pre-formed DSAs and the resultant immunological milieu likely account for this higher incidence. Despite the heightened risk of AMR, rigorous triple immunosuppressive management (Tacrolimus, prednisone, and mycophenolate) and close clinical monitoring contributed to controlling these episodes and preventing detrimental effects on graft function and patient survival. This underscores that while desensitization can enable transplantation in sensitized patients, it necessitates vigilant post-transplant follow-up to detect and treat rejection promptly.

Our results demonstrate that lung transplantation in sensitized patients with desensitized protocol including eculizumab is feasible and can yield survival rates comparable to those of non-sensitized patients. Historically, the presence of pre-formed DSAs has been a major concern, as it predisposes recipients to early graft failure or hyperacute rejection [17, 18]. However, advances in immunosuppression, plasmapheresis, and

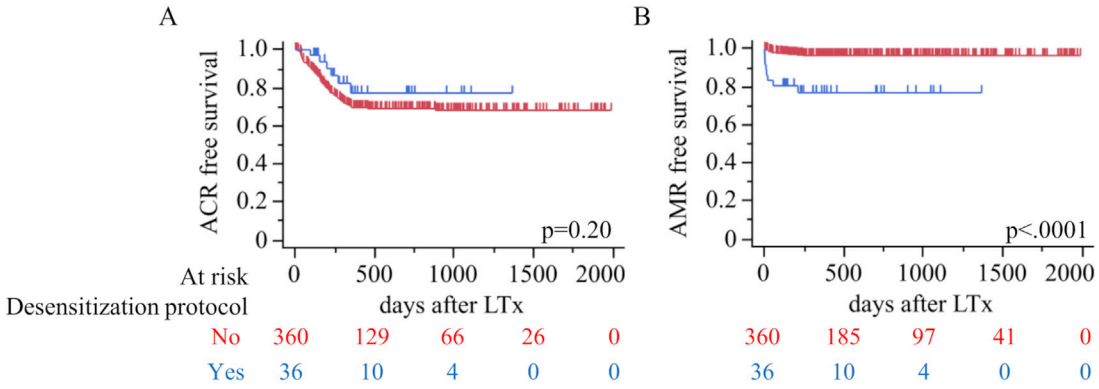


**FIGURE 2 |** Rejection outcomes by desensitization status. Kaplan–Meier estimates of **(A)** ACR-free survival and **(B)** AMR-free survival after LTx (red, No; blue, Yes). Log-rank p-values are shown on each panel; tick marks indicate censoring. Numbers at risk are provided below the x-axis.

targeted biological therapies have paved the way for more aggressive desensitization protocols [19]. Our data align with emerging evidence from other centers, which likewise suggests that, while sensitized patients carry an elevated risk profile, this risk does not necessarily translate into inferior overall survival if managed appropriately [20–22]. The finding that one-year and overall survival did not differ significantly between the two groups is particularly noteworthy. This indicates that the immunologic risks that are traditionally associated with high sensitization status may be mitigated through our specialized perioperative and postoperative regimens including eculizumab. Such findings are significant for transplant centers that may otherwise exclude sensitized patients from lung transplant candidacy, offering a viable approach to expand access to transplantation for this challenging population. Importantly, our desensitization regimen is unique in two respects. First, we employ perioperative complement inhibition with eculizumab (C5 blockade), a strategy pioneered in kidney transplantation to prevent antibody-mediated injury but not previously reported in large lung transplant cohorts. Second, we combine interleukin-2 receptor blockade (basiliximab) and polyclonal T-cell depletion (rabbit ATG) during induction—agents that are normally used as alternatives but here are used synergistically to blunt both cellular and humoral alloimmunity. While these intensifications carry a theoretical increased risk of opportunistic infections and cytopenias, our data show that CMV and other infection rates remain manageable (see **Table 3**; **Supplementary Table S3**), and no cases of refractory AMR were observed. Taken together, the marked reduction in early AMR and the preservation of one-year and overall survival suggest that the benefits of this two-pronged, complement-targeted approach outweigh the risks in this high-risk, sensitized population. One caveat of our approach is that therapeutic plasma exchange (PLEX) can remove circulating eculizumab, since the monoclonal antibody is itself an IgG. In our protocol we therefore administer eculizumab immediately after each PLEX session to partially offset this loss, but studies in other fields estimate that a single PLEX can clear 40%–60% of infused antibody. As a result, trough complement activity may transiently rebound between exchange and dosing. Although we did not measure $CH_{50}$ or free eculizumab levels in this series, future work should incorporate pharmacodynamic monitoring to optimize the timing and dosing of eculizumab around PLEX and ensure continuous complement blockade.

One of the more concerning complications in sensitized patients is the potential for AMR, which could lead to CLAD development. Our study revealed that AMR occurred significantly more frequently in the desensitization group (22.2% vs. 3.3%, p = 0.0001), consistent with pre-existing DSAs that can drive humoral immune responses against graft. Given these patients' substantial immunologic burden, it is not entirely surprising that AMR rates were elevated even though perioperative desensitized protocol. However, despite the higher frequency of AMR events, these episodes were manageable with augmented immunosuppression and close clinical follow-up, preventing a negative impact on CLAD rate and overall survival. In contrast, ACR rates did not differ significantly between the two groups. This implies that the cellular immunologic pathways underlying ACR may be effectively controlled by standard immunosuppressive regimens, which typically include calcineurin inhibitors and anti-proliferative agents alongside steroids. The heightened concern for AMR in this subset reinforces the need for close surveillance of DSA titers and incorporating protocolized biopsies to ensure timely detection and intervention.

Given the potent immunosuppressive therapies employed, an essential aspect of managing sensitized patients is balancing the risk of rejection against the risk of infection [5]. In our cohort, the overall incidence of infections, excluding cytomegalovirus, was not significantly different between the desensitization and non-desensitization groups. Respiratory infections, including bacterial pneumonia and recurrent infections, were similarly frequent, suggesting that the standard infection prophylaxis regimens are effective in both populations. However, CMV infections were notably more common in the desensitization group. As shown in **Supplementary Table S3**, multivariable logistic regression demonstrated that the desensitization protocol

**TABLE 4 |** Univariate and multivariate cox proportional hazard analysis as a predictor of PGD.

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95% CI | p value | Hazard Ratio | 95% CI | p value |
| Recipient factors | | | | | | |
| Age, years | 0.98 | 0.97–1.00 | 0.051 | | | |
| Female | 1.31 | 0.84–2.03 | 0.24 | | | |
| BMI, kg/m2* | 1.05 | 1.00–1.11 | 0.037 | 1.08 | 1.02–1.14 | 0.0072 |
| BSA, m2* | 1.89 | 0.78–4.63 | 0.16 | | | |
| Smoking history | 1.07 | 0.69–1.66 | 0.75 | | | |
| Hypertension | 1.15 | 0.74–1.79 | 0.55 | | | |
| Diabetes | 0.93 | 0.57–1.49 | 0.76 | | | |
| CKD | 1.13 | 0.50–2.39 | 0.77 | | | |
| Bilateral | 1.67 | 1.05–2.70 | 0.032 | 1.09 | 0.55–2.19 | 0.80 |
| PRA | 1.71 | 1.10–5.67 | 0.017 | 1.64 | 1.02–2.65 | 0.042 |
| preformed DSA | 1.86 | 0.99–3.41 | 0.053 | | | |
| Desensitization protocol | 1.72 | 0.83–3.46 | 0.14 | | | |
| Etiology | | | | | | |
| ILD | 0.78 | 0.49–1.23 | 0.28 | | | |
| COPD | 0.53 | 0.27–0.97 | 0.038 | 0.82 | 0.41–1.64 | 0.57 |
| PAH | 2.53 | 1.10–5.76 | 0.029 | 2.39 | 0.98–5.84 | 0.056 |
| COVID-19 | 2.32 | 1.23–4.31 | 0.0094 | 1.20 | 0.54–2.69 | 0.66 |
| Laboratory | | | | | | |
| Hemoglobin, g/dL* | 0.91 | 0.84–0.99 | 0.037 | 1.03 | 0.92–1.17 | 0.60 |
| WBC, 1,000/mm3* | 1.03 | 0.97–1.09 | 0.34 | | | |
| Platelets, 1,000/mm3* | 1.00 | 1.00–1.00 | 0.86 | | | |
| Sodium, mEq/L | 1.03 | 0.97–1.10 | 0.32 | | | |
| BUN, mg/dL | 1.01 | 0.98–1.03 | 0.43 | | | |
| Creatinine, mg/dL | 1.39 | 0.59–3.24 | 0.45 | | | |
| ALT, U/L* | 1.00 | 0.99–1.01 | 0.93 | | | |
| AST, U/L* | 1.00 | 0.99–1.02 | 0.46 | | | |
| Albumin, g/dL* | 0.60 | 0.40–0.91 | 0.016 | 0.61 | 0.38–0.98 | 0.043 |
| Total bilirubin, mg/dL | 1.20 | 0.81–1.73 | 0.34 | | | |
| INR | 2.43 | 0.81–8.04 | 0.12 | | | |
| Arterial blood gas | | | | | | |
| pH | 2.63 | 0.11–65.75 | 0.55 | | | |
| PaCO2 | 1.01 | 0.99–1.02 | 0.48 | | | |
| PaO2 | 1.00 | 1.00–1.00 | 0.028 | 1.00 | 1.00–1.00 | 0.74 |
| Donor | | | | | | |
| Age, years | 1.02 | 1.00–1.04 | 0.052 | | | |
| Female | 1.21 | 0.76–1.91 | 0.43 | | | |
| Intraoperative outcome | | | | | | |
| Operative time (hours) | 1.20 | 1.07–1.33 | 0.0010 | 1.03 | 0.86–1.23 | 0.75 |
| Intra-op blood transfusion (unit) | | | | | | |
| pRBC | 1.07 | 1.03–1.15 | 0.0012 | 1.08 | 0.94–1.25 | 0.25 |
| FFP | 1.11 | 1.02–1.21 | 0.014 | 0.96 | 0.80–1.15 | 0.69 |
| Plt | 1.17 | 1.00–1.37 | 0.050 | | | |
| Ischemic time (hours) | 1.04 | 0.98–1.11 | 0.19 | | | |
| VA-ECMO use | 1.85 | 1.16–3.01 | 0.0096 | 1.27 | 0.68–2.37 | 0.46 |

*BMI, body mass index; BSA, body surface area; CKD, chronic kidney disease; PRA, panel reactive antibody; DSA, donor specific antibody; ILD; interstitial lung disease; COPD, chronic obstructive pulmonary disease; PAH, pulmonary arterial hypertension; WBC, white blood cell; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; pRBC, packed red blood cells; FFP, fresh frozen plasma; Plt, platelets; VA ECMO, veno-arterial extracorporeal membrane oxygenation. *Unknown cases were excluded.*

independently increased CMV infection risk more than fourfold (OR 4.23, 95% CI 1.90–9.20; $p < 0.001$), whereas serologic mismatch itself was not a significant predictor (OR 0.69, 95% CI 0.29–1.47; $p = 0.36$). The interaction term between mismatch and desensitization was also non-significant (OR effectively 0.00; $p = 0.98$), indicating that intensified immunosuppression, rather than mismatch status, drives the elevated CMV risk across all desensitized patients. This higher incidence reflects the intensified immunosuppressive approach and the frequent use of additional agents, such as eculizumab and anti-thymocyte globulin, which further compromise antiviral immunity. Early onset of CMV infection in these patients (median onset at 262.0 days post-transplantation, IQR: 97.8–401 days) underscores the importance of robust CMV surveillance strategies, which may include routine viral load monitoring, prophylactic or preemptive antiviral therapy, and meticulous follow-up. Previously, we reported that CMV infection remains a critical complication in lung transplant recipients, particularly those with serological mismatch [23]. At our center, CMV prophylaxis is routinely administered for up to

**TABLE 5 |** Univariate and multivariate cox proportional hazard analysis as a predictor of CLAD.

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95% CI | p value | Hazard Ratio | 95% CI | p value |
| Recipient factors | | | | | | |
| Age, years | 0.99 | 0.97–1.01 | 0.16 | 0.99 | 0.97–1.01 | 0.24 |
| Female | 0.84 | 0.49–1.46 | 0.55 | | | |
| BMI, kg/m2* | 1.08 | 1.02–1.15 | 0.01 | | | |
| BSA, m2* | 2.53 | 0.81–7.96 | 0.11 | | | |
| Smoking history | 1.16 | 0.68–1.98 | 0.58 | | | |
| Hypertension | 1.03 | 0.61–1.75 | 0.92 | | | |
| Diabetes | 1.38 | 0.80–2.39 | 0.25 | | | |
| CKD | 1.33 | 0.52–3.40 | 0.54 | | | |
| Bilateral | 0.84 | 0.49–1.46 | 0.54 | | | |
| PRA | 0.95 | 0.55–1.64 | 0.86 | | | |
| preformed DSA | 1.28 | 0.61–2.72 | 0.51 | | | |
| Desensitization protocol | 1.80 | 0.71–4.55 | 0.21 | 1.75 | 0.67–4.57 | 0.26 |
| Etiology | | | | | | |
| ILD | 0.94 | 0.54–1.64 | 0.82 | | | |
| COPD | 1.07 | 0.76–1.48 | 0.71 | | | |
| PAH | 0.99 | 0.72–1.36 | 0.95 | | | |
| COVID-19 | 0.97 | 0.80–1.17 | 0.74 | | | |
| Laboratory | | | | | | |
| Hemoglobin, g/dL* | 1.14 | 1.02–1.27 | 0.02 | | | |
| WBC, 1,000/mm3* | 0.98 | 0.92–1.05 | 0.65 | | | |
| Platelets, 1,000/mm3* | 1.00 | 1.00–1.00 | 0.91 | | | |
| Sodium, mEq/L | 0.99 | 0.91–1.07 | 0.72 | | | |
| BUN, mg/dL | 1.01 | 0.98–1.04 | 0.36 | | | |
| Creatinine, mg/dL | 1.48 | 0.46–4.74 | 0.51 | | | |
| ALT, U/L* | 1.00 | 0.99–1.02 | 0.71 | | | |
| AST, U/L* | 1.00 | 0.98–1.01 | 0.73 | | | |
| Albumin, g/dL* | 1.45 | 0.93–2.27 | 0.10 | | | |
| Total bilirubin, mg/dL | 1.19 | 0.81–1.75 | 0.38 | | | |
| INR | 1.07 | 0.26–4.36 | 0.92 | | | |
| Arterial blood gas | | | | | | |
| pH | 0.15 | 0.00–6.34 | 0.32 | | | |
| PaCO2 | 0.98 | 0.96–1.01 | 0.14 | | | |
| PaO2 | 1.00 | 1.00–1.00 | 0.67 | | | |
| Donor | | | | | | |
| Age, years | 1.01 | 0.99–1.03 | 0.50 | | | |
| Female | 0.78 | 0.43–1.42 | 0.42 | | | |
| Intraoperative outcome | | | | | | |
| Operative time (hours) | 0.95 | 0.83–1.09 | 0.46 | | | |
| Intra-op blood transfusion (unit) | | | | | | |
| pRBC | 0.97 | 0.90–1.04 | 0.42 | | | |
| FFP | 1.00 | 0.91–1.11 | 0.97 | | | |
| Plt | 0.94 | 0.75–1.17 | 0.57 | | | |
| Ischemic time (hours) | 1.01 | 0.87–1.18 | 0.85 | | | |
| VA-ECMO use | 0.98 | 0.57–1.68 | 0.93 | | | |
| Postoperative outcomes | | | | | | |
| *de novo* DSA | 1.19 | 0.61–2.30 | 0.61 | | | |
| PGD | | | | | | |
| any grade | 0.72 | 0.41–1.26 | 0.25 | | | |
| grade>=2 | 0.73 | 0.37–1.41 | 0.35 | 0.63 | 0.31–1.23 | 0.20 |
| grade3 | 0.69 | 0.25–1.93 | 0.48 | | | |
| AKI | 0.61 | 0.34–1.10 | 0.10 | | | |
| Dialysis | 0.76 | 0.30–1.91 | 0.55 | | | |
| Respiratory infection | 1.59 | 0.90–2.82 | 0.11 | | | |
| Positive aspergillus galactomannan antigen | 1.35 | 0.74–2.46 | 0.33 | | | |
| CMV infection | 1.01 | 0.45–2.23 | 0.99 | 0.88 | 0.39–1.97 | 0.75 |
| ACR | 1.54 | 0.90–2.66 | 0.12 | | | |
| AMR | 2.40 | 0.95–6.05 | 0.06 | 2.21 | 0.87–5.62 | 0.097 |

*CLAD, chronic lung allograft dysfunction; BMI, body mass index; BSA, body surface area; CKD, chronic kidney disease; PRA, panel reactive antibody; DSA, donor specific antibody; ILD; interstitial lung disease; COPD, chronic obstructive pulmonary disease; PAH, pulmonary arterial hypertension; WBC, white blood cell; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; pRBC, packed red blood cells; FFP, fresh frozen plasma; Plt, platelets; VA ECMO, veno-arterial extracorporeal membrane oxygenation; DSA, donor specific antibody; PGD, primary graft dysfunction; AKI, acute kidney injury; CMV, cytomegalovirus; ACR, acute cellular rejection; AMR, Antibody-mediated rejection. *Unknown cases were excluded.*

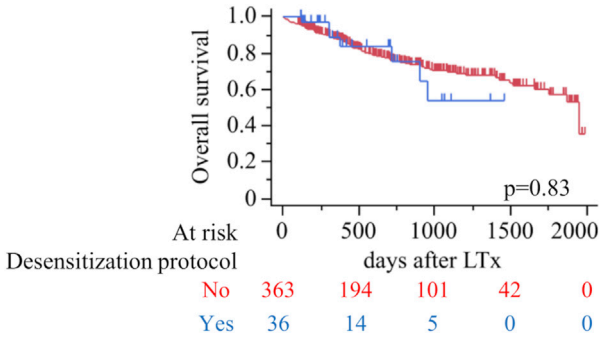


**FIGURE 3 |** Overall survival after lung transplantation according to desensitization protocol (red, No; blue, Yes). Kaplan–Meier curves with numbers at risk shown below; tick marks indicate censoring. Log-rank p=0.83.

1 year post-transplantation, utilizing valganciclovir as the primary agent. This protocol has significantly reduced CMV-related morbidity; however, the risk of late-onset CMV infection following the cessation of prophylaxis persists. Based on our previous study, the median onset of CMV infection after lung transplantation was reported to occur at approximately 395 days (IQR: 264–453) for all patients and at 425 days (IQR: 405–456) after completing prophylaxis in serological mismatch cases. The recurrence rate highlights the importance of tailoring CMV management strategies, particularly in high-risk cohorts. Encouragingly, while CMV infections were more frequent, this did not compromise overall survival, suggesting that aggressive diagnosis and treatment protocols can mitigate most adverse outcomes. Moreover, the fact that other infection rates—such as aspergillus galactomannan antigen positivity, bacteremia, and fungal bloodstream infections—remained similar between groups indicates that the increased susceptibility is largely CMV-specific, supporting targeted adjustments to CMV prevention rather than broad-spectrum antimicrobial changes. It is also worth noting that other infection rates—such as aspergillus galactomannan antigen positivity, bacteremia, or fungal bloodstream infections—did not differ significantly. This finding reassures that enhanced immunosuppression in desensitized patients may not universally increase susceptibility to all pathogens but rather select agents like CMV.

A noteworthy point in our analysis is the higher use of VA-ECMO intraoperatively in the desensitization group compared to the non-desensitization group. This could reflect either a preference for more aggressive intraoperative support in patients perceived to be at higher risk or an actual clinical necessity due to their heightened perioperative instability. VA-ECMO use could introduce risks such as bleeding, thrombotic events, and inflammatory cascade activation that might contribute to PGD [24, 25]. Interestingly, PGD severity at 72 h did not differ significantly between the groups, though there was a trend toward higher PGD grades in the desensitization group. Prior literature has consistently identified both donor- and recipient-related factors contributing to PGD, including high BMI, pulmonary hypertension, and the presence of DSAs [26, 27]. The association of bilateral transplantation with increased PGD likely reflects the greater surgical insult, longer ischemic times, and higher transfusion requirements inherent to bilateral procedures. Conversely, bilateral grafts confer superior long-term pulmonary mechanics, ventilation–perfusion matching, and reserve—factors that ultimately translate into a survival advantage despite a higher early PGD risk. Thus, the short-term vulnerability to reperfusion injury does not negate the medium- and long-term benefit of bilateral allografts. Multivariate analysis in our study confirmed that higher BMI, elevated PRA, and lower albumin levels were independent predictors of PGD. While our data do not definitively implicate the desensitization protocol with eculizumab alone as a driver of PGD, sensitized patients may come to transplants with more challenging clinical profiles overall.

The findings of this study reinforce the notion that sensitized patients can undergo successful lung transplantation if adequately managed. The elevated risk of AMR, CMV infection, and additional resource utilization does not appear to compromise long-term survival. Thus, the standard of care may evolve to include routine evaluation of patients previously excluded solely based on high sensitization statuses. In comparison to studies using alternative desensitization strategies, such as the protocol described by Aversa et al [8], our protocol—with the addition of eculizumab—represents a more aggressive immunosuppressive approach. Importantly, the critical role of complement activation in graft injury strongly supports the use of eculizumab. Evidence from our prior study, demonstrated a clear temporal correlation between post-reperfusion complement deposition and severe primary graft dysfunction in lung allografts [28]. This finding underscores that complement-mediated injury is a key driver of graft dysfunction in this setting, making complement inhibition not merely an adjunct but an essential therapeutic component.

Several limitations should be acknowledged. First, this was a single-center cohort study, and the desensitization protocol with eculizumab may not be universally implemented or standardized. Protocol variations across centers could result in different outcomes, thus limiting the generalizability of our findings. Second, although our analysis included a substantial number of lung transplants, the proportion of desensitized patients was relatively small, reflecting the lower prevalence of sensitized candidates. This disparity may introduce some statistical limitations in detecting small but meaningful differences. Third, we did not collect systematic post-transplant DSA clearance data beyond routine monthly surveillance, so we cannot directly correlate pfDSA kinetics with clinical outcomes. Fourth, we did not perform a comprehensive cost-effectiveness evaluation. However, it is clear from our results that the desensitization group incurred higher resource utilization, at least in terms of transfusions and possibly extended ICU stays. Finally, our median follow-up was shorter in the desensitized cohort (367 vs. 567 days; p = 0.06), which may limit the detection of CLAD—an outcome that typically accumulates over several years. Longer follow-up will therefore be required to fully assess the impact of desensitization on long-term CLAD risk. In addition, our institutional protocol used

**TABLE 6 |** Univariate and multivariate cox proportional hazard analysis as a predictor of overall survival.

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95% CI | p value | Hazard Ratio | 95% CI | p value |
| Recipient factors | | | | | | |
| Age, years | 1.01 | 0.99–1.03 | 0.26 | | | |
| Female | 1.07 | 0.71–1.61 | 0.74 | | | |
| BMI, kg/m2* | 1.04 | 0.99–1.08 | 0.12 | | | |
| BSA, m2* | 1.10 | 0.47–2.59 | 0.82 | | | |
| Smoking history | 1.05 | 0.70–1.57 | 0.81 | | | |
| Hypertension | 1.07 | 0.71–1.60 | 0.74 | | | |
| Diabetes | 1.40 | 0.93–2.12 | 0.11 | | | |
| CKD | 1.99 | 1.10–3.59 | 0.022 | 1.34 | 0.71–2.53 | 0.37 |
| Bilateral | 0.58 | 0.39–0.87 | 0.0078 | 0.44 | 0.28–0.69 | 0.0004 |
| PRA | 1.12 | 0.74–1.68 | 0.59 | | | |
| preformed DSA | 1.36 | 0.78–2.36 | 0.28 | | | |
| Desensitization protocol | 1.36 | 0.78–2.36 | 0.28 | | | |
| Etiology | | | | | | |
| ILD | 1.09 | 0.72–1.65 | 0.69 | | | |
| COPD | 1.59 | 1.01–2.52 | 0.047 | 1.46 | 0.88–2.43 | 0.14 |
| PAH | 0.83 | 0.36–1.90 | 0.65 | | | |
| COVID-19 | 0.77 | 0.41–1.45 | 0.41 | | | |
| Laboratory | | | | | | |
| Hemoglobin, g/dL* | 1.00 | 0.92–1.08 | 0.94 | | | |
| WBC, 1,000/mm3* | 0.98 | 0.92–1.03 | 0.36 | | | |
| Platelets, 1,000/mm3* | 1.00 | 1.00–1.00 | 0.96 | | | |
| Sodium, mEq/L | 1.03 | 0.97–1.09 | 0.37 | | | |
| BUN, mg/dL | 1.55 | 0.14–12.06 | 0.70 | | | |
| Creatinine, mg/dL | 2.70 | 1.19–5.87 | 0.015 | | | |
| ALT, U/L* | 1.01 | 1.00–1.01 | 0.16 | | | |
| AST, U/L* | 1.01 | 1.00–1.01 | 0.073 | | | |
| Albumin, g/dL* | 0.18 | 0.035–0.95 | 0.043 | 0.14 | 0.030–0.64 | 0.012 |
| Total bilirubin, mg/dL | 1.17 | 0.85–1.50 | 0.26 | | | |
| INR | 1.25 | 0.41–3.14 | 0.67 | | | |
| Arterial blood gas | | | | | | |
| pH | 0.14 | 0.0087–2.54 | 0.18 | | | |
| PaCO2 | 1.00 | 0.98–1.01 | 0.70 | | | |
| PaO2 | 1.00 | 1.00–1.00 | 0.65 | | | |
| Donor | | | | | | |
| Age, years | 1.01 | 1.00–1.03 | 0.097 | | | |
| Female | 1.16 | 0.76–1.77 | 0.48 | | | |
| Intraoperative outcome | | | | | | |
| Operative time (hours) | 0.97 | 0.87–1.07 | 0.53 | | | |
| Intra-op blood transfusion (unit) | | | | | | |
| pRBC | 1.00 | 0.94–1.04 | 0.88 | | | |
| FFP | 1.01 | 0.92–1.08 | 0.89 | | | |
| Plt | 1.03 | 0.88–1.17 | 0.65 | | | |
| Ischemic time (hours) | 0.93 | 0.81–1.04 | 0.24 | | | |
| VA-ECMO use | 1.10 | 0.73–1.67 | 0.65 | | | |
| Postoperative outcomes | | | | | | |
| *de novo* DSA | 0.8 | 0.45–1.42 | 0.45 | | | |
| PGD | | | | | | |
| any grade | 1.49 | 0.99–2.26 | 0.056 | | | |
| grade>=2 | 1.98 | 1.31–2.98 | 0.0012 | 1.76 | 1.11–2.80 | 0.017 |
| grade3 | 3.33 | 2.12–5.25 | <0.0001 | | | |
| AKI | 2.24 | 1.48–3.37 | 0.0001 | 1.82 | 1.15–2.88 | 0.011 |
| Dialysis | 3.25 | 2.10–5.05 | <0.0001 | | | |
| CVA | 3.19 | 1.29–7.89 | 0.012 | 3.48 | 1.33–9.09 | 0.011 |
| Bowel ischemia | 11.31 | 4.51–28.36 | <0.0001 | 3.04 | 1.06–8.71 | 0.039 |
| Digital ischemia | 5.74 | 2.32–14.18 | 0.0002 | 6.48 | 2.45–17.11 | 0.0002 |

*BMI, body mass index; BSA, body surface area; CKD, chronic kidney disease; PRA, panel reactive antibody; DSA, donor specific antibody; ILD; interstitial lung disease; COPD, chronic obstructive pulmonary disease; PAH, pulmonary arterial hypertension; WBC, white blood cell; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; pRBC, packed red blood cells; FFP, fresh frozen plasma; Plt, platelets; VA ECMO, veno-arterial extracorporeal membrane oxygenation; DSA, donor specific antibody; PGD, primary graft dysfunction; AKI, acute kidney injury; CVA, cerebrovascular attack. *Unknown cases were excluded.*

PRA >40% as the threshold for initiating desensitization, regardless of the presence of preformed DSA. While this approach maximized safety, it may have resulted in overtreatment of patients without pfDSA. Future protocols may refine these criteria to target desensitization more precisely. Another important limitation is the absence of repeated IVIG maintenance infusions in our protocol, which may have contributed to the higher incidence of AMR observed in the desensitized group.

In conclusion, our study supports that lung transplantation in sensitized patients is feasible and safe with appropriate desensitization protocols and vigilant postoperative care. However, these patients are at higher risk for certain complications—most notably AMR and CMV infections—and their overall survival rates are comparable to non-sensitized recipients. Future research directions include multi-institutional trials to validate our findings and further refine desensitization protocol, investigate long-term graft function beyond the first year, and develop biomarkers or diagnostic tools to detect impending AMR earlier. Ultimately, our results underscore the importance of expanding lung transplant eligibility to include sensitized patients who can benefit substantially from transplantation when managed with an optimized, individualized immunosuppressive approach.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on reasonable request by a qualified investigator for three years after the date of publication from the corresponding author.

## ETHICS STATEMENT

The studies involving humans were approved by the study was approved by the Institutional Review Board of Northwestern University (STU00207250, STU00213616, and STU00217958). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because The need for patient consent for data collection was waived by the institutional review board due to the retrospective nature of this study.

## AUTHOR CONTRIBUTIONS

YM and CK established the study design. YM and TK performed literature research. YM and TK collected the data and performed the statistical analyses. YM and CK wrote the initial draft of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2025.15040/full#supplementary-material

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REVIEW
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# State of Art of Dose Individualization to Support tacrolimus drug monitoring: What's Next?

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Tacrolimus is an immunosuppressant with a narrow therapeutic index and a high intra- and inter-patient variability showing significant challenges in optimal dosing and monitoring. Historically, pre-dose concentration monitoring and simplified area under the curve measurements have been the standard approach. However, recent advances in pharmacokinetic modeling have improved individualized dosing strategies, moving beyond empirical methods. This review explores the evolving landscape of Tacrolimus therapeutic drug monitoring, focusing on advanced modeling techniques that support personalized dosing. Key methodological approaches include Population Pharmacokinetic (PopPK) modeling, Bayesian prediction, Physiologically-Based Pharmacokinetic (PBPK) modeling, and emerging machine learning and artificial intelligence technologies. While no single method provides a perfect solution, these approaches are complementary and offer increasingly sophisticated tools for dose individualization. The review critically examines the potential and limitations of current modeling strategies, highlighting the complexity of translating advanced statistical and mathematical techniques into clinically accessible tools. A significant challenge remains the gap between sophisticated modeling techniques and the practical usability for healthcare professionals. The need for user-friendly platforms is emphasized, with recognition of existing commercial solutions while also noting their inherent limitations. Future directions point towards more integrated, intelligent systems that can bridge the current technological and practical gaps in personalized immunosuppressant therapy.

**Keywords: kidney transplantation, pharmacogenetics, pharmacokinetics, population pharmacokinetics, tacrolimus, mathematical modeling**

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## INTRODUCTION

The landscape of solid organ transplantation witnessed a transformative shift during the 1990s, with the new immunosuppressive strategies significantly changing short-term graft and patient survival [1]. Despite these advances, long-term outcomes continue to front challenges, with tacrolimus remaining the cornerstone of post-transplant immunosuppression [1]. Tacrolimus pharmacokinetic is characterized by a narrow therapeutic window and high variability between and within patients underscoring the critical importance of personalized therapeutic drug monitoring (TDM). Transplant medicine represents a delicate balance between immunological management and pharmacological precision. The current clinical paradigm presents a critical

challenge: preventing organ rejection while simultaneously avoiding the risks of over-immunosuppression. Current standard practices, particularly weight-based dosing, are a poor predictor of tacrolimus exposure, with only 20%–35% of transplant recipients achieving target therapeutic levels at first steady state [2–5]. During this period, accurate adjustments of immunosuppressants are vital to prevent risks such as allograft rejection, nephrotoxicity, and therapeutic failure [6–10].

Several studies have demonstrated that tacrolimus levels below the therapeutic target are associated with an increased risk of allograft rejection within the first 3–6 months post-transplantation [11, 12]. Careful management of immunosuppression is crucial, as under-immunosuppression can lead to acute rejection, while over-immunosuppression increases the risks of infections and malignancies. As transplant patient life expectancy continues to improve, the focus has evolved from preventing early graft rejection to managing the long-term consequences of prolonged immunosuppressive therapy and its associated adverse effects [13]. Adjusting both under- and over-exposure remains a significant challenge due to the considerable variability among transplant recipients [14, 15].

Population pharmacokinetic (PopPK) modeling has emerged as a promising bridge research insights and clinical application, offering a sophisticated approach for drug dosing that incorporates multiple variables affecting drug metabolism and distribution. The integration of single Nucleotide polymorphisms (SNP), particularly CYP3A variants, provides opportunities for more precise dosing strategies. Guidelines from both the Clinical Pharmacogenetic Implementation Consortium (CPIC), the Dutch Pharmacogenomics Working Group, and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) have emphasized the importance of genetic variants in tacrolimus metabolism. However, a significant gap remains between these theoretical frameworks and their practical implementation in clinical care.

Current TDM approaches rely on a trial-and-error method that can take up to 3 weeks to achieve target drug levels, leaving patients vulnerable to potential complications. Recent modeling advances have expanded the variables considered in tacrolimus pharmacokinetics, including clinical factors such as age, body composition, albumin levels, demographic characteristics like ethnicity, and SNPs affecting drug transport and metabolism. The concentration-to-dose (C/D) ratio has emerged as a valuable tool for ongoing dose adjustment [16–18], while Bayesian modeling approaches show promise for more precise initial dosing strategies.

This review aims to explore the complex landscape of tacrolimus pharmacokinetic variability by critically analyzing PopPK models and advanced modeling strategies. These include Bayesian prediction, Physiologically-Based Pharmacokinetic (PBPK) modeling, and machine learning technologies as innovative tools for individualizing immunosuppressive therapy. The authors seek to bridge sophisticated mathematical techniques with clinical implementation, highlighting the need for user-friendly platforms that can translate complex statistical methodologies into accessible clinical tools for therapeutic optimization.

# CONVENTIONAL THERAPEUTIC DRUG MONITORING OF TACROLIMUS

Traditional TDM protocols for tacrolimus starting dose fail to account for the multifaceted nature of tacrolimus pharmacokinetics. This conventional approach runs under the presumption that a linear relationship exists between body weight and both drug clearance and volume of distribution–an assumption that has proven to not be the best tool to apply in clinical practice [15]. Tacrolimus maintenance dosing is usually adjusted based on pre-dose trough levels (C0), a widely accepted parameter for TDM due to its presumed strong correlation with the area under the curve (AUC) [19].

## Pre-Dose Concentration Versus AUC

The measurement of C0 has emerged as the standard of care in transplant centers globally. However, the correlation between C0 and AUC has shown varying degrees of reliability across different studies [19]. Recent real-world data analysis of patients in their second and third post-transplant years demonstrated that while both C0 and AUC correlated with BPAR incidence, AUC proved superior in identifying patients with exposure irregularities despite apparently adequate C0 levels [20]. The C0/dose ratio has emerged as a valuable predictor of CNI nephrotoxicity, with studies by Thölking et al. [16, 21, 22] and others [23, 24] demonstrating its prognostic value for renal function outcomes. Fast metabolizers, identified by lower C0/dose ratios, showed higher peak concentrations despite similar trough levels, suggesting that C0 monitoring alone might miss important exposure patterns [17, 25].

## Sources of Variability in Tacrolimus Pharmacokinetics

Numerous factors have been identified that impact tacrolimus pharmacokinetics, contributing to the inter-patient variability [26–28]. Tacrolimus displays variable absorption in the gastrointestinal tract, with factors like gastric pH, motility, and the presence of food impacting its bioavailability. Reduced absorption can be observed in conditions such as delayed gastric emptying or gastrointestinal inflammation, leading to subtherapeutic drug levels. Gastrointestinal motility disorders, particularly diarrhea, can markedly enhance tacrolimus absorption, potentially leading to toxic levels in certain patients [29–33]. Lemahieu et al mentioned a decreased intestinal p-glycoprotein activity as a potential cause for higher absorption of tacrolimus. Moreover, the accelerated movement through the intestinal tract results in increased tacrolimus exposure to both the distal portion of the small intestine and colonic tissue, where absorption can occur [34]. This drug is extensively metabolized in the liver by cytochrome P450 enzymes, primarily CYP3A4 and CYP3A5, with hepatic function variations significantly altering drug clearance.

Pharmacokinetic variability is further complicated by physiological factors like erythrocyte binding, where lower hematocrit levels result in higher free drug concentrations and increased clearance [27]. Alterations in albumin levels and

hematocrit enhance tacrolimus elimination and dosing requirements, although these changes do not substantially impact the unbound drug fraction [27, 35–41].

Patient demographics play a crucial role, with pediatric patients requiring higher doses due to enhanced hepatic enzyme activity, while elderly individuals (≥65 years) experience slower metabolism from age-related liver and kidney function decline, potentially leading to up to 50% higher tacrolimus exposure despite lower dose-to-body weight ratios [42–47].

Drug metabolism through oxidative pathways predominantly involves the Cytochrome P450 (CYP) 3A subfamily, which significantly influences tacrolimus concentrations [44, 45]. *CYP3A5*1* (**1* allele expressers) (rs776746) demonstrate markedly increased tacrolimus clearance, requiring approximately 50% higher doses to achieve therapeutic levels compared to non-expressors (**3/*3* genotype) [50–54]. This pharmacogenetic effect underscores the importance of CYP3A5 genotyping in optimizing tacrolimus therapy [2, 50–52, 55]. In contrast, the *CYP3A4*22* variant also demonstrates clinical relevance. Carriers of the T variant allele exhibit reduced CYP3A4 activity [56], requiring approximately 33% lower tacrolimus doses [57]. The combined influence of CYP3A4/5 SNPs according to metabolizer phenotypes have significant impact on tacrolimus pharmacokinetic. Different studies have demonstrated that integrating both CYP3A5/4 genotypes can explain over 60% of observed variability in tacrolimus concentrations [57, 58]. Current clinical guidelines from CPIC and IATDMCT [15] recommend increasing doses by 1.5–2 times for patients with enhanced metabolism, highlighting the practical application of this genetic information in personalizing tacrolimus therapy.

Tacrolimus transport is primarily mediated by P-glycoprotein (Pgp), an efflux pump encoded by the ABCB1 gene, which facilitates drug movement across multiple physiological barriers including intestinal epithelium, hepatic tissue, blood-brain barrier, renal tubules, pancreatic cells, and lymphocytic membranes [59]. The ABCB1 gene's widespread distribution is crucial in determining tacrolimus pharmacokinetics, particularly in absorption, distribution, and elimination [49]. Over 50 ABCB1 SNPs have identified with three key variants in clinical research: 3435C>T (rs1045642), 1236C>T (rs1128503), and 2677G>T/A (rs2032582). These SNPs exist in linkage disequilibrium, suggesting potential coordinated effects on Pgp function. However, despite theoretical expectations of decreased Pgp activity associated with these variants, multiple clinical investigations have failed to demonstrate consistent correlations between these polymorphisms and systemic tacrolimus concentrations [60–65].

Drug-drug interactions with tacrolimus, primarily mediated by CYP3A4 and Pgp, are well-documented [66]. Co-administration of drugs that interact with ABCB1 and/or CYP3A can significantly alter the bioavailability and metabolism of tacrolimus [67]. This may result in high levels of immunosuppression, increasing the risk of toxicity, or in levels that are too low, raising the likelihood of organ rejection [68]. Inhibitors like azole antifungals, calcium channel blockers (e.g., verapamil, diltiazem), HIV protease inhibitors (e.g., ritonavir), macrolides (excluding azithromycin), amiodarone, and nefazodone increase tacrolimus exposure. While azole antifungals are strong inhibitors of tacrolimus metabolism, others, such as azithromycin, have minimal clinical effects. In contrast, inducers like rifampicin, anticonvulsants, and corticosteroids significantly decrease tacrolimus levels. Therefore, in addition to making dosage adjustments, therapeutic drug monitoring (TDM) is essential in clinical practice for transplant patients, especially when changes to their treatment regimen are necessary.

# OVERVIEW OF PHARMACOKINETIC MODELS FOR TACROLIMUS: POPULATION PHARMACOKINETIC (POPPK) MODELS, PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS, AND MACHINE LEARNING (ML) APPROACHES

Currently, the two primary approaches for describing the pharmacokinetics of tacrolimus and predicting its concentrations in transplant patients are population pharmacokinetic (PopPK) and physiologically based pharmacokinetic (PBPK) models. Recently, a new approach, machine learning (ML), has also emerged. While PopPK and PBPK models use differential equations, ML relies on statistical relationships between variables to make predictions.

It is worth noting that PopPK and PBPK models each have unique strengths and limitations, and they are not mutually exclusive; instead, they can be used complementarily. **Table 1** summarizes the main differences between these two approaches, meanwhile **Table 2** summarizes the limitations of each one.

## Population Pharmacokinetic Models

The PopPK approach aims to identify the sources of variability in the pharmacokinetic profile of a drug within the target population, but sufficient data are required. This is a necessary step in the successful clinical translation of any drug. The number of subjects included in the study determines the precision and clinical relevance of the effect of a covariate. PopPK models are compartmental models that describe the dose–concentration relationship from all available data by building a model with structural and statistical components that fits the data (**Figure 1**). PopPK modeling enables us to optimize the dose regimens, based on the predictive factors of PK variability in the target population.

### Model-Informed Precision Dosing

Model-Informed Precision Dosing (MIPD) is an advanced quantitative approach used to optimize individualized dosing. This method combines TDM measurements with PopPK models to individualize treatment regimens by applying Bayesian forecasting [69, 70].

**TABLE 1 |** Summary of Characteristics of each approach, Pop-PK and PBPK models.

| Feature | PBPK modeling | Pop-PK modeling |
|---|---|---|
| **Methodolgy** | Mechanistic | Empirical/Statistical |
| **Sparse Data analysis** | Less efficient than Pop-PK | Very useful and efficient |
| **Extrapolation capability** | Interspecies, age, disease states | Descriptive capability. Extrapolation only within the range of variation of the identified covariates in the target population |
| **Drug-Drug interactions (DDI) Prediction** | Powerful | Limited |
| **Special Population Suitability** | High suitability for pediatric, geriatric, disease states | Aims at identifying factors of variability within a given population |
| **Regulatory Acceptance** | High, especially for DDIs and special populations | High, widely used for dose recommendations |
| **Real-World Application** | Limited as it requires detailed physiological parameters to be available | Useful for clinical PK studies and as support tool during the therapeutic drug monitoring (TDM) using bayesian prediction |

**TABLE 2 |** Summary of limitations of each approach, Pop-PK and PBPK models.

| PBPK modeling | Pop-PK modeling |
|---|---|
| **Model complexity and computational complexity** due to the multiple interconnected compartments and the differential equations required to define the system | **Computational complexity:** Typically, mathematically less complex than PBPK because less parameters are involved, **but large datasets and complex non-linear mixed-effects models can still require long computing times** |
| **Requires knowledge of species-specific anatomical, physiological and biochemical parameters** such as tissue volumes, blood flow, metabolic enzyme and transporter expression and also **drug specific** such as partition coefficients<br>Not all these parameters can be experimentally measured, and then they have to be estimated from other data | |
| **Variability**: Physiological parameters (Flows, Volumes…) can vary across populations or disease states, leading to uncertainty and variability | **Large population studies are required:** Pop-PK modeling aims at identifying the sources of PK variabilty to optimize the dose regimens in the target population<br>If the range and effect of a physiological parameter observed in the target population is small, it will be misleading to identify this as an influential covariate within the study, even though the parameter may be truly influential.Therefore it requires large population studies to capture variability, but data collection limitations may restrict the range of accuracy of covariates that are physiologically meaningful to explain PK variability in the target population |
| **Oversimplifications under certain circumstances:**<br>i) Lack of homogeneity within the same compartment exists (i.e., Brain)<br>ii) Lack of PK linearity occurs<br>iii) Changes of physiological conditions with time | **Oversimplifications of the real-world drug processes** that have an impact on model predictions |
| **Software Limitations:** Lack of flexibility of some platforms to handle highly complex or non-standard models, requiring modelling expertise | **There are commercially available powerful softwares but they require expertise modelers in pharmacometrics, biostatistics and non-linear mixed-effects models** which may not be available in all clinical or research settings |

MIPD is a promising alternative to conventional dosing approaches. It enables faster initial dose titration through *a priori* MIPD based on baseline covariate values that predict variability. It also improves subsequent achievement of $C_0$ or AUC targets via *a posteriori* MIPD based on prior pharmacokinetic assessments and updated covariate information over time [71, 72].

Widespread use of MIPD is currently limited by several challenges, including limited clinical modelling expertise, limited generalizability and harmonization of models across different patient populations, and a lack of conclusive evidence that it actually improves outcomes [73]. Despite these barriers, considerable progress has been made, providing a valuable source of evidence to support and guide future clinical pharmacometrics efforts in the context of renal transplantation [72]. As mentioned above, tacrolimus by concentration-guided dose titration has certain limitations and the MIPD represents a viable alternative to optimize the individualized dosing regimen in transplant TDM [35, 74].

## Model-Informed Precision Dosing Modeling Software

Several software programs have been developed to enhance the prediction of patient drug concentrations and provide individualized dose recommendations to minimize PK variability. Notably, Fuchs et al., followed by Del Valle-Moreno et al., conducted extensive reviews to catalog MIPD software tools, offering detailed descriptions of their primary features. These reviews place particular emphasis on selecting the most appropriate software tools to align with specific clinical needs [70, 75].

The use of MIPD software continues to grow, driven by its precision, advancements in PopPK models, and the expanding set of drugs that can benefit from optimization. This trend reflects an increasing awareness of the importance of dose individualization for vulnerable populations, such as elderly patients, individuals

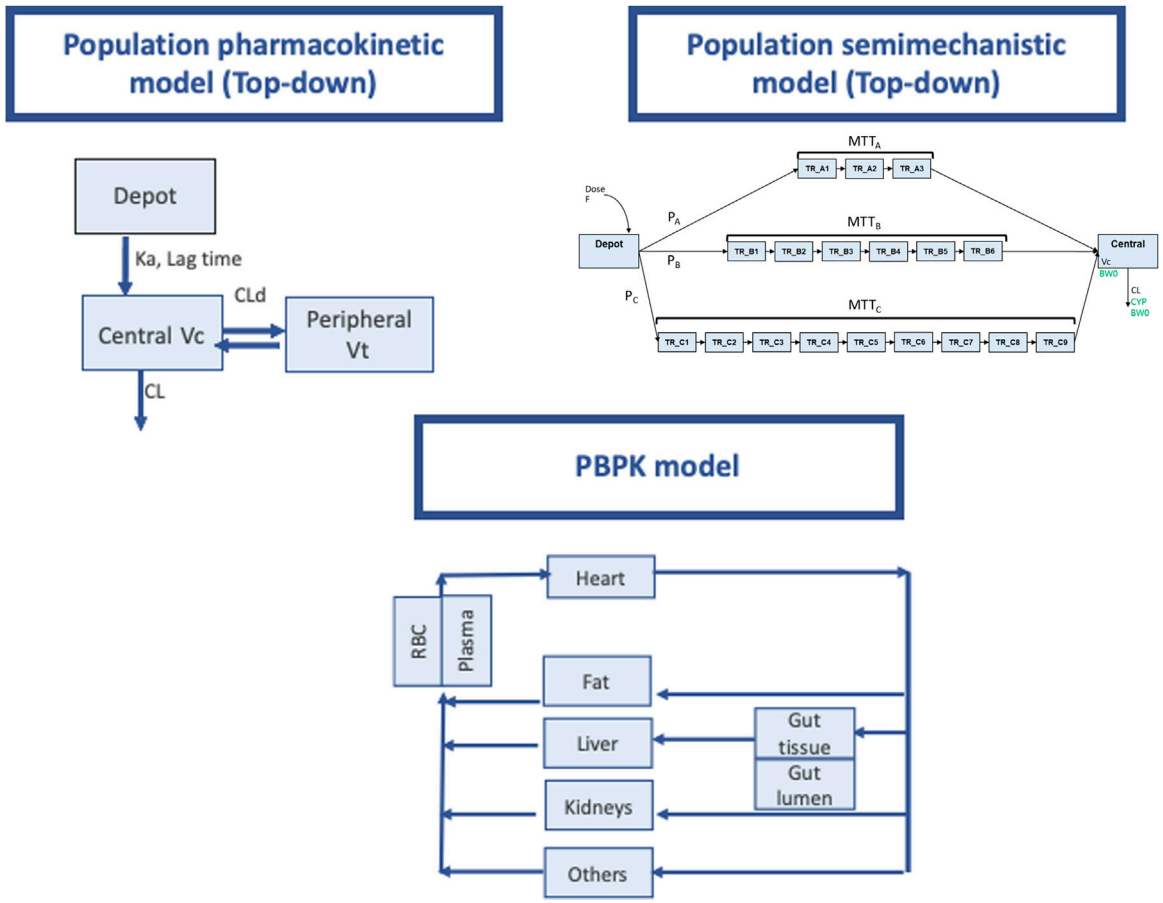


**FIGURE 1 |** Pharmacokinetic modeling approaches used for PK prediction of tacrolimus. Upper left pannel: Schematic representation of a population pharmacokinetic model with a deport compartiment and two open compartments (central and peripheral). Ka absorption rate constnat, Vc and Vp central and peripheral distribution volumes. CLd distribution clearance, CL elimination clearance. "Central" and "peripheral" compartments, do not represent actual physiological tissues and provide only empirical descriptions of drug pharmacokinetics in the body. The model building process starts with simple models and increases in complexity depending on the complexity of the pharmacokinetic behavior of the drug under study. This approach based on observed data, is widely known as "top-down". Upper right panel (Taken from Henin et al, with permission): Schematic representation of LCPT model structure. F relative bioavailability; $P_A$, $P_B$, $P_C$ proportion of dose following fast (chain A), medium (chain B) and slow (chain C) absorption processes respectively; $MTT_A$ mean transit time for chain A (fast absorption); $MTT_B$ mean transit time for chain B (medium absorption); $MTT_C$ mean transit time for chain C (slow absorption); TR_AX (X being from 1 to 3) Xth transit compartment in chain A; TR_BX (X being from 1 to 6) Xth transit compartment in chain B; TR_CX (X being from 1 to 9) Xth transit compartment in chain C; V C volume of central compartment; CL clearance; BW0 body weight at baseline (covariate on V C and CL); CYP CYP3A5 single nucleotide polymorphism (covariate on CL). Lower panel: PBPK model (adapted form Prado-velasco et al 2019 with permission). Physiological plausibility is present in this approach flow diagram for TAC PBPK model with 4 flow-limited tissues (fat, kidneys, liver and others) and 2 membrane-limited tissues (gut and blood). The blood compartment is defined through the red blood cell- plasma component. The gastric system is comprised of a gut lumen where the TAC form is liberated following a zero-order kinetic with sink condition, a one-order absorption membrane and gut tissue perfused with blood.

with renal or hepatic impairment, pregnant women, critically ill patients, and children. Consequently, these computer programs have become indispensable tools in routine clinical practice [70, 75].

Hoffert et al. identified seven software tools currently utilized in clinical settings to guide tacrolimus dosing for renal transplant patients: Rx Studio, PrecisePK, InsightRx Nova, MwPharm, DoseMeRX, BestDose, and ISBA [76].

PrecisePK, MwPharm, DoseMeRX, and BestDose underwent prospective validation of their tacrolimus modules prior to their integration into clinical practice. Software tools designed for clinical decision-making may obtain CE marking, which signifies compliance with European Union regulations, although this certification is not mandatory. These tools serve as decision-support systems, providing dosing recommendations to clinicians, who ultimately make the final therapeutic decisions.

For tacrolimus dosing, CE marking has been obtained by PrecisePK, InsightRx Nova, MwPharm, and DoseMeRX. Additionally, some software modules offer multiple PopPK models to facilitate MIPD for renal transplant patients. However, only InsightRx Nova and MwPharm support MIPD for pediatric populations [76].

## Population Pharmacokinetic Models for Tacrolimus

Four comprehensive reviews of tacrolimus PopPK models have been published [74, 76–78]. Brooks et al. and Kirubakaran et al.

compiled detailed information on models for solid organ transplant recipients, including transplant type, formulations, sampling times, and bioanalytical methods [74, 78]. Nanga et al. proposed a meta-model applicable across different populations [77], while Hoffert et al. reviewed MIPD software modules and covariate impacts on exposure [76].

Most studies focused on the first post-transplant year, with patients on tacrolimus, mycophenolate, and corticosteroids [74, 76–78]. For kidney transplants, models primarily covered immediate-release formulations, with fewer studies on extended-release versions like Envarsus® [79–82]. NONMEM was the predominant modeling software, though some studies used non-parametric approaches like Pmetrics [74, 76–78].

Two-compartment models were most common, particularly with intensive sampling data, while trough concentration studies typically used one-compartment models. Various absorption models were tested, reflecting tacrolimus' complex absorption patterns [47]. Most models derived from White populations, potentially limiting their applicability to other ethnic groups. Hispanic patients showed 40% lower apparent clearance compared to non-Hispanic populations [83].

Key factors affecting tacrolimus clearance include CYP3A5 genotype, hematocrit, and post-transplant time [74, 77, 78]. *CYP3A5*3/*3* variant carriers show lower clearance and higher dose requirements than CYP3A5 expressors [15, 84]. Studies also examined CYP3A4, ABCB1, ABCC2, and POR28 polymorphisms [79, 85–94]. Long-term administration shows decreasing dose requirements due to reduced corticosteroids, improved CYP3A5/CYP3A4 activity, and increasing hematocrit [78]. Størset et al. standardized concentrations to 45% hematocrit for better pharmacokinetic assessments [95].

Body composition significantly affects distribution volume. Fat-free mass better predicts tacrolimus clearance than total body weight, as demonstrated by Holford and Størset [35, 95]. Overweight patients risk overexposure with weight-based dosing [96]. Bio-impedance spectroscopy studies suggfance variability [97]. Model validation remains limited compared with the high rate of published models, with few studies including external cohort validation. Zhao et al carried out external evaluation of 16 models developed in kidney transplant recipients with data from 52 external patients [72]. According to the authors, the published models were unsatisfactory in prediction- and simulation-based diagnostics, thus inappropriate for direct extrapolation correspondingly. However, Bayesian forecasting could improve the predictability considerably with priors.

## Physiologically-Based Pharmacokinetic Models

PBPK models represent a significant advancement over traditional PopPK approaches in their ability to predict drug concentrations across multiple organs. These models integrate both physicochemical properties and physiological characteristics, creating a comprehensive framework based on physiologically meaningful compartments interconnected through blood circulation. The mathematical foundation relies on mass-balance differential equations that precisely define drug movement throughout the system [98].

The architecture of PBPK models demonstrates remarkable flexibility in compartment selection, adapting to specific study objectives. In tacrolimus modeling, particular emphasis is placed on pharmacokinetically significant tissues such as red blood cells, fat, liver, and intestinal tissues, while other less relevant tissues may be consolidated into broader compartments.

Three distinct approaches have emerged in PBPK modeling, each offering unique advantages. The bottom-up approach predicts pharmacokinetics by leveraging drug physicochemical characteristics and *in vitro* ADME data. This strategy proves particularly valuable when clinical data is limited, with flexibility to be adapted to different populations through physiological parameter adjustments. In contrast, the top-down approach relies heavily on clinical data for model optimization, providing high accuracy for studied populations but with limited extrapolation capabilities. The middle-out approach bridges these methodologies, combining mechanistic and clinical data to enable iterative model refinement.

Model evaluation follows rigorous criteria as outlined in regulatory frameworks [98]. These include detailed comparisons of simulations with experimental concentration-time profiles, utilizing both graphical representations and error function analyses. Models must demonstrate consistency across various scenarios, including different doses, species, populations, and similar compounds. Sensitivity analysis plays a crucial role in identifying key parameters and establishing their plausible ranges.

The importance of PBPK modeling in drug development and clinical applications has been recognized by regulatory bodies, with both the EMA and FDA issuing comprehensive guidance documents for model evaluation. These guidelines, while primarily focused on regulatory applications, provide valuable frameworks that inform broader research applications in human drug modeling.

### Physiologically-Based Pharmacokinetic Modeling Software

Once the entire system is defined and all relevant tissue compartments are established according to the study's objectives, the model's equations must be coded to enable simulations or parameter estimation, depending on the study's goals. This coding can be done using general mathematical modeling software, commonly used by engineers, or specialized PBPK modeling software. Most of these options are commercial products [99, 100]. Generally, none of these tools are particularly beginner-friendly but offer an exponential learning curve (**Table 3**).

### Physiologically-Based Pharmacokinetic Models for Tacrolimus

Despite the established history of PopPK models in tacrolimus dosing support, PBPK modeling adoption faces several challenges. The complexity of drug disposition mechanisms in transplantation and limitations of closed-code software packages necessitate more complex models, requiring flexible platforms and specialized expertise.

**TABLE 3 |** Summary of some fo the most commonly used PBPK softwares and characteristics.

| | General mathematical modelling softwares not specific to PBPK (open softwares) | Characteristics (model structure not defined *a priori*) |
|---|---|---|
| | **Company** | |
| MATLAB, Berkeley Madonna, ModelMaker, acslX | http://www.mathworks.com/products/matlab/, http://www.modelkinetix.com/modelmaker/, http://www.berkeleymadonna.com/, http://www.acslX.com | Very flexible but require programming skills and modelling expertise |
| Phys-PK | https://www.physpk.com/ | Not free programme. Very flexible. Require programming skills **but it also allows interface model building**. Exponential learning curve. User-customisation management for simulation of special populations (paediatrics, geriatrics, and hepatic and renal impairment). This is achieved by adjusting physiological and pharmacokinetic parameters according to the demographic and physiological characteristics of each group. Drug-Drug interactions |
| | **PBPK specific softwares (Designed softwares)** | **Characteristics (Model structure typically defined *a priori*)** |
| | **Company** | Less flexible but require less mathematical modelling expertise |
| GastroPlus | Simulation Plus https://www.simulations-plus.com/ | Exponential learning curve. Not free programme. Customised user management for simulations in pediatrics, geriatrics and pregnancy. Also focused on dissolution, formulation development and virtual bioequivalence. Advanced compartment absorption and transit (ACAT) model to predict oral bioavailability. Drug-Drug inteactions |
| Phoenix-WinNonlin | Certara https://www.certara.com | Not specific for PBPK modeling and simulation, but it can be also used for this purpose. Not free programme |
| PK-Sim and Mobi$ | Open system Pharmacology https://www.open-systems-pharmacology.org/ | Exponential learning curve. Free program. Customised user management for simulations in special populations (pediatrics, geriatrics and hepatic and renal impairment, pregnancy and obesity), genetic variability.Absorption compartment models GI-Sim to predict oral bioavailability. Drug-Drug inteactions |
| Simcyp | Certara https://www.certara.com/software/simcyp-pbpk/ | Exponential learning curve. Not free programme. Customised user management for simulations in special populations (pediatrics, geriatrics hepatic and renal impairment, pregnancy and obesity), genetic variability, reduced cardiac output). Also focused on dissolution, formulation development and virtual bioequivalence, food effect. ADAM model: Advanced dissolution, absorption metabolism model, to predict oral bioavailability. Drug-Drug interactions. Mechanistic transdermal absorption model |

*(*) In general, all them allow the simulation of different clinical scenarios, such as dose changes, chronic administration, or enzymatic variability, which is useful for optimizing therapy and assessing possible drug-drug interactions. This table highlights key characteristics of the software solutions, including whether they are free or paid software and the specific capabilities they offer are showed.*

*$Mobi allows custom models using programming approaches within PK-Sim.*

PBPK models for tacrolimus must address multiple factors contributing to patient variability. Critical considerations include low and variable bioavailability due to poor solubility, first-pass effects influenced by CYP3A5 and P-glycoprotein transport, and elimination pathways particularly relevant in transplant patients. Models must also account for hematocrit's influence on blood-plasma partitioning and distribution across tissues, including liver, kidneys, adipose tissue, and blood cells.

Among PBPK modeling publications for tacrolimus, four significant studies focused on kidney transplantation. Emoto et al. developed a comprehensive Simcyp-based model using a middle-out approach [101]. Their work confirmed the impact of CYP3A4 abundance, hematocrit, and serum albumin levels on tacrolimus pharmacokinetics, though P-glycoprotein contributions were not considered. The model successfully explored pediatric populations, attributing age-dependent changes primarily to CYP3A ontogeny.

Prado-Velasco et al. advanced the field by investigating circadian modulation in pediatric patients using Phys-PK [102]. Their model, incorporating major organ compartments and demographic variables, demonstrated superior predictions compared to PopPK approaches. They applied Poulin and Theil methods for tissue-plasma partitioning [103], revealing significant intra-patient variability during formulation transitions.

A minimal PBPK model by Itohara et al. using Simcyp focused on absorption parameters [104], though it excluded critical factors like solubility and P-glycoprotein polymorphisms. Van der Veken et al. later addressed these limitations by incorporating mechanistic absorption modeling [105]. Their work revealed that amorphous solid dispersion causes tacrolimus to behave as a BCS class 1 rather than class 2 compound, suggesting absorption may not be the primary source of variability in exposure. Recent advances include El-Khateef et al.'s work combining therapeutic drug monitoring with PBPK modeling to investigate chronic kidney disease effects [106]. The approach has also expanded to other transplant types, including liver [107], lung [108], and heart [109], with applications extending to pregnancy populations [110].

PBPK modeling has emerged as a valuable tool for understanding tacrolimus pharmacokinetics across diverse populations and conditions. While these models demonstrate

promise in optimizing dosing strategies and predicting drug interactions, external validation remains crucial for broader clinical implementation. These insights are particularly valuable for special populations, where personalized dosing strategies significantly impact therapeutic outcomes.

## Machine Learning

Machine learning (ML) is a branch of artificial intelligence (AI) that allows computers to learn and make predictions from data without being explicitly programmed to perform each task [111]. Instead of following pre-defined instructions, ML systems use algorithms that analyze data and look for patterns to improve their performance on specific tasks autonomously. The modeling steps consist of: i) data collection and clearing of data for inconsistencies, ii) selection of the best algorithm suitable for the specific purpose (supervised learning, unsupervised learning and reinforcement learning algorithms), iii) training of the model with training data to adjust parameters and learn, iv) performance evaluation of the model with unseen test data, v) optimization of parameters and model deployment in a real-world environment where it can adapt and improve with new data.

PBPK modeling approach offers the possibility of minimizing the animal studies and only using drug-related input parameters for PK predictions in humans. The evaluation of the prediction performance of different software packages as a function of data availability and software options, in a bottom-up approach, showed that predictions are not always within the acceptable range. Moreover, model prediction could not be improved with modeling strategies, but with unbiased parameters used to inform the model [111, 112]. ML is already available to generate unbiased and optimized parameters to be used in bottom-up PBPK modeling approach [113]. The top-down and middle-out approaches can also benefit from AI and ML. For example, AI can contribute to identifying all published PK data of the literature for a drug. Also, these approaches can contribute to optimizations of parameters in the middle-out approach such as tissue Kp values, specific enzyme intrinsic clearance values, or unbound fractions among others. Parameter optimization is particularly labour-intensive and typically not automated, relying heavily on the modeler's expertise to identify the best-fit parameters. AI and ML could help in this process with ML algorithms. These technologies can test numerous combinations at a speed far beyond human capabilities. Therfore, AI could identify the optimal model configuration that best fits all available clinical data.

ML is still evolving, so that its contribution to advances in MIPD is still scarce. Few ML models have been developed for tacrolimus in renal transplantation with good predictions in both cases. Tang et al [114] used ML to predict stable dose in a large Chinese cohort (N = 1,045 recruited patients, 80% used for the derivation cohort and 20% used for the validation cohort). Among all the ML models, regression tree performed best in both derivation and validation cohorts. Covariates statistically significant in the derivation cohort were CYP3A5 genotype, hypertension and use of omeprazole. Sanchez-Herrero et al also applied ML to predict tacrolimus blood concentrations in a paediatric cohort of renal transplant patients (N = 21) [115]. The ExtraTrees Regressor algorithm had superior performance than the other algorithms tested. In both studies the authors reported acceptable values of metrics used to evaluate the accuracy of predictions. Woillard et al investigated whether ML models (Xgboost) accurately estimated tacrolimus AUC in transplant patients using sparse concentration data [116] and also explored the training of Xgboost ML models on simulated tacrolimus concentration-time profiles [117]. Xgboost machine learning models trained on simulated concentration-time profiles from literature PopPK models enable precise tacrolimus AUC estimation based on sparse concentration data. Further studies are still required to advance on the application of ML on MIPD.

## Other Tools for a More Efficient Modeling With NONMEM: ChatGPT and Gemini Large Language Models for Generating Initial Codes Templates of NONMEM

Shin et al evaluated the utility of the ChatGPT4.0 and Gemini Ultra 1.0 large language models for NONMEM coding tasks relevant to pharmacometrics and clinical pharmacology [118]. Their conclusions were that these tools could be useful in the earlier steps to obtain early versions of the codes, but that these codes still require careful checking for errors and improvements before implementation.

# CONCLUSION

In conclusion, understanding the predictive factors of variability in tacrolimus pharmacokinetics is essential for achieving precision dosing and optimizing therapeutic outcomes. Factors such as genetic polymorphisms (e.g., CYP3A5 expression), demographic characteristics, comorbidities, drug-drug interactions, and physiological changes significantly influence tacrolimus absorption, distribution, metabolism, and clearance. Recognizing these variables allows for more accurate dose adjustments, reducing the risk of underdosing or overdosing and minimizing associated adverse effects or graft rejection.

The integration of these predictive factors into MIPD frameworks, supported by advanced PopPK models and decision-support software, enables individualized treatment strategies tailored to each patient's unique profile. This approach not only enhances the safety and efficacy of tacrolimus therapy but also underscores the importance of personalized medicine in improving outcomes for vulnerable populations, including pediatric, elderly, and critically patients.

MIPD is endorsed by tacrolimus PopPK modelling of tacrolimus. Population and PBPK models, together with individualized adjustment tools such as Bayesian prediction, allow for more accurate drug management. However, challenges such as high variability and integration of complex clinical covariates remain. Future research aims to integrate more detailed physiological models and pharmacogenetic approaches to further optimize therapy. None of these approaches replace the others, rather they complement each other.

Despite the promise of MIPD in optimizing therapeutic drug monitoring, several hurdles must be addressed to facilitate its

implementation in clinical practice. Key challenges include limited availability of robust data for model validation, unclear regulatory pathways for endorsing MIPD tools, and the high costs associated with software licenses and training healthcare professionals. Additionally, the complexity of MIPD models and tools can hinder their practical use, requiring user-friendly interfaces and continuous updates to maintain relevance and accuracy. Prospective clinical studies demonstrating improved outcomes, such as reduced toxicity or enhanced efficacy, would be valuable. Furthermore, collaborative efforts involving diverse stakeholders -such as researchers, clinicians, regulators, and patient groups- could support model validation and integration into routine care. Education and training programs tailored to healthcare providers will enhance trust and adoption of MIPD approaches. By addressing these challenges through targeted studies and multistakeholder collaboration, the widespread implementation of MIPD can become feasible and impactful.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.

## GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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# Use of Normothermic Perfusion Machines in Lung Transplantation: Consensus Statement of the Italian Society of Organ and Tissues Transplantation Group With DELPHI Method

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**Background:** *Ex vivo* lung perfusion (EVLP) is a technique for graft preservation, evaluation and treatment, that could expand donor pool for transplantation. Nevertheless, the wide spectrum of available platforms has generated disparities in use, outcome, and costs. This study is an attempt to create a national consensus on EVLP use by a group of experts from the Italian Society of Organ Transplantation.

**Methods:** The 9-member promoting committee was divided into 3 groups to propose statements. Using the DELPHI method 27 experts (three from each of the 9 lung transplant centres) voted agreement to each statement in 3 rounds. The cutoff for acceptance was set at 80% agreement.

**Results:** In the first vote, 52 statements were proposed, and an agreement was reached for 20 of them (38%). After revision, the second round resulted in a quorum for 36 out of 40 statements proposed (90%). At the third vote, agreement was confirmed for 36 statements (8 indications for use, 19 modalities for use, 13 evaluation parameters).

**Abbreviations:** DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; EVLP, *Ex vivo* lung perfusion; SITO, Italian Society of Organ and Tissues Transplantation; LTx, Lung transplantation; OCS, Organ Care System; REDCap, Research Electronic Data Capture platform.

**Conclusion:** The statements outlined in this document do not represent absolute guidelines, but rather recommendations. The statements selected and presented are therefore aimed to assist Italian clinicians in the use of an *ex vivo* normothermic perfusion platform in the right context.

**Keywords: EVLP, consensus paper, lung transplantation, methodology, Delphi**

## INTRODUCTION

Lung transplantation (LTx) is the preferred treatment option for patients with end-stage lung disease that has become unresponsive to medical therapy [1]. However, this treatment is still limited by the scarcity of suitable grafts (approximately 15%–30% of donors), which results in a significant mortality rate on the waiting list, estimated to be between 8%–13% [2]. In recent years, several strategies have been implemented to increase the donor pool. These include the use of lungs from extended-criteria donors [3] and DCDs [4].

The increased utilisation of non-standard grafts has been facilitated by the integration of *ex vivo* lung perfusion (EVLP) into clinical practice [5]. This procedure offers a potentially useful time window for both graft preservation and the evaluation and, possibly, reconditioning of lungs with questionable function [6, 7].

However, a range of protocols and devices are available for EVLP performance, including Lung Assist™ by Organ Assist®, XVIVO Perfusion System (XPS)™ by XVIVO®, Vivoline LS1™ by Vivoline Medical®, OCS™ by TransMedics® and the TorEX Lung Perfusion System by Traferox®. The clinical potential of these machines is still under investigation [8]. This characteristic determines a wide heterogeneity of EVLP use in clinical practice between different centres [9], making comparison impossible.

The absence of recommendations or guidelines that can be utilised at a national level engenders challenges in the realm of reimbursement for device utilisation. Presently, the financial burden of these devices falls exclusively upon the budget allocated by transplant centres. The objective of this study is to deliberate and achieve a consensus on the utilisation of EVLP in Italy, with the aim of producing evidence-based recommendations to standardise clinical practice and minimise the cost-benefit ratio.

## METHODS

The present study was initiated by a working group of the Italian Society of Organ and Tissue Transplantation (SITO) with a view to developing a national consensus on the use of EVLP platforms. The Delphi method was employed to gather expert opinions and structure the recommendations, a technique that has gained wide recognition for its systematic approach to achieving consensus among diverse expert groups [10, 11]. The Delphi standard methodology and the limited availability of comparative randomised controlled trials (RCTs) precluded the application of a formal evidence grading system. A promoting committee was established, comprising nine experts selected according to criteria described in **Table 1** from various disciplines, including five thoracic surgeons, two anaesthesiologists, one cardiac surgeon and one pulmonologist. These individuals represent five Italian lung transplant centres: Milan, Padua, Palermo, Siena and Turin. The committee was divided into three subgroups of three members each, tasked with drafting statements in three main categories: indications for use, operational methods, and evaluation parameters. Directors from the nine Italian lung transplant centres (see **Figure 1**; **Table 2**) nominated 27 experts (thoracic surgeons, anaesthesiologists and pulmonologists) to participate in the consensus process.

The proposed statements were evaluated using a four-point Likert scale in the first two rounds, followed by a dichotomous response (agreement/disagreement) in the third round. Furthermore, participants were granted the opportunity to provide commentary and substantiate their selections. The Delphi method's structured feedback cycles are particularly well-suited to areas with limited empirical evidence, such as evolving practices in EVLP [12, 13]. The data were collected and managed via a survey developed in the REDCap (Research Electronic Data Capture) platform, which is hosted at the Unit of Biostatistics, Epidemiology, and Public Health, Department of Cardiac-Thoracic-Vascular Sciences and Public Health at the University of Padua [14, 15]. The Unit of Biostatistics, Epidemiology, and Public Health provided comprehensive support for the entire data collection and analysis process. REDCap is a secure, web-based software platform designed to support data capture for research studies. It provides an intuitive interface for validated data capture, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads, and procedures for data integration and interoperability with external sources. A consensus threshold of 80% was established for the acceptance of the statement. Statements that did not meet the required standard were subjected to a process of refinement, informed by in-depth discussions and a review of the relevant literature. This iterative process was undertaken to ensure scientific rigour and alignment with best practices in consensus methodologies [16, 17].

In order to avoid the introduction of bias, responses were collected anonymously. Furthermore, participant demographics (e.g., educational background and workplace) were processed exclusively in aggregate form, with the purpose of describing the panel of experts. Prior to commencing the survey, the participants were provided with a comprehensive overview of the data processing procedures. Statistical analyses, performed using R software, calculated agreement percentages and assessed response consistency. These tools are frequently employed in health research to validate consensus processes and quantify agreement [18]. However, it is important to acknowledge the

**TABLE 1 |** Selection criteria for expert committee.

| Selection criteria |
|---|
| Clinical activity more than 5 years |
| Participation in at least 10 EVLP procedures |
| At least 5 publications in the field of lung transplantation |

limitations of the Delphi method. Firstly, there is the issue of expert bias, which arises from the selection of experts. This selection may influence the generalisability of the findings. Secondly, there is the lack of external validation, which arises from the method's reliance on shared expert opinions and knowledge without direct experimental verification. Notwithstanding these limitations, the Delphi method continues to be recognised as a established approach for generating expert-driven recommendations in fields with limited robust evidence [10, 19]. A flowchart illustrating the activities undertaken can be found in **Figure 2**.

## RESULTS

All 27 experts took part in the three votes. Following extensive deliberations, a consensus was reached on a total of 36 statements, encompassing 8 indications for use, 16 on methods of use, and 12 on graft assessment. A total of 52 statements were proposed during the first vote (see **Supplementary Tables S1–S3**), and agreement was reached for 20 of them (38%). It is evident from **Supplementary Figures S1–S3** that none of the proposed statements achieved a disagreement rate of more than 80% among the voting experts. The 32 statements that did not reach the required agreement were then reformulated by the respective committees (see **Supplementary Tables S4–S6**). In the subsequent vote, 40 statements were submitted with supporting literature: 8 on indications for use, 19 on methods of use and 13 on graft assessment parameters. The results of the vote established a quorum for a total of 36 statements, with four statements failing to reach the requisite level of agreement (see **Supplementary Figures S4–S6**). In the most recent dichotomous vote (see **Supplementary Figures S7–S9**), consensus was reached for a total of 36 statements (see **Tables 3–5**).

## DISCUSSION

Normothermic perfusion platforms are assuming an increasing role in lung transplantation as they represent an option for graft preservation, evaluation and possible reconditioning [20]. However, the broad spectrum of indications and protocols can prove perplexing and give rise to considerable divergence within the domain of evaluation modalities, outcomes and management costs. This is of particular importance in Italy, where a reimbursement procedure for the use of the device has not yet been implemented and the lack of shared recommendations

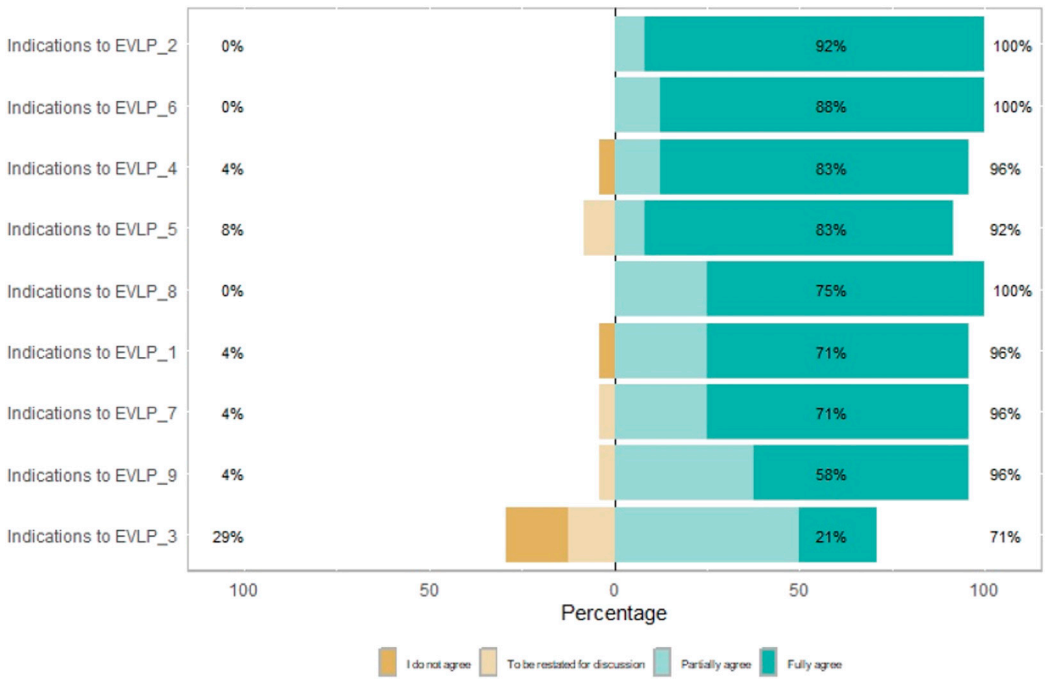


**FIGURE 1 |** Representative map of the 9 lung transplant centres in Italy. The size of the blue circle is proportional to the number of transplants performed in the year 2023.

**TABLE 2 |** EVLP activity for each centre.

| Transplant centre | Year of EVLP activity beginning | Volume activity |
|---|---|---|
| Bergamo | 2017 | 15 |
| Bologna | 2019 | 9 |
| Milano | 2011 | 71 |
| Padova | 2011 | 62 |
| Palermo | 2015 | 2 |
| Pavia | 2017 | 4 |
| Roma | 2012 | 6 |
| Siena | 2016 | 16 |
| Torino | 2011 | 49 |

limits the legislator. Consequently, the pre-eminent authorities in the domain of lung transplantation within the nation have determined the imperative to embark upon the formulation of a consensus on the utilisation of this apparatus, encompassing its indications, methodologies of application, and evaluation criteria for lungs subjected to EVLP.

The authors elected to prioritise percentage consensus rates over hierarchical levels of evidence in their methodological approach. Please refer to the supplementary materials for a comprehensive overview of the consensus results and to the following references for a detailed mapping of the sources.

## Indications to EVLP

Experts have agreed that, according to the available literature, EVLP has shown to allow extending the preservation window, ranging from few hours (4–6 h) [21–23], to extended durations (exceeding 12 h) [24]; Reported clinical experiences show a rate of graft unsuitability after EVLP <25% and comparable post-transplant outcome in EVLP treated graft recipients [25].

EVLP is also indicated in lung graft evaluation [7, 20, 26, 27], both from DBD [28] and DCD donors [27, 29–34]. In particular, the utilisation of EVLP should be contemplated in instances of doubtful or non-assessable organ function at retrieval [30, 35, 36]. In this context, EVLP platforms have also been shown to be effective in highlighting graft issues (infections, inflammation) that are not apparent in initial donor evaluation [36–38]. Furthermore, their utilisation should be contemplated in instances where logistical or clinical concerns have the capacity to prolong ischemic times [39], thereby facilitating a comprehensive evaluation and optimising the suitability of grafts for transplantation, even across substantial geographical distances [40, 41].

It was determined by the collective opinion of the experts that the decision to utilise EVLP should be made irrespective of the condition of the recipient, given that the utilisation of EVLP has already been documented for both standard [41] and urgent recipients [42]. In the absence of exclusion criteria for the utilisation of EVLP for recipients [43], as outlined in referral guidelines [44, 45], lungs from donors exhibiting significant infection, such as full-blown pneumonia, purulent discharge or overt signs of aspiration during bronchoscopy, and severe irreversible structural damage to the graft, should be excluded from *ex vivo* perfusion [6, 43].

Finally, despite the plethora of reported successes in the literature [30, 35, 37, 46], experts concur that the role of EVLP in active lung graft reconditioning remains unrecognised, largely due to conflicting results [23, 26, 47]. The necessity for prospective multicentre randomised studies is evident in order to achieve a more precise definition of this issue.

## Methods of Use

It has been posited by experts in the field that there are three primary EVLP protocols (Lund, Toronto and OCS) [6, 9, 20, 48], though at present, there is an absence of studies that directly compare the relative merits of these protocols. The impact of the individual parameters of each device and protocol on organ function after EVLP, PGD development and post-operative outcome has yet to be evaluated. The optimal approach remains to be determined, as the debate surrounding the superiority of early versus delayed normothermic perfusion persists [49, 50]. The ambiguity arises from the ongoing discourse surrounding the optimal atrium configuration, namely, whether to employ an open or closed approach [21, 51–53]. The prevailing consensus is that the decision regarding the selection of the EVLP system to be employed rests with the individual transplant centre, contingent upon its preferences, experience, and accessibility. Furthermore, at this time, the results of studies comparing different perfusion solutions (cellular vs. acellular) remain inconclusive [54–56]. For short perfusion times, perfusion solutions with the addition of blood might offer an advantage for lung assessment [57]. Conversely, in prolonged EVLP, the use of acellular solutions might be advantageous in order to avoid the harmful effects of haemolysis [58].

A consensus was achieved on the modalities of circulation and ventilation, with particular reference to the timing of achieving the target flow [22, 28, 59–66] and the initiation of ventilation [6, 20, 67], as illustrated in **Table 4**. Specifically, a standardised protocol should be established to concurrently increase pulmonary blood flow and graft core temperature at the initiation of EVLP, in accordance with the target flow rate intended for maintenance during the procedure. Maintaining pulmonary arterial pressures below 15–20 mmHg was also recommended in order to avoid the development of oedema [53, 58, 66, 68, 69]. Furthermore, it was advised that low tidal volume (below 8 mL/kg predicted body weight) and a respiratory rate always below 20 acts/minute should be maintained to avoid ventilator-induced lung injury [6, 70–77]. Two other statements make specific recommendations for portable or static systems: In the context of portable EVLP (early normothermic perfusion), experts do not perceive a requirement to maintain lung inflation at the conclusion of retrieval as is customary [24]. This is due to the fact that hypothermic transport prior to graft insertion in the machine is not anticipated [60, 68], thereby circumventing the risk of barotrauma injury [78]. Conversely, for static EVLP, it is advised to execute recruitment manoeuvres prior to graft

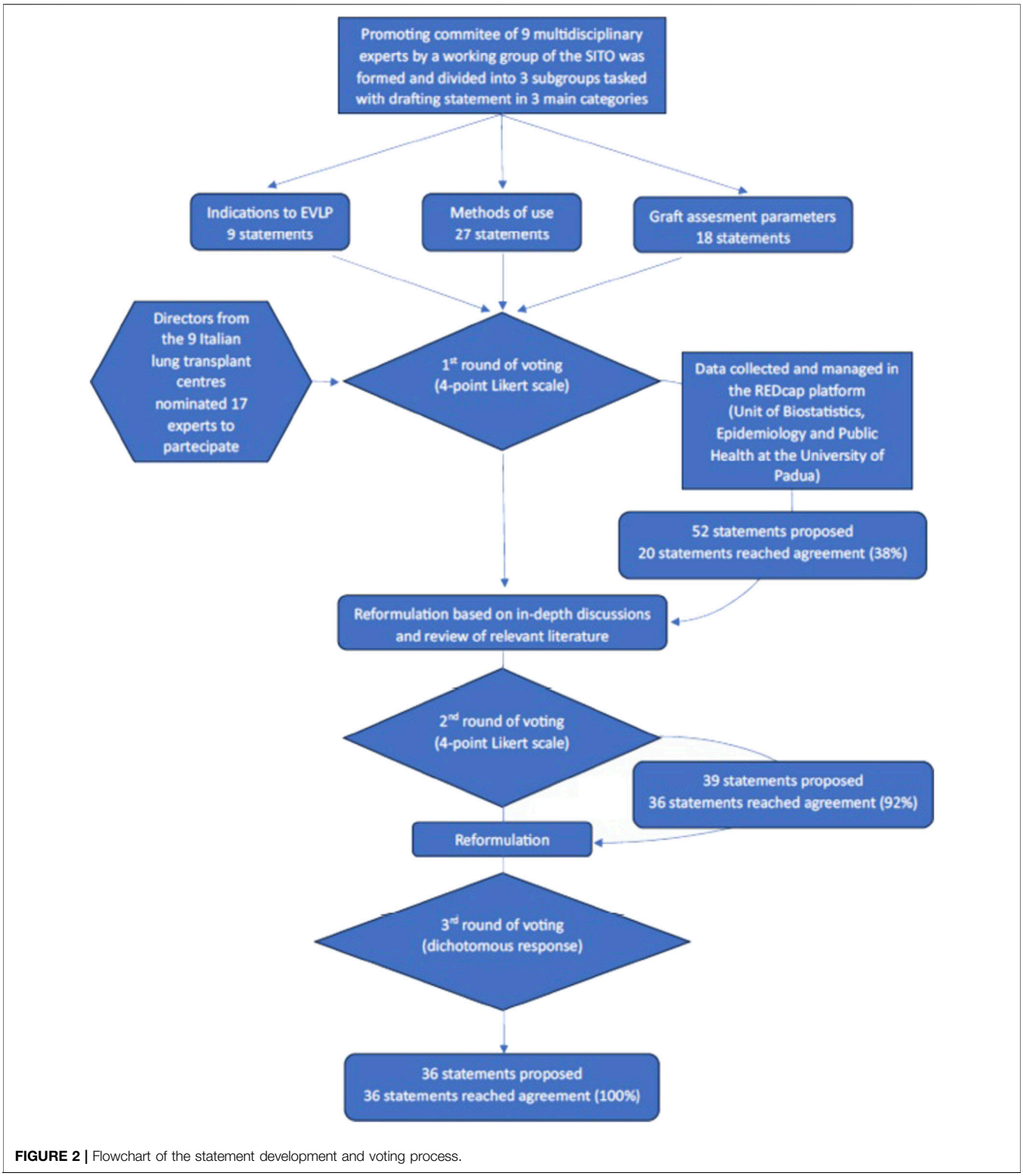


**FIGURE 2 |** Flowchart of the statement development and voting process.

function evaluation in order to ensure the homogenisation of ventilation distribution [6, 79, 80]. However, it has been specified that there is an absence of evidence to suggest that one recruitment modality is superior to another. In instances where air leakage from the parenchyma does not complicate recruitment, the repair of breaches with sutures or staplers is generally discouraged. This is due to the experience accumulated by experts over the years, which has shown that

**TABLE 3 |** Indications to EVLP.

| Statement | Consensus |
|---|---|
| EVLP can be used as an effective technique for organ preservation | 88.9% |
| EVLP is a useful platform for organ assessment | 100.0% |
| There is currently no strong clinical evidence for a role of EVLP in active organ reconditioning | 92.6% |
| The use of EVLP may find indication in both DBD and DCD donation of any class | 100.0% |
| EVLP can be used for graft, regardless of the clinical condition of the recipient | 96.3% |
| The use of EVLP is proposed in cases of donors with questionable organ function, or not evaluable at harvest | 100.0% |
| EVLP is usable in the case of logistical or clinical issues that have the potential to increase ischemia time | 100.0% |
| EVLP is not recommended for use in case of irreversible structural damage of the graft | 100.0% |

such procedures can cause lung damage, which in turn can exacerbate the progression of EVLP.

In the event of lung deflation, once potential causes associated with the circuit itself have been excluded, it is advised to undertake a flexible bronchoscopy to ascertain the presence of secretions and to aspirate them, if necessary. In the absence of other causative factors, the occurrence of deflation is a salient factor in the potential for graft injury.

In order to further improve the procedure, experts recommend considering the pronation of the lungs in EVLP, if safely possible; in fact, this could improve graft function, avoiding the development of oedema in the declivous regions [81, 82]. A wide range of options is available with respect to the type and dosage of antimicrobials, as no superior treatment has been identified [36, 37, 83–88].

It is evident that, upon ascertaining the suitability of organs, the optimal temporal parameters for lung separation remain to be elucidated. Indeed, some platforms allow the perfusion of one lung to continue during the implantation of the contralateral lung [89], further reducing cold ischemia periods. However, the potential benefit of this procedure [90] has yet to be demonstrated by comparative studies, and experts have agreed that further investigation is required. In any case, experts concur that, in the event of a split during EVLP or mono-pulmonary perfusion, adjustment of ventilation and circulation parameters is imperative [91, 92].

## Graft Assessment Parameters in EVLP

The expert emphasised that graft assessment during EVLP is based on multiple parameters, since one parameter alone is not sufficient to guarantee graft suitability for transplantation. Moreover, it is imperative to acknowledge that the trend over time holds greater significance than the absolute value (best or worst) in relation to all evaluation parameters. All available

**TABLE 4 |** Methods of use.

| Statement | Consensus |
|---|---|
| There are three most widely used of EVLP in clinical practice (Lund, Toronto, OCS), but no evidence exists, at present, regarding the superiority of one over the others | 100.0% |
| There are, at present, no differences in clinical results obtained between perfusions with acellular and cellular solution with concentrated hematins | 100.0% |
| Achievement of target flow must occur in a congruent time concomitant with lung rewarming | 100.0% |
| It is recommended to maintain pulmonary arterial pressures less than 15–20mmHg to reduce the risk of developing pulmonary oedema | 100.0% |
| In case of lung split during machine reperfusion, as well as in monopulmonary reperfusion, adjustment of target flow to the monopulmonary condition is mandated, maintaining control of mean PAP and pulmonary resistances as much as possible | 100.0% |
| It is recommended that lung ventilation should not begin until temperatures between 32 °C–34 °C have been reached | 100.0% |
| During the reperfusion process, it is recommended to maintain a respiratory rate of 7–12 acts/minute, and in any case always less than 20 acts/minute | 100.0% |
| Regarding static EVLP, it is recommended to assess lung function after a recruitment manoeuvre having the purpose of reopening collapsed lung regions | 100.0% |
| When performed for the purpose of portable EVLP (early normothermic perfusion), it is not strictly necessary to keep the lungs inflated at the end of retrieval, as hypothermic transport prior to graft insertion in the machine is not provided | 96.3% |
| When performed during EVLP, there is no evidence of superiority of one mode of recruitment over another | 100.0% |
| Pronation of the lungs during EVLP can be considered | 100.0% |
| In case of “minor” air leakage from the lung parenchyma that does not complicate parenchymal recruitment and organ evaluation, attempted breach repair with sutures or staplers is not recommended | 96.3% |
| In case of lung parenchyma deflation or failure to achieve adequate recruitment in the absence of problems with the ventilatory system, having verified proper circuit closure and the absence of frank areas of parenchymal air leakage, flexible bronchoscopy through the dedicated operative canal is recommended to check for secretions and aspirate them | 100.0% |
| In the case of lung split during machine reperfusion, or in the case of monopulmonary reperfusion, reduction of tidal volume from defined criteria for bipulmonary reperfusion is critical | 100.0% |
| Once organ suitability is defined, there is, at present, no evidence of the best timing and mode of lung separation and preservation of the second lung (hypothermia vs. EVLP continuation) | 100.0% |
| Since there is, at present, no evidence to support a better outcome with the use of one class of antimicrobials than the others, the decision on the use of the type and dosage of antimicrobials during EVLP is deferred to the experience of the transplant centre | 100.0% |

**TABLE 5 |** Graft assessment parameters in EVLP.

| Statement | Consensus |
|---|---|
| Evaluation of graft quality during EVLP is based on multiple standard physiological and objective parameters. One parameter alone is not sufficient to assess graft quality. In addition, at least two endobronchial assessments during *ex vivo* reperfusion phases are desirable | 100.0% |
| For all evaluation parameters, the trend over time should be considered more relevant than the absolute value (best or worst) | 100.0% |
| Flexible bronchoscopy through dedicated Bronco-Port is recommended to assess the presence of foamy fluid (oedema), haemorrhagic fluid, repletion with purulent secretions, or signs of aspiration | 100.0% |
| It is not recommended to use grafts in which it is verified through bronchoscopy during EVLP of frank plasmorrhea and signs of aspiration, or repletion of purulent secretions | 100.0% |
| Visual inspection at the end of lung parenchyma recruitment is recommended to detect features such as haemorrhagic infarction, appearance of infarct areas, and other abnormalities that may affect lung function and its suitability for transplantation | 100.0% |
| Palpatory inspection of the graft at the end of lung parenchyma recruitment is recommended to detect features such as decreased elasticity of the parenchyma itself or increased weight of the various areas, appearance of areas of thickening, and other abnormalities that may affect lung function and its suitability for transplantation | 96.3% |
| The assessment of PaO2/FiO2 value, in isolation, is never sufficient for the definitive evaluation of the goodness of the graft | 100.0% |
| At the end of adequate recruitment period and performance of hemogasanalysis in EVLP, PaO2/FiO2 values 350mmHg (or 300mmHg in case of using cellular solution) indicate doubtful graft performance. Notwithstanding, we defer to the experience of the transplant centre to evaluate graft quality according to all the multiple physiological and objective parameters necessary for this evaluation | 100.0% |
| Evaluation of pulmonary vascular resistance trends during the procedure is recommended. An increase in resistances should cause organ damage to be considered | 100.0% |
| Continued evaluation of perfusate leakage in the bell is recommended. Once anastomotic defects or frank parenchymal injury have been excluded, evolution to pulmonary oedema should be considered. Where feasible, assessment of weight change during the procedure may be indicative of possible organ oedema | 100.0% |
| Evaluation of static compliance of the isolated organ is recommended. Values of less than 70mL/cmH2O at the end of the evaluation, or worsening over time, should be considered doubtful graft performance | 96.3% |
| Radiography is recommended, if possible, to better define any regionality of organ damage (signs of oedema, imbibition, interstitial overload, parenchymal lesions); radiography alone cannot preclude organ use, only guide the decision | 100.0% |

protocols [60, 93–96] recommend that the decision regarding implantation should be made subsequent to consideration of the stability of lung perfusion and ventilation parameters, the PaO2/FiO2 ratio, and finally the organ condition based on visual and tactile examination.

With regard to the PaO2/FiO2 value, it is widely accepted among experts that a single sample is never sufficient for the definitive assessment of graft quality. Furthermore, a PaO2/FiO2 ratio of less than 350 mmHg (or less than 300 mmHg when using a cellular solution) should raise suspicion of poor graft performance. It has been posited that PaO2 does not always reflect the condition of the lung graft [97] and that the PaO2/FiO2 confidence interval for acceptance can vary greatly depending on the type of solution used (cellular or acellular) [98], due to the linearization of the relationship between oxygen content and PaO2 that occurs with acellular perfusate.

It has been demonstrated that a repeated objective examination during EVLP may result in the identification of areas of the lung parenchyma that are more susceptible to the accumulation of hydrostatic fluid, which can potentially lead to the development of pulmonary oedema [99]. The rate of fluid consumption in the reservoir, and, where feasible, the assessment of weight change along the procedure, should be considered a marker of organ oedema development [100].

Conversely, experts have recommended considering a questionable graft performance in cases of increased vascular resistance, static compliance with values below 70mL/cmH2O at the conclusion of the evaluation, or a deterioration of these parameters over time [80]. The findings of numerous studies [101, 102] demonstrate that these parameters serve as effective quantitative indicators of lung function, providing a valuable addition to the existing body of research.

Instrumental examinations have been considered equally fundamental: flexible bronchoscopy is useful for assessing the presence of bronchorrhea and signs of aspiration, or the repletion of purulent secretions that contraindicate the use of the graft for transplantation [95, 103, 104] and at least two endobronchial assessments during *ex vivo* reperfusion would be desirable. With regard to X-ray examinations, a special compartment for safely performing X-rays is provided in static platforms [95], but feasibility has also been described for portable platforms [105]. Finally, it must be acknowledged that, in contrast to conventional chest radiographs whose usefulness has been called into question [106–109], EVLP radiographs offer isolated images of the donor lungs with enhanced contrast. This allows for radiographic findings in EVLP that are associated with the outcome of lung transplantation [110, 111].

In light of the aforementioned factors, it is recommended by experts that an X-ray be performed in EVLP. However, it is also stressed by these experts that the imaging results should be considered as only one part of the evaluation. As previously stated, radiographs have the capacity to yield confounding data [107], and consequently, they must be evaluated and interpreted in conjunction with the extensive array of decision-making values provided by EVLP platforms.

## CONCLUSION

In conclusion, this consensus statement, which was reached by the Delphi method, represents a shared agreement among 27 experts from nine Italian lung transplant centres regarding normothermic *ex vivo* lung perfusion. In view of the paucity of multicentre randomised prospective studies comparing the three major EVLP protocols in use, it is imperative to emphasise that the statements outlined in this document do not represent absolute guidelines, but rather recommendations that are the direct expression of the experts' shared opinion and knowledge. The statements selected and presented are therefore aimed at assisting Italian clinicians in the complex decision to reject an organ, accept it for transplant after a period of cold ischemia, or use an *ex vivo* normothermic perfusion platform in the right context, with shared modalities and evaluation criteria. However, it is imperative for practitioners to acknowledge that, by their very nature, these statements cannot be regarded as definitive, as this is a newly introduced and evolving field with considerable potential and future prospects. Furthermore, the consensus presented in our manuscript reflects perspectives from a single national context. While the implementation of an external validation process to assess the transferability of the consensus across diverse healthcare systems and cultural contexts would undoubtedly enhance the robustness, generalizability and applicability of the findings, such an endeavour was beyond the scope and resources of the present project. Nevertheless, this may represent a significant direction for future research.

Notwithstanding the aforementioned limitations, this inaugural national document on the utilisation of *ex vivo* perfusion systems for lung transplantation has the potential to serve as a valuable clinical instrument. Moreover, it could serve as a unifying point for the pursuit of economic reimbursement for such procedures, a factor that presently imposes significant constraints on the dissemination of this pivotal technology within the transplant domain.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.2025.14762/full#supplementary-material

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# Kidney Transplant Outcomes With Non-Depleting Antibody Induction Therapy in Human Leucocyte Antigen Sensitised Recipients

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Lymphocyte depleting induction is recommended for kidney transplant recipients (KTRs) at high immunological risk, which traditionally includes those with detectable anti-human leucocyte antigen antibodies. Data to support this approach in the modern era of histocompatibility testing are limited. We investigated outcomes in KTRs who underwent Basiliximab induction between 2012–2023 in the UK. We stratified outcomes by levels of sensitisation and T cell epitope mismatch (PIRCHE-II) scores. 1348 KTRs were included; 859 (63.7%) were unsensitised, 351 (26.0%) sensitised (calculated reaction frequency [cRF] 1%–84%), and 138 (10.3%) highly sensitised (cRF 85%–100%). Patient survival, allograft survival, and death-censored graft survival (DCGS) were 97%, 94%, and 97% at 1 year, and 88%, 78%, and 84% at 5 years respectively. There were no differences in outcomes between unsensitised and sensitised recipients; graft survival was lower in highly sensitised patients. T cell epitope mismatch scores were higher in those with rejection at 1 year (ln[PIRCHE+1] 3.94 ± 1.01 no rejection vs. 4.25 ± 0.58 rejection, p = 0.02) and epitope mismatch was associated with early rejection in multivariable analyses (Odds Ratio 1.58, 95% CI 1.01–2.62). Hence, non-depleting induction provides good outcomes in unsensitised and sensitised KTRs. T cell epitope mismatches inform rejection risk in the first post-transplant year.

**Keywords: rejection, survival, Epitopes, HLA allosensitization, Basiliximab**

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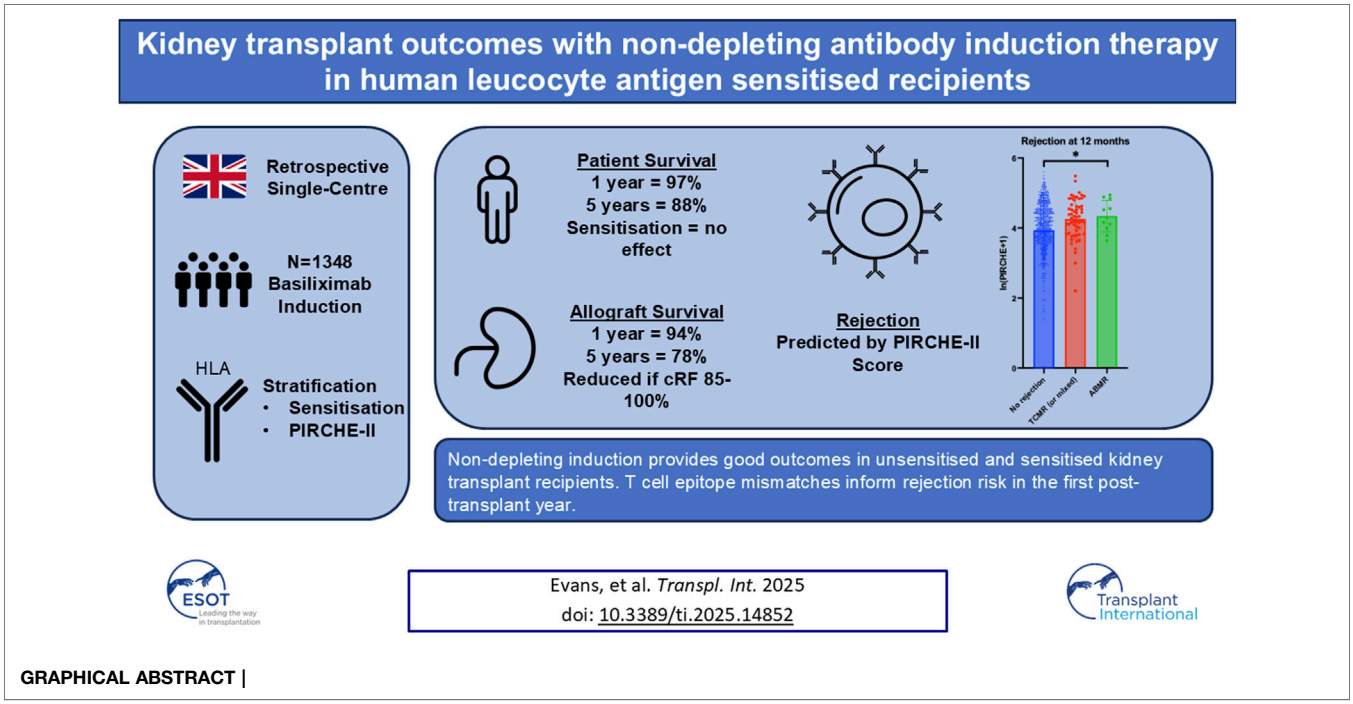
**Abbreviations:** ABMR, Antibody mediated rejection; ATG, Anti-thymocyte globulin; CI, Confidence interval; CMV, Cytomegalovirus; cPRA, calculated panel reactive antibodies; cRF, calculated reaction frequency; DBD, Donor after brain death; DCD, Donor after cardiac death; DCGS, Death censored graft survival; DSA, Donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, Human leucocyte antigen; HR, Hazard ratio; IL2-RA, Interleukin-2 receptor antagonist; IQR, Interquartile range; KDIGO, Kidney Disease Improving Global Outcomes; KTR, Kidney transplant recipient; OR, Odds ratio; PIRCHE, Predicted Indirectly ReCognizable HLA Epitope; SD, Standard deviation; TCMR, T cell mediated rejection; UK, United Kingdom; US, United States.




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Induction immunosuppression in the form of antibody therapy is utilised in most kidney transplant procedures. These agents primarily modulate the T cell response to foreign human leucocyte antigens (HLAs). This results in reduced rates of acute rejection and allows for a reduction in the use of other immunosuppressive agents, such as calcineurin inhibitors and corticosteroids, that have unwanted side effects when used at high dose [1].

Induction antibody therapy may be classified into agents that deplete T cells (e.g., Antithymocyte globulin [ATG] and Alemtuzumab [Campath]), B cells or complement, and agents that are non-depleting, which act by inhibiting cytokine signalling important in T cell activation and proliferation, e.g., IL-2 receptor antagonists (IL2-RAs) such as Basiliximab. In general, depleting agents provide more profound immunosuppression which is counterbalanced by increased infectious and malignant complications as well as an increased cost [2].

The choice of which induction agent to use continues to be a source of debate amongst transplant professionals globally, with marked variation in practice between centres even within the same country [3–5]. International guidelines published by Kidney Disease Improving Global Outcomes (KDIGO) recommend basing the choice of induction agent on an assessment of immunological risk, with IL2-RAs recommended as first line, and depleting antibodies used in cases at increased risk [6]. This approach is supported by guidelines from the United Kingdom (UK) [7].

One of the key determinants of immunological risk is the presence of preformed antibodies to HLAs. Traditionally, the presence of such antibodies was detected and identified using a panel of lymphocytes, with the relatively non-specific and subjective complement dependent cytotoxicity test, which was then reported as percentage panel reactive antibodies (PRA) [8]. Significant advances in histocompatibility methods, including the development of single antigen bead testing using Luminex based technology, have meant the identification of HLA antibodies now occurs with exquisite sensitivity and specificity [9]. The presence of antibodies is now quantified by the calculated PRA (cPRA), or the calculated reaction frequency (cRF) in the UK, with immunological risk primarily due to antibodies that are donor specific [10, 11]. These advancements in HLA antibody identification methodology have occurred in parallel with advancements in molecular HLA typing methods which have enabled the HLA typing of transplant pairs at all loci to a high resolution. Subsequent computational algorithms have been developed to inform HLA matching according to differences in structure at the epitope level [12, 13].

The KDIGO guidelines, published 13 years ago, are primarily based on studies that pre-date these advancements in histocompatibility and immunogenetics [6]. For example, there have been 2 large trials comparing ATG with IL2-RA induction in patients at increased immunological risk, and inclusion was based on the historic assessment of HLA sensitisation with PRA in both [14, 15]. Moreover, the pivotal study by Brennan et al. compared ATG to Basiliximab in the setting of maintenance immunosuppression with cyclosporin, subsequently shown to be inferior to tacrolimus based regimens [16]. As such, the relevance of these historic guidelines to the contemporary management of kidney transplant recipients should be questioned, and cohorts reporting outcomes in patients

managed in the modern era of histocompatibility testing are required.

For over a decade, our centre protocol has been to use IL2-RA induction for all kidney transplant recipients. This provides a unique opportunity for the assessment of kidney transplant outcomes when non-depleting induction therapy is used across a range of immunological risk. In this study, we determine patient and allograft outcomes of kidney transplants undertaken with Basiliximab induction. We assess outcomes stratified by current standard and novel measures of immune risk.

# PATIENTS AND METHODS

## Study Design, Setting, and Participants

We undertook a single-centre, observational, cohort study of kidney transplant recipients who underwent transplantation at the Royal Free Hospital, London, UK. Adult patients (aged >18 years) who underwent kidney alone transplantation and had induction therapy with Basiliximab between 1st January 2012 and 31st December 2022 were included. Patients who underwent multiorgan transplant, and those who had induction with depleting antibodies, or in whom the induction agent was unclear, were excluded.

Our centre provides kidney transplant services to a multi-ethnic population from a large geographical area in north central London and Hertfordshire. Approximately 130 kidney alone transplants are undertaken each year. For the entire study period, the unit protocol was for all patients to undergo induction with Basiliximab, 20 mg administered intravenously on the day of transplant, repeated on postoperative day 4. The maintenance immunosuppression and infectious prophylaxis protocols are outlined in **Supplementary File 1 [1]**. Ultimately around 70% of recipients are managed steroid free long term [17]. We follow a pre-emptive strategy for the management of cytomegalovirus (CMV) and protocol biopsies are not performed. HLA antibodies are routinely measured at 1-, 3-, 6- and 12-month after transplant, and yearly thereafter. Biopsies are performed if an HLA antibody is donor specific and its development is associated with evidence of graft dysfunction (e.g., change in creatinine or development of proteinuria).

## Variables, Data Sources and Measurement

Data were documented prospectively within electronic health records and retrospectively analysed. Clinical variables related to the donor (age, sex, and donor type), recipient (age, sex, ethnicity, and cause of end stage kidney disease), and the transplant (pre-emptive, previous transplant, and mismatch at HLA-A, -B, and -DR loci) were recorded.

### Assessment of HLA Sensitisation

Patients were grouped according to levels of HLA sensitisation. Levels of sensitisation were determined using the cRF at the time of transplantation. This measure represents the percentage of the previous 10,000 blood group identical kidney donors against whom the recipient has HLA antibodies. The inclusion of blood group distinguishes the cRF from the assessment of sensitisation using the calculated panel reactive antibody, which is the predominant method outside of the UK [18]. Details of the techniques used for antibody identification and HLA typing are outlined in **Supplementary File 1 [2]**. Patients were categorised as unsensitised (cRF 0%), sensitised (cRF 1%–84%), and highly sensitised (cRF 85%–100%); a subgroup analysis was undertaken in patients who were very highly sensitised (cRF 98%–100%).

### Assessment of T cell Epitope Mismatch

In a subset of patients with the necessary molecular HLA typing, T cell epitope mismatches were determined. T cell epitope mismatches were quantified using Predicted Indirectly ReCognizable HLA Epitope (PIRCHE-II) scores.[1] This scoring system employs a computational algorithm using *in silico* antigen presentation pathway analysis to predict the number of mismatched HLA peptides that can be presented in the context of recipient HLA class II [13]. The PIRCHE-II score is the sum of all donor-derived candidate peptides that have a predicted binding affinity to the recipients HLA class II of less than 1000 nM [19]. Scores were transformed using the natural logarithm for analysis [20], with a score of 1 added to all recipients to allow inclusion of patients with a PIRCHE-II score of 0, as has been undertaken in previous analyses [21].

## Outcome Measures

Patient and allograft outcomes were recorded at 12-, 36-, and 60-month after transplantation. Primary outcome measures were patient survival, graft survival, and death-censored graft survival. We also determined graft function (creatinine and estimated glomerular filtration rate [eGFR]), rates and type of biopsy proven rejection, rejection free allograft survival, infectious complications including CMV and BK viremia, and the development of malignancy. Outcomes were stratified by cRF and PIRCHE-II scores.

## Statistical Methods

Data are reported as number and percentages for categorical variables and mean and standard deviation (SD) or median and interquartile range (IQR) for numerical variables depending on data distribution. Categorical variables were compared using the Fisher's exact or Chi-squared test. Numerical variables were compared between 2 groups using the Mann–Whitney or an unpaired *t* test, and across greater than 2 groups with a one-way analysis of variance. Kaplan-Meier survival curves were plotted for patient and allograft outcomes, with differences between groups assessed using the log-rank test. Multivariable logistic regression analyses were undertaken to determine clinical variables associated with rejection at 12-month. Odds ratios (OR) and 95% confidence intervals (CIs) were determined for each variable. Multivariable cox regression analyses were undertaken to determine clinical variables associated with patient survival, graft survival, DCGS, and rejection-free

[1]https://www.pirche.com

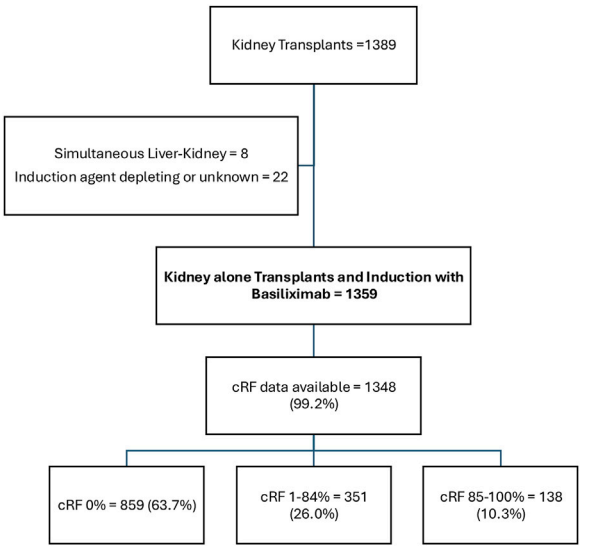



**FIGURE 1 |** Cohort Description.

allograft survival over 60-month of follow-up. Hazard ratios (HR) and 95% CIs were determined for each variable. Variables included in multivariable models were recipient demographic variables and clinical variables with a p value of <0.05 in univariable analyses. These included recipient age, sex, and ethnicity, transplant type (live/DBD/DCD; pre-emptive or not; first or subsequent graft), HLA-mismatch, cRF, and ln(PIRCHE+1). Models were developed with cRF and ln(PIRCHE+1) as both continuous and categorical variables. Analysis was performed using GraphPad Prism version 10.[2] A *p*-value of ≤0.05 was considered statistically significant.

## Ethics Statement

The study involved the retrospective analysis of routinely collected clinical data and, as such, was exempt from formal review board approval.

# RESULTS

## Cohort Description

1389 kidney transplants were undertaken during the study period. Of these, 1359 (97.8%) were kidney alone transplants that underwent induction with Basiliximab and were included in the analysis (**Figure 1**). Recipients had a mean age of 50.0 ± 14.0 years, 494 (36.7%) were female, 615 (45.3%) were of white ethnicity, and diabetes was the cause of ESKD in 307 (22.6%) patients (**Table 1**). 374 (27.5%) patients underwent living donor kidney transplant, and 985 (72.5%) patients underwent deceased donor kidney transplant. Transplants were pre-emptive in 323 (23.8%) patients and represented a first kidney transplant in 1168 (86.0%) cases.

[2]http://www.graphpad.com

cRF data were available in 1348 (99.2%) patients. 859 (63.7%), 351 (26.0%), and 138 (10.3%) patients had cRFs of 0% (unsensitised), 1%–84% (sensitised), and 85%–100% (highly sensitised) respectively. 59 (4.4%) patients had a donor specific antibody (DSA) detectable at the time of transplant (antibodies against all HLA loci were represented, median fluorescence intensity ranged 971–8000), and 36 (2.8%) patients had a DSA detectable in historical sera. Highly sensitised patients were younger, more commonly female, less commonly white, less frequently underwent living or pre-emptive kidney transplantation, and had a better total match at HLA-A, -B, and -DR loci.

## Patient and Allograft Outcomes

1080 (79.5%) patients were followed up to 12 months, 844 (62.1%) patients to 36 months, and 659 (48.5%) patients to 60 months. In the entire cohort, patient survival was 96.9%, 93.6%, and 87.6%, allograft survival was 93.9%, 88.4%, and 78.3%, and DCGS was 96.9%, 92.1%, and 84.5% at 12-, 36-, and 60-month respectively (**Table 2**). Patient survival was not different according to cRF categories, whereas allograft survival and DCGS were lower in highly sensitised patients compared to other cRF groups (**Figure 2**). Subgroup analyses of outcomes in very highly sensitised patients (cRF 98%–100%) and in deceased donor kidney transplants are outlined in **Supplementary File 1 [3], [4]**; patient and allograft outcomes followed similar trends to the wider cohort, albeit did not reach statistical significance for all outcomes. There was no difference in patient or allograft survival between sensitised patients with and without a preformed DSA (detected either at the time of transplant or historically).

## Graft Function, Rejection, Infection and Malignancy

Median (IQR) creatinine of all patients was 125 (102–158) μmol/L, 129 (102–175) μmol/L, and 130 (102–187) μmol/L at 12-, 36-, and 60-month post-transplant; eGFR was 50 (38–64) mL/min, 47 (33–64) mL/min, and 48 (32–64) mL/min at the same timepoints respectively (**Table 3**; **Supplementary File 1 [5]**). There were no differences in GFR between cRF categories at any of the follow-up time points (**Table 3**, **Supplementary File 1 [6]**).

Rejection rates (cumulative) were 9.35%, 10.31%, and 11.53% in patients followed-up to 12-, 36-, and 60-month. Rejection was more common in highly sensitised patients at all time points and there was more antibody mediated rejection (ABMR) within the first 12 months as levels of sensitisation increased (**Table 3**). 11 (61%) of 18 highly sensitised patients that experienced rejection at 12-month were very highly sensitised (cRF 98%–100%); outcomes in very highly sensitised patients are summarised in **Supplementary File 1 [7]**. As with highly sensitised patients, rejection was higher in very highly sensitised compared to other cRF categories at all time points, and ABMR occurred more frequently, albeit T cell mediated rejection (TCMR) remained the commonest type of rejection overall. Rejection free allograft survival was worse in highly sensitised and very highly sensitised patients compared to other cRF groups, primarily

**TABLE 1 |** Clinical characteristics of the cohort.

| Clinical Variable | Whole population | cRF 0% | cRF 1%–84% | cRF 85%–100% | P-value |
|---|---|---|---|---|---|
| Number of patients | 1359 | 859 | 351 | 138 | |
| Donor Variables | | | | | |
| Age (mean; SD) | 48.30 (14.67) | 48.86 (14.60) | 47.77 (14.31) | 45.99 (15.81) | 0.07 |
| Donor type | | | | | |
| Live | 374 (27.5) | 254 (29.6) | 102 (29.1) | 18 (13.1) | **<0.0001** |
| Donor after Brain Death (DBD) | 628 (46.2) | 367 (42.7) | 167 (47.6) | 86 (62.3) | |
| Donor after Cardiac Death (DCD) | 357 (26.3) | 238 (27.7) | 82 (23.4) | 34 (24.6) | |
| Recipient Variables | | | | | |
| Age (mean; SD) | 49.95 (13.98) | 50.51 (14.39) | 49.43 (13.34) | 47.28 (13.00) | **0.032** |
| Sex (n = female; %) | 494 (36.4) | 249 (29.0) | 166 (47.3) | 73 (52.9) | **<0.0001** |
| Ethnicity | | | | | |
| White (n; %) | 615 (45.3) | 414 (48.2) | 146 (41.6) | 51 (37.0) | **0.0063** |
| Asian (n; %) | 408 (30.0) | 255 (29.7) | 113 (32.2) | 38 (27.5) | |
| Black (n; %) | 336 (24.7) | 190 (22.1) | 92 (26.2) | 49 (35.5) | |
| Cause of ESKD | | | | | |
| Diabetes (n; %) | 307 (22.6) | 217 (25.3) | 64 (18.2) | 23 (18.2) | **0.0094** |
| Polycystic kidney (n; %) | 105 (7.7) | 73 (8.5) | 22 (8.5) | 10 (6.3) | |
| Other/unknown (n: %) | 947 (69.7) | 569 (66.2) | 265 (66.2) | 105 (75.5) | |
| Transplant Variables | | | | | |
| Pre-emptive (n; %) | 323 (23.8) | 219 (25.5) | 85 (22.9) | 16 (11.5) | **0.0008** |
| First transplant (n; %) | 1168 (86.0) | 821 (95.6) | 288 (82.1) | 51 (37.0) | **<0.0001** |
| Total HLA-A, -B, -DR Mismatch (mean; SD) | 2.99 (1.37) | 3.08 (1.31) | 2.96 (1.37) | 2.41 (1.49) | **<0.0001** |
| Total HLA-A, -B, -DR Mismatch 0–3 (n; %) | 900 (66.2) | 561 (65.3) | 231 (65.8) | 105 (76.1) | **0.0396** |

*Significant results are highlighted in bold.*

**TABLE 2 |** Patient and Allograft outcomes at 12-, 36-, and 60-month in the whole population, and in unsensitised (cRF 0%), sensitised (cRF 1%–84%), and highly sensitised (cRF 85%–100%) patients.

| Outcome | Whole population | cRF 0% | cRF 1%–84% | cRF 85%–100% | P-value (comparing all cRF categories) | P-value (cRF 0% vs. cRF 1%–84%) | P-value (cRF 0% vs. cRF 85%–100%) | P-value (cRF 1%–84% vs. cRF 85%–100%) |
|---|---|---|---|---|---|---|---|---|
| Patient survival | | | | | | | | |
| 12 months | 1047/1080 (96.94) | 653/669 (97.61) | 290/298 (97.32) | 104/113 (92.04) | 0.16 | 0.82 | **0.0054** | **0.02** |
| 36 months | 790/844 (93.60) | 482/514 (93.77) | 240/252 (95.24) | 68/78 (87.18) | 0.39 | 0.51 | 0.054 | **0.019** |
| 60 months | 577/659 (87.56) | 349/400 (87.25) | 183/202 (90.59) | 45/57 (78.95) | 0.34 | 0.28 | 0.10 | **0.02** |
| Graft Survival | | | | | | | | |
| 12 months | 1014/1080 (93.89) | 635/669 (94.92) | 284/298 (95.30) | 95/113 (84.07) | **0.0018** | 0.87 | **0.001** | **0.0004** |
| 36 months | 746/844 (88.39) | 455/514 (88.52) | 229/252 (90.87) | 62/78 (79.49) | 0.16 | 0.38 | **0.04** | 0.0092 |
| 60 months | 516/659 (78.30) | 306/400 (76.50) | 170/202 (84.15) | 40/57 (70.18) | **0.027** | **0.034** | 0.32 | **0.02** |
| Death Censored Allograft Survival | | | | | | | | |
| 12 months | 1014/1047 (96.85) | 635/653 (97.24) | 284/290 (97.93) | 95/104 (91.35) | 0.09 | 0.66 | **0.007** | **0.0053** |
| 36 months | 746/810 (92.10) | 455/490 (92.86) | 229/244 (93.86) | 62/76 (81.58) | **0.0324** | 0.76 | **0.0033** | **0.0024** |
| 60 months | 516/611 (84.45) | 306/364 (84.07) | 170/191 (89.01) | 40/56 (71.43) | **0.0079** | 0.13 | **0.04** | **0.0024** |

*Significant results are highlighted in bold.*

driven by increased rejection in the first post-transplant year (**Figures 3A,B**). Rejection free allograft survival was also worse in sensitised patients with a preformed DSA detected either at the time of transplant or in historical sera, compared to sensitised patients without a preformed DSA (**Figure 3C**).

CMV viremia occurred in 259 (23.98%) patients within the first post-transplant year. BK viremia of any level occurred in 137 (12.69%) patients and BK viremia $>10^4$ copies/mL occurred in 65 (6.02%) patients. There was no difference in the prevalence of either infection between cRF categories (**Table 3**). At 60-month

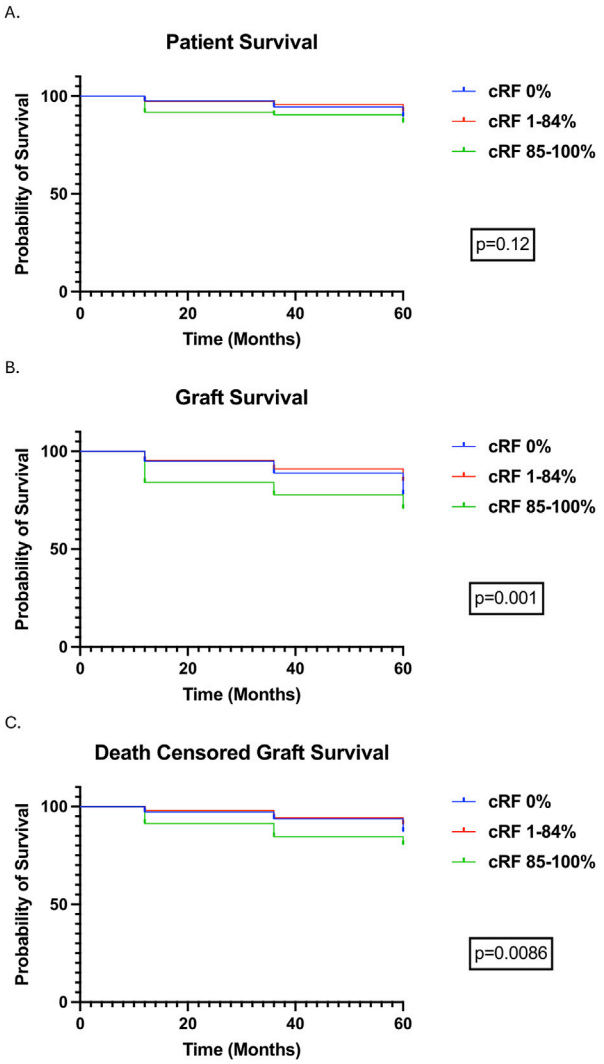



**FIGURE 2 |** Transplant Outcomes over a follow-up period of 5 years stratified by cRF. Survival curves were compared using the logrank test. **(A)** Patient Survival, **(B)** Allograft Survival, **(C)** Death Censored Allograft Survival.

post-transplant, 53 (8.04%) patients had developed a malignancy, and 39 (5.91%) patients had experienced a cardiovascular event. There were also no differences in these events between cRF categories (**Table 3**).

## T cell Epitope Mismatch and Rejection

T cell epitope mismatch data were available in 825 patients; the baseline clinical characteristics of these patients are outlined in **Supplementary File 1 [8]**. 740 (89.7%) and 335 (40.6%) patients completed follow-up to 12 and 60 months respectively. Mean ln(PIRCHE+1) scores were 4.249 ± 0.583 and 3.973 ± 1.005 in patients with and without rejection at 12-month ($p = 0.022$), and 4.102 ± 0.669 and 3.909 ± 0.987 in patients with and without rejection at 60-month ($p = 0.27$) (**Figures 4a, b**).

Patients were divided into PIRCHE score quartiles with quartile 1 (Q1 PIRCHE) having the lowest and quartile 4 (Q4 PIRCHE) having the highest PIRCHE scores. Rejection of any type at 12 months occurred in 9 (5.63%) patients in Q1 PIRCHE and 16 (10.74%) patients in Q4 PIRCHE ($p = 0.29$); TCMR occurred in 8 (5.56%) and 15 (11.81%) patients in Q1 and Q4 PIRCHE respectively ($p = 0.27$). There was a stepwise increase in rejection (both any rejection and TCMR) with each increase in PIRCHE quartile but this did not reach statistical significance (**Figure 4c**). There were no differences in DCGS or rejection free allograft survival when patients were stratified by PIRCHE scores (**Figure 4d**).

Multivariable logistic regression analyses were undertaken to determine clinical variables associated with rejection at 12-month (**Table 4**). Ln(PIRCHE+1) was associated with rejection at 12-month (Odds Ratio 1.576, 95% CI 1.006–2.618) whereas cRF was not.

## Multivariable Analyses of Patient and Graft Outcomes

Cox regression analyses were undertaken to determine clinical variables associated with patient survival, graft survival, DCGS, and rejection-free allograft survival (**Table 5**; **Supplementary File 1 [9], [10]**). A higher PIRCHE score (HR 1.350, 95% CI 1.028–1.817) was associated with worse rejection-free allograft survival, whereas cRF was not associated with any outcome.

# DISCUSSION

## Key Results

Induction immunosuppression is widely used in kidney transplantation but there is marked variation in which induction regimen is used. The relevance of guidelines that recommend depleting antibody induction based on traditional assessments of immune risk to the contemporary management of kidney transplant recipients is unknown. We therefore assessed outcomes in kidney transplant recipients who underwent induction with non-depleting antibody therapy in the modern era of histocompatibility testing, with outcomes stratified by HLA sensitisation determined by single antigen bead testing, and T cell epitope mismatches determined by PIRCHE-II scores.

We included an ethnically diverse cohort of 1359 kidney transplant recipients who underwent induction with Basiliximab. Just over one third of the cohort were sensitised, and 1 in 10 recipients were highly sensitised to HLA antigens. Patient survival, graft survival and DCGS were 88%, 78%, and 84% at 5 years respectively, representing favourable outcomes compared to registry data [22, 23]. There were no differences in these primary outcomes in sensitised compared to unsensitised recipients; a reduction in graft survival and DCGS were restricted to highly sensitised recipients but remained 70% and 71% at 5 years. The 12-month rejection rate was 9% overall and the major rejection type was TCMR. As was seen with the outcome data, there was no difference in rejection rates in sensitised compared to unsensitised recipients; there was an increase in rejection restricted to the highly sensitised group at all follow-up timepoints, with a cumulative rejection rate of 26% in highly sensitised recipients who were followed up to 5 years. Rejection free allograft survival was worse in sensitised patients with a preformed DSA. The association of T cell epitope mismatches with outcomes was assessed in

**TABLE 3 |** Outcomes 12-, 36-, and 60-month in unsensitised (cRF 0%), sensitised (cRF 1%–84%), and highly sensitised (cRF 85%–100%) recipients.

| Outcomes | Whole population | cRF 0% | cRF 1%–84% | cRF 85%–100% | P-value (comparing all cRF categories) | P-value (cRF 0% vs. cRF 1%–84%) | P-value (cRF 0% vs. cRF 85%–100%) | P-value (cRF 1%–84% vs. cRF 85%–100%) |
|---|---|---|---|---|---|---|---|---|
| 12-month outcomes | | | | | | | | |
| Creatinine (μmol/l; median, IQR) | 125 (102–158) | 129 (104–162) | 118 (98–145) | 126 (96–166) | **0.0009** | **0.0005** | >0.99 | 0.26 |
| eGFR (ml/min; median, IQR) | 50 (38–65) | 50 (38–64) | 52 (40–66) | 47 (36–69) | 0.19 | 0.29 | >0.99 | 0.51 |
| Rejection (n; %) | 101/1080 (9.35) | 63/669 (9.42) | 20/298 (6.71) | 18/113 (15.93) | **0.021** | 0.17 | **0.044** | **0.0068** |
| TCMR (n; % of rejection) | 81/101 (80.20) | 56/63 (88.89) | 13/20 (65.00) | 12/18 (66.67) | **0.014** | **0.03** | **0.03** | >0.99 |
| ABMR (n; % of rejection) | 9/101 (8.91) | 2/63 (3.17) | 4/20 (20.00) | 3/18 (16.67) | **0.020** | **0.03** | **0.07** | >0.99 |
| Mixed/Both TCMR and ABMR (n; % of rejection) | 11/101 (10.89) | 5/63 (7.94) | 3/20 (15.00) | 3/18 (16.67) | 0.42 | 0.39 | 0.37 | >0.99 |
| CMV viremia (n; %) | 259/1080 (23.98) | 154/669 (23.02) | 77/298 (25.84) | 27/113 (23.89) | 0.63 | 0.37 | 0.81 | 0.80 |
| BK viremia (any level) (n; %) | 137/1080 (12.69) | 90/669 (13.45) | 30/298 (10.07) | 17/113 (15.04) | 0.24 | 0.17 | 0.66 | 0.17 |
| BK viremia (>10,000 copies/mL) (n; %) | 65/1080 (6.02) | 44/669 (6.58) | 13/298 (4.36) | 8/113 (7.08) | 0.35 | 0.24 | 0.84 | 0.31 |
| 36-month outcomes | | | | | | | | |
| Creatinine (μmol/l; median, IQR) | 129 (102–175) | 132 (107–179) | 121 (96–165) | 145 (102–193) | **0.0039** | **0.006** | >0.99 | 0.07 |
| eGFR (ml/min; median, IQR) | 47 (33–64) | 47 (32–62) | 50 (35–68) | 41 (28–69) | 0.089 | 0.21 | >0.99 | 0.19 |
| Rejection (n; %) | 87/844 (10.31) | 50/514 (9.73) | 17/252 (6.75) | 20/78 (25.64) | <**0.0001** | 0.22 | **0.0002** | <**0.0001** |
| TCMR (n; % of rejection) | 66/87 (75.86) | 43/50 (86.00) | 9/17 (52.94) | 14/20 (70.00) | **0.020** | **0.015** | 0.17 | 0.32 |
| ABMR (n; % of rejection) | 7/87 (8.05) | 1/50 (2.00) | 3/17 (17.65) | 3/20 (15.00) | **0.026** | **0.048** | 0.07 | >0.99 |
| Mixed/Both TCMR and ABMR (n; % of rejection) | 14/87 (16.09) | 6/50 (12.00) | 5/17 (29.41) | 3/20 (15.00) | 0.22 | 0.13 | 0.71 | 0.43 |
| Malignancy (n; %) | 52/844 (6.16) | 37/514 (7.20) | 10/252 (3.97) | 5/78 (6.41) | 0.21 | 0.11 | >0.99 | 0.36 |
| Cardiovascular event (n; %) | 34/844 (4.03) | 19/514 (3.70) | 12/252 (4.76) | 3/78 (3.85) | 0.74 | 0.56 | >0.99 | >0.99 |
| 60-month outcomes | | | | | | | | |
| Creatinine (μmol/l; median, IQR) | 130 (102–187) | 132 (105–192) | 128 (98–171) | 122 (96–201) | 0.15 | 0.19 | >0.99 | >0.99 |
| eGFR (ml/min; median, IQR) | 48 (32–64) | 47 (31–63) | 50 (32–66) | 46 (28–72) | 0.64 | >0.99 | >0.99 | >0.99 |
| Rejection (n; %) | 76/659 (11.53) | 45/400 (11.25) | 16/202 (7.92) | 15/57 (26.32) | **0.0016** | 0.25 | **0.005** | **0.0007** |
| TCMR (n; % of rejection) | 55/76 (72.37) | 33/45 (73.33) | 10/16 (62.50) | 12/15 (80.00) | 0.56 | 0.53 | 0.74 | 0.43 |
| ABMR (n; % of rejection) | 8/76 (10.53) | 5/45 (11.11) | 1/16 (6.25) | 2/15 (13.33) | 0.88 | >0.99 | >0.99 | 0.60 |
| Mixed/Both TCMR and ABMR (n; % of rejection) | 13/76 (17.11) | 7/45 (15.56) | 5/16 (31.25) | 1/15 (6.67) | 0.22 | 0.27 | 0.67 | 0.17 |
| Malignancy (n; %) | 53/659 (8.04) | 34/400 (8.50) | 13/202 (6.44) | 6/57 (10.53) | 0.51 | 0.42 | 0.62 | 0.39 |
| Cardiovascular event (n; %) | 39/659 (5.91) | 23/400 (5.75) | 14/202 (6.93) | 2/57 (3.51) | 0.67 | 0.59 | 0.76 | 0.54 |

*Significant results are highlighted in bold.*

825 recipients and there was an increase in PIRCHE-II scores in those with rejection in the first year. T cell epitope mismatch, but not cRF, was associated with early rejection and rejection free allograft survival in multivariable analyses.

## Interpretation

The 2012 KDIGO guidelines on the management of kidney transplant recipients recommends basing the choice of induction therapy on an assessment of immunological risk.

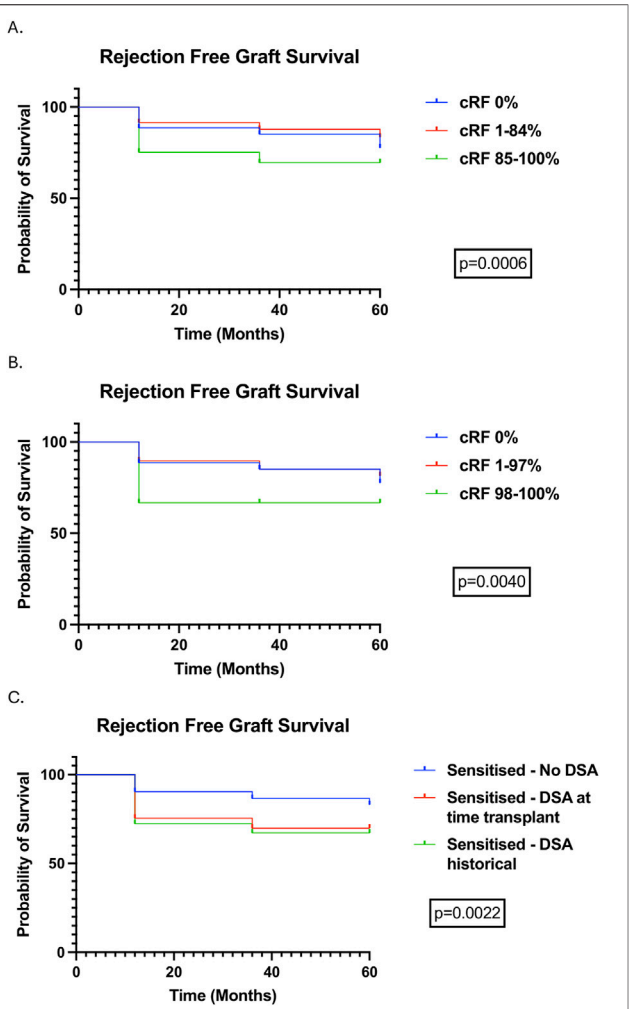



**FIGURE 3 |** Rejection free allograft survival (censored for death) stratified by cRF categories, demonstrating outcomes in highly sensitised **(A)** and very highly sensitised **(B)** patients, and stratified by the presence of DSAs, detectable at the time of transplant and historically **(C)**.

Non-depleting antibody therapy is recommended first line, and depleting antibody induction is recommended for patients at high immunological risk, defined by several factors including any level of HLA sensitisation [6]. This recommendation is underpinned by a meta-analysis published shortly before guideline development that highlighted a 25% reduction in graft loss at 1 year with IL2-RA compared with no antibody induction, and a 30% increase in risk of biopsy-proven acute rejection at 1 year when IL2-RA was compared to ATG; this came at the cost of a 75% increase in malignancy and 32% increase in CMV disease [2]. Two multicentre randomised studies have compared IL2-RA induction with ATG specifically in patients at increased immunological risk, with one of these studies making this comparison in the setting of cyclosporin based immunosuppression [14, 15]. Both studies demonstrated a reduction in rejection with ATG compared to IL2-RA at 1- and 5-years, but importantly no difference in patient or allograft outcomes were demonstrated, with follow up now reported out to 10 years [24–26]. A comparison of IL2-RA with ATG induction coupled with early steroid withdrawal in a predominantly white low immunological risk population was made in the Harmony study, which demonstrated no difference in rejection rates, patient or allograft outcomes at 1- and 5-years between the arms [27, 28]. A more recent pilot study demonstrated no difference between depleting and non-depleting induction in sensitised recipients without preformed DSAs [29]. The lack of proven benefit of depleting antibody induction on hard outcomes (i.e., patient and allograft survival), coupled with a more adverse side effect profile and cost, underlies our unit policy for Basiliximab induction in all. In this study we provide unique real-world data on outcomes from the use of this uniform approach in a large contemporary cohort of patients undergoing kidney transplantation across a range of immunological risk.

The outcomes of this strategy are summarised in **Supplementary File 2** and outlined alongside those seen in previous large randomised controlled trials of induction therapy that include high [14, 15, 24–26], low [16, 27, 28, 30, 31], and mixed [32, 33] immunological risk populations, in addition to recent registry data from the US [22] and the UK [23], and other large registry analyses [34, 35]. Patient survival in our cohort was 96.9% at 1-year and 87.6% at 5-years (96.1% and 85.4% in deceased donors), similar to patient outcomes previously reported. For example, patient survival was 95%–97% at 1-year and 85%–90% at 5-years in the low immunological risk Harmony population [27, 28]; UK-wide patient survival after deceased donor kidney transplant is currently 96% and 85% at 1- and 5-years respectively [23]. Graft survival (censored for death) was 96.9% and 84.5% at 1- and 5-years (96.0% and 82.1% in deceased donors), providing favourable outcomes compared to recently reported 5-year US graft survival of 66.1%–82.2% after deceased donor kidney transplantation [22]. Graft survival in our cohort did not differ in sensitised compared to unsensitised recipients, and in highly sensitised recipients was 91.4% and 71.4% at 1- and 5-years. These graft outcomes are similar to the outcomes in the high immunological risk population that underwent induction with ATG in the TAXI study, where graft survival was 85% and 76% at 1- and 5-years [15, 25]. Hence, the use of non-depleting antibody induction in our cohort in recipients across a range of immunological risk provided comparable patient and graft outcomes to previous cohorts where depleting antibody induction has been used. This occurred in an ethnically diverse population, which was predominantly managed steroid free.

Acute rejection rates have steadily declined over the last 2 decades. In 2000, rejection within the first year occurred in 17%–24% of kidney transplant recipients in the US [35], and early rejection occurred in 15%–16% of high immunological risk patients managed with depleting antibodies and 26%–27% of patients managed with non-depleting antibodies in the older clinical trials [14, 15]. 1-year rejection rates reduced to 8%–10% in the US in 2012, and the most recent data demonstrate rates of 5.7% and 6.0% in patients undergoing induction with

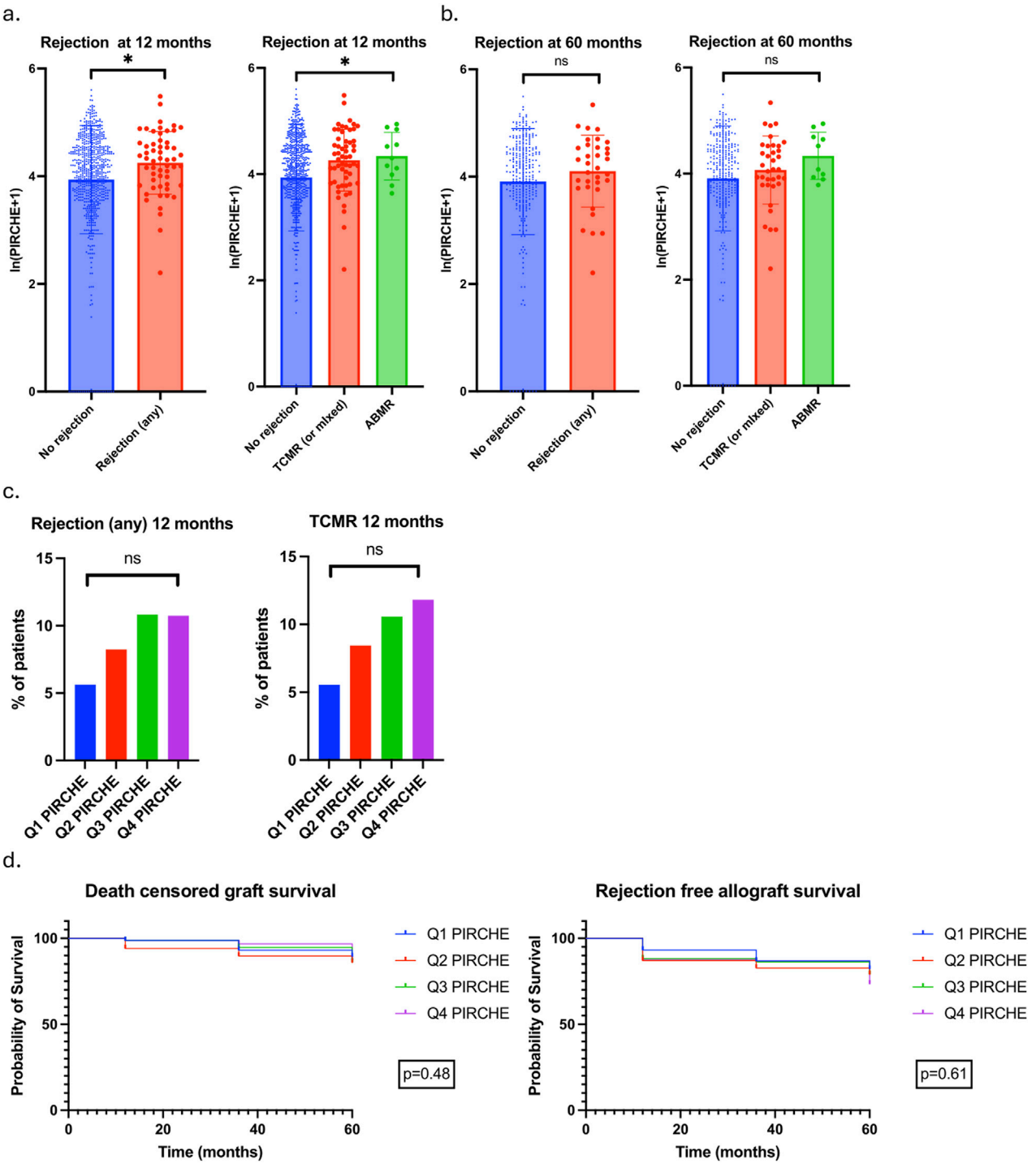



**FIGURE 4 |** Association between PIRCHE scores and rejection. **(a)** ln(PIRCHE+1) scores in patients with no rejection, any rejection, TCMR/mixed rejection and ABMR at 12 months. Mean and SD values plotted. No rejection 3.937 ± 1.005, Any rejection 4.249 ± 0.583, TCMR/mixed 4.262 ± 0.581, ABMR 4.330 ± 0.447. P value for unpaired T-test comparing No rejection to any rejection 0.022; P value for one-way ANOVA comparing no rejection and TCMR/mixed and ABMR 0.024. **(b)** ln(PIRCHE+1) scores in patients with no rejection, any rejection, TCMR/mixed rejection and ABMR at 60 months. Mean and SD values plotted. No rejection 3.909 ± 0.987, Any rejection 4.102 ± 0.669, TCMR/mixed 4.067 ± 0.644, ABMR 4.336 ± 0.444. P value for unpaired T-test comparing No rejection to any rejection 0.27; P value for one-way ANOVA comparing no rejection and TCMR/mixed and ABMR 0.27. **(c)** 12-month rejection rates in patients divided into PIRCHE score quartiles. Rates of any rejection and TCMR/mixed rejection plotted. **(d)** DCGS and rejection free allograft survival in patients stratified by PIRCHE quartile.

**TABLE 4 |** Multivariable logistic regression analyses of clinical variables associated with rejection (any type) at 12-month. Odds ratios and 95% confidence intervals are provided for each variable included within the model. cRF is included as a categorical variable and ln(PIRCHE+1) as a continuous variable.

| Clinical Variable | Odds Ratio for Rejection at 12-month | 95% confidence interval |
|---|---|---|
| Age at Transplant | 0.9844 | 0.9629 to 1.006 |
| Male Sex [reference = female] | 0.7775 | 0.4319 to 1.423 |
| Ethnicity [black; reference = white] | 0.9112 | 0.4449 to 1.825 |
| Ethnicity [Asian; reference = white] | 0.7587 | 0.3572 to 1.548 |
| DBD transplant [reference = live transplant] | 0.5766 | 0.2690 to 1.246 |
| DCD transplant [reference = live transplant] | 0.9076 | 0.4140 to 2.005 |
| Total HLA Mismatch at HLA-A, -B, -DR loci | 1.060 | 0.8121 to 1.382 |
| cRF 1%–84% (sensitised) [reference = unsensitised) | 0.6298 | 0.2819 to 1.296 |
| cRF 85%–100% (highly sensitised) [reference = unsensitised) | 1.856 | 0.7282 to 4.475 |
| Pre-emptive transplant [reference = not pre-emptive] | 1.336 | 0.6619 to 2.586 |
| Multiple grafts [reference = first graft] | 1.271 | 0.5613 to 2.708 |
| ln(PIRCHE+1) | **1.576** | **1.006 to 2.618** |

*Significant results are highlighted in bold.*

**TABLE 5 |** Cox regression analyses of clinical variables associated with patient survival, graft survival, death-censored graft survival and rejection-free allograft survival. Hazard ratios and 95% confidence intervals are provided for each variable included within the model. cRF is included as a categorical variable and ln(PIRCHE+1) as a continuous variable.

| Clinical Variable | Patient survival | | Graft survival | | Death-censored graft survival | | Rejection-free allograft survival | |
|---|---|---|---|---|---|---|---|---|
| | Hazard ratio | 95% confidence interval | Hazard ratio | 95% confidence interval | Hazard ratio | 95% confidence interval | Hazard ratio | 95% confidence interval |
| Age at Transplant | **1.069** | **1.040 to 1.102** | **1.025** | **1.008 to 1.042** | 1.002 | 0.9805 to 1.025 | 0.9884 | 0.9735 to 1.003 |
| Male Sex [reference = female] | 1.168 | 0.6102 to 2.357 | 0.7787 | 0.5059 to 1.211 | **0.5220** | **0.2934 to 0.9274** | **0.6102** | **0.4110 to 0.9089** |
| Ethnicity [black; reference = white] | **0.2849** | **0.09454 to 0.7038** | 0.7934 | 0.4692 to 1.318 | 1.392 | 0.7266 to 2.690 | 1.051 | 0.6621 to 1.658 |
| Ethnicity [Asian; reference = white] | 0.8578 | 0.4248 to 1.673 | 0.7817 | 0.4656 to 1.287 | 0.7682 | 0.3573 to 1.590 | 0.7088 | 0.4213 to 1.164 |
| DBD transplant [reference = live transplant] | 1.456 | 0.5695 to 4.503 | 1.639 | 0.8542 to 3.423 | 1.837 | 0.7645 to 5.155 | 0.9129 | 0.5388 to 1.579 |
| DCD transplant [reference = live transplant] | 0.9462 | 0.3476 to 3.023 | 1.772 | 0.8911 to 3.802 | **2.906** | **1.179 to 8.311** | 1.340 | 0.7796 to 2.346 |
| Total HLA Mismatch at HLA A-, B-, and DR-loci | 0.9653 | 0.7345 to 1.266 | 0.8341 | 0.6869 to 1.011 | 0.7680 | 0.5869 to 1.002 | 0.9243 | 0.7672 to 1.111 |
| cRF 1%–84% [reference = cRF 0%) | 0.9219 | 0.4399 to 1.828 | 0.8069 | 0.4804 to 1.315 | 0.7633 | 0.3803 to 1.461 | 0.6852 | 0.4154 to 1.097 |
| cRF 85%–100% [reference = cRF 0%) | 0.6697 | 0.1315 to 2.542 | 1.138 | 0.4991 to 2.407 | 1.348 | 0.5105 to 3.231 | 1.600 | 0.8361 to 2.918 |
| Pre-emptive transplant [reference = not pre-emptive] | **0.07196** | **0.004035 to 0.3371** | **0.3434** | **0.1504 to 0.6822** | 0.5652 | 0.2242 to 1.228 | 1.123 | 0.6878 to 1.779 |
| Multiple grafts [reference = first graft] | 1.747 | 0.5514 to 4.799 | 1.093 | 0.5253 to 2.128 | 0.9386 | 0.3539 to 2.181 | 1.277 | 0.7167 to 2.181 |
| ln(PIRCHE+1) as continuous variable | 1.197 | 0.7712 to 1.942 | 1.140 | 0.8571 to 1.551 | 1.124 | 0.7819 to 1.679 | **1.350** | **1.028 to 1.817** |

*Significant results are highlighted in bold.*

non-depleting and depleting induction respectively [22]. The current low rates of acute rejection encountered in routine clinical practice may lead us to question whether this outcome remains as relevant in contemporary analyses and clinical trials. Rejection within the first year in our cohort occurred in 9.4% of patients, consistent with US registry data from the last decade, and the 11% acute rejection rate seen in Harmony when Basiliximab induction was combined with a steroid free regimen [27]. Clinically relevant BK viremia (>$10^4$ copies/mL) was relatively infrequent (6%) in our cohort, and the non-depleting induction facilitated a pre-emptive approach to CMV management with acceptable rates of viremia. Cumulative 5-year rejection rates were 15% in Harmony [28], and 24.2% when IL2-RA induction was combined with steroid withdrawal in the Astellas corticoid study group trial [30, 31], with both these studies investigating populations at low immunological risk. Biopsy proven rejection within the first 5 years occurred in 11.5% of our cohort, despite the inclusion of patients across a range of HLA sensitisation. Two thirds of our cohort had a total HLA A-, B-, DR-mismatch of 3 or less, and our outcomes would support the current UK practice of including HLA matching within allocation schemes, especially in younger

recipients. Our data would suggest that acceptable rejection rates and good graft outcomes are achievable with non-depleting induction in all when HLA antibody detection occurs using sensitive methods and efforts are made to HLA match transplant pairs.

Stratification of immunological risk in transplantation has traditionally involved an assessment of the total level of sensitisation to HLA antibodies [36]. Guidelines suggest that any level of sensitisation leads to increased risk and the need for depleting antibody induction, but our data would not support this given no difference in rejection rates or graft outcomes between unsensitised and sensitised recipients. Moreover, cRF was not associated with rejection or graft outcomes in multivariable models. An increase in rejection and a reduction in graft survival was seen, however, in highly sensitised recipients.

Donor specificity of antibodies is a key determinant of immunological risk and previous studies provide conflicting evidence regarding outcomes on the use of Basiliximab in the setting of pre-existing DSAs. Some reports demonstrate that in the absence of a preformed DSA, sensitisation does not impact rejection and outcomes after Basiliximab induction [29, 37], whereas others have found that it does [38]. Our data demonstrate worse rejection free allograft survival in the presence of a preformed DSA, but no difference in other patient or allograft outcomes. Whether depleting induction therapy would have improved outcomes in the highly sensitised subgroup or in patients with a preformed DSA is not answered by this study.

Given most rejection episodes were T cell mediated across all cRF categories, we investigated if T cell epitope mismatches, determined using PIRCHE scores, were associated with rejection. These mismatches predict the risk of *de novo* T cell alloimmune responses, and higher mismatch scores have previously been associated with the development of DSAs, rejection, and graft survival in kidney transplantation [20, 39, 40]. Moreover, scores have been shown to provide additive information to traditional HLA matching [41], especially in sensitised recipients, and they may inform outcomes in patients with TCMR [42, 43]. Our data support a role for T cell epitope mismatch scores in the immunological assessment of transplant pairs undergoing non-depleting antibody induction. Scores were higher in patients with rejection at 1 year and were associated with early rejection and rejection free allograft survival in multivariable models, whereas levels of sensitisation were not.

### Limitations

In this study, we provide unique data on the use of non-depleting antibody induction in a large, ethnically diverse, cohort of kidney transplant recipients with outcomes stratified by HLA sensitisation at the time of transplant and T cell epitope mismatch scores. We report these outcomes from a centre that manages most patients steroid free, albeit we lack data on the maintenance immunosuppression regimens used at the individual level. We provide medium term outcomes to 5 years but lack outcome data thereafter. We anticipate our outcomes are generalizable to many healthcare systems, albeit the ethnic diversity of the cohort, the inclusion of HLA matching within organ allocation, and the ability to perform HLA typing at medium to high resolution may mean it is not applicable to all settings. Moreover, our results should be interpreted in the knowledge that there were relatively few patients in the highly sensitised group, and a larger number of patients would be required to confirm the findings of multivariable analyses. We assess the impact of T cell epitope mismatches in a large proportion of the cohort, but not all. We report rejection that was clinically apparent, but our lack of protocol biopsies may underestimate true prevalence [44]. Moreover, we lack data on the development of DSAs, which has been shown to be impacted by choice of induction therapy, especially in sensitised recipients [45]. We provide data on some complications of immunosuppression, including infection, malignancy, and cardiovascular disease, but we lack more granular data on the development of diabetes, hyperlipidaemia and blood pressure control, which are increasingly relevant to the transplant population we manage. Moreover, we lack patient reported outcomes on the uniform use of this non-depleting induction strategy.

## CONCLUSION

In summary, non-depleting antibody induction provides good outcomes for kidney transplant recipients managed using contemporary histocompatibility techniques across a range of immunological risk. Depleting antibody induction may not be necessary in all patients who are sensitised to HLA antigens, but may be considered in highly sensitised recipients and those with a preformed DSA. T cell epitope mismatch scores provide useful information during the immunological assessment of transplants being undertaken with non-depleting antibody induction. We propose that guidelines for induction therapy in kidney transplantation should be reviewed and updated.

## DATA AVAILABILITY STATEMENT

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

## ETHICS STATEMENT

Ethical approval was not required for the studies involving humans because The study involved the retrospective analysis of routinely collected clinical data and, as such, was exempt from formal review board approval. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal

guardians/next of kin in accordance with the national legislation and institutional requirements because The study involved the retrospective analysis of routinely collected clinical data and, as such, was exempt from formal review board approval.

## AUTHOR CONTRIBUTIONS

Participated in research conception and design: RN, RE, RF, AS, MH, and GJ. Undertook data acquisition, analysis and interpretation: RN, KB, NT, AH, AG, MJ, FK, AK, AN, GS, HS, SV, FT, NM, SF, RF, and RE. Drafted the initial manuscript: RN and RE. All authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTEREST

RE has received honorarium from Chiesi and Therakos.

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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.2025.14852/full#supplementary-material

**SUPPLEMENTARY FILE S2 |** Summary of outcomes in the cohort in this study, and in cohorts from previous randomised trials and registry analyses.

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# Impact of Induction Therapy in Low Immunological Risk Simultaneous Pancreas-Kidney Transplantation

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T-cell depleting agents and IL-2 receptor blockers are the most common induction therapies in simultaneous pancreas-kidney transplantation (SPKT), but the optimal choice remains debated. Here, we perform a retrospective, single-center study with SPKT recipients from 2000 to 2023. Basiliximab was used between 2008 and 2013, and thymoglobulin in other periods. Patients with prior transplants, calculated PRA >20%, pre-SPKT Donor-Specific Antibodies or graft primary non-function because technical reasons, were excluded. An Inverse Probability of Treatment Weighting (IPTW) was performed to adjust for confounding variables. 305 SPKT recipients were included, of which 172 (56%) received thymoglobulin and 133 (44%) basiliximab. Recipient (86% vs. 80%), pancreas (86% vs. 83%) and kidney (84% vs. 89%) death-censored graft survival at 20 years were comparable between groups. Basiliximab was not associated with an increased risk of patient death [HR 1.47 (0.69–3.14), P = 0.32], pancreas [HR 1.08 (0.55–2.10), P = 0.83] or kidney graft failure [HR 0.80 (0.38–1.70), P = 0.56] compared to thymoglobulin. Basiliximab did not significantly increase the risk of pancreas [OR 1.49 (0.84–2.63), P = 0.37] or kidney graft rejection [OR 1.31 (0.54–3.15), P = 0.20]. However, it was associated with significantly lower risk of CMV [OR 0.41 (0.23–0.72), P = 0.002] and BK virus infections [OR 0.31 (0.12–0.80), P = 0.02]. No significant difference was found in new-onset malignancy incidence. These results were maintained even after IPTW

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**Abbreviations:** ABMR, Antibody-Mediated Rejection; CIT, Cold Ischemia Time; CMV, Cytomegalovirus; cPRA, Calculated Panel Reactive Antibody; DBD, Donor After Brain Death; DCD, Donor after Circulatory Death; DGF, Delayed Graft Function; DM, Diabetes Mellitus; DSA, Donor Specific Antibodies; ESKD, End-Stage Kidney Disease; IPTW, Inverse Probability of Treatment Weighting; IQR, Interquartile Range; MFI, Mean Fluorescence Intensity; PDRI, Pancreas Donor Risk Index; PTLD, Post-Transplant Lymphoprolipherative Disorders; SD, Standard Deviation; SPKT, Simultaneous Pancreas-Kidney Transplantation; TCMR, T Cell-Mediated Rejection.

adjustment. In SPKT recipients with low immunological risk, basiliximab provides comparable long-term patient and graft outcomes to thymoglobulin while reducing the incidence of opportunistic infections.

**Keywords: simultaneous kidney pancreas transplantation, thymoglobulin, basiliximab, opportunistic infections, neoplasm**

## INTRODUCTION

Simultaneous Pancreas-Kidney Transplantation (SPKT) has proven to be an effective therapy for patients with End-Stage Kidney Disease (ESKD) and insulin-dependent Diabetes Mellitus (DM), reducing the incidence of major cardiovascular events while improving patient survival and quality of life [1–5].

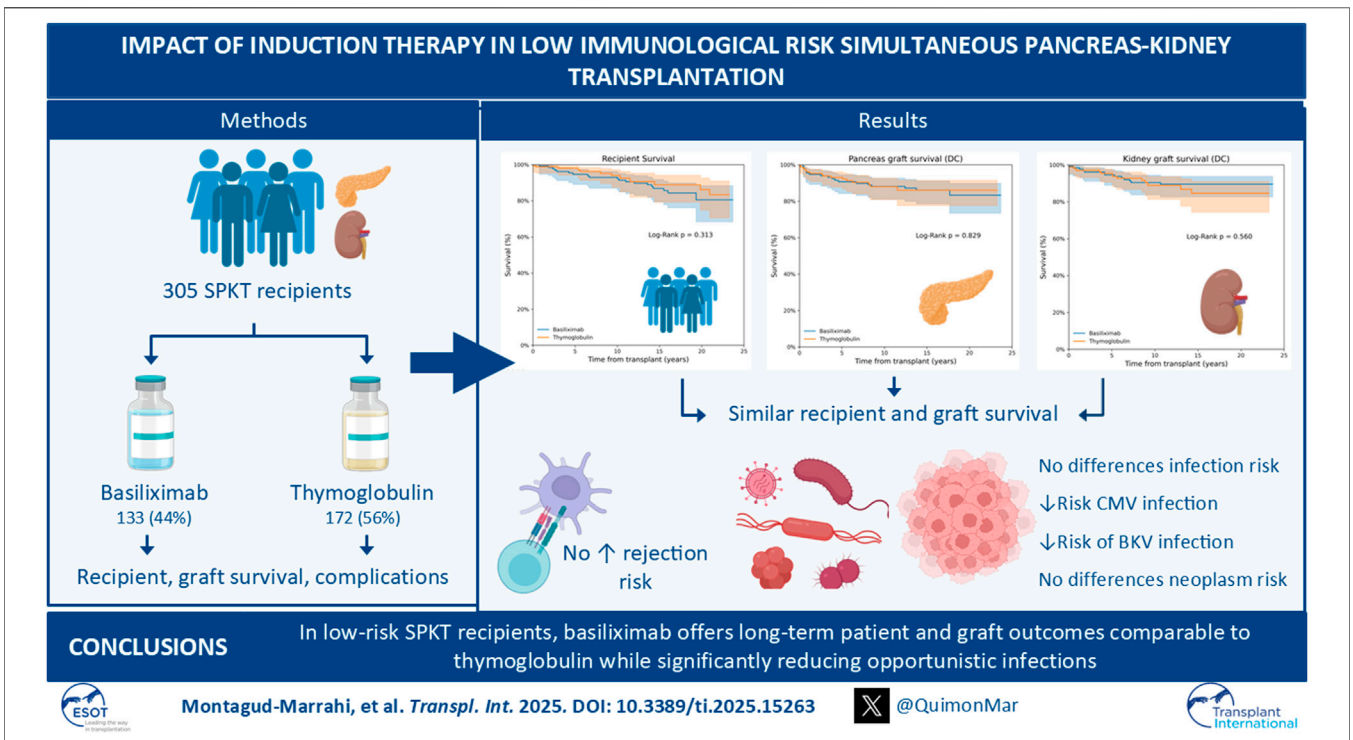
Despite significant advances in immunosuppressive therapy in recent years, allograft rejection remains one of the most common causes of graft loss, especially after 1 year post-transplant [5–7]. Immunosuppressive regimen for SPKT includes induction therapy, typically with either a T-cell depleting agent (e.g., thymoglobulin) or an IL-2 receptor blocker (e.g., basiliximab) administered in the immediate post-transplant period, followed by maintenance therapy, which usually consists of a combination of steroids, calcineurin inhibitors, and an antiproliferative agent (such as mycophenolate or mTOR inhibitors) [8]. Most transplant centers use T-cell depleting agents for SPKT as induction therapy, regardless of the recipient's immunological risk prior to transplantation [9, 10]. However, evidence comparing SPKT outcomes between T-cell depleting agents and basiliximab is controversial, particularly in patients with low immunological risk [8–10]. Identifying the most appropriate induction therapy for SPKT recipients is increasingly important, as T-cell depleting agents have been linked to higher rates of opportunistic infections and *de novo* malignancies, negatively impacting on recipient survival [10–12].

In the present study we compare post-transplant outcomes in SPKT recipients receiving either thymoglobulin or basiliximab as induction therapy. Specifically, we analyze patient and graft survival, rejection rates, incidence of infections, and the occurrence of *de novo* malignancies following transplantation.

## MATERIALS AND METHODS

### Study Design

We conducted a longitudinal retrospective single center study including all SPKT performed at Hospital Clínic Barcelona from January 1st, 2000 until December 31st, 2023 (n = 385). Patients with ≥1 previous transplant of any type (n = 19), pre-transplant calculated Panel Reactive Antibody (cPRA) >20% and/or Donor Specific Antibodies (DSAs) (n = 54), and those with a kidney or




**GRAPHICAL ABSTRACT**

pancreas graft primary non-function for technical reasons (n = 7) were excluded. In total, 305 SPKT recipients were included.

According to our immunology laboratory, a bead in the Single Antigen assay was considered positive when the mean fluorescence intensity (MFI) was ≥1,000. However, this threshold was subject to minor patient-specific adjustments based on the background MFI observed in non–donor-specific beads, which could result in a slightly higher or lower effective cut-off.

Data was collected until 31st December 2024. The clinical and research activities being reported were consistent with the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. The study protocol was approved by the Research Ethics Committee (HCB/2025/0613).

## Immunosuppression

Induction immunosuppression therapy was used in all patients, with two doses of basiliximab of 20 mg at day 0 and at day +4 after surgery between January 2008 until July 2013. Before January 2008, and after July 2013, rabbit anti-human lymphocytes polyclonal antibodies (either Thymoglobulin® 1.25 mg/kg/day or ATG® 2.5 mg/kg/day, for 4 consecutive days) was administered as induction therapy. The first dose was administered intraoperatively, and the subsequent three doses on consecutive days following surgery. Dosage was adjusted according to leukocyte and platelet counts: it was reduced by 50% if the leukocyte count was <3.000/mL and/or the platelet count was <75,000/mL. If the leukocyte count fell below 1.500/mL and/or the platelet count below 50.000/mL, the dose was postponed (not discontinued) until the following day to try to reach a total cumulative dose of 5 mg/kg (10 mg/kg for ATG).

Maintenance immunosuppression protocol was based on triple therapy with calcineurin inhibitor (cyclosporine A until 2005, and thereafter tacrolimus), mycophenolate or mTOR inhibitors, and steroids (methylprednisolone in the immediate post-transplant period, followed by oral prednisone). The first dose of the calcineurin inhibitor was administered immediately before surgery. Administration was not postponed in cases of kidney Delayed Graft Function (DGF), and dosage adjustments were made solely based on trough levels. Therefore, all patients received the same regimen regardless of kidney DGF occurrence and induction therapy.

## Anticoagulation

Anticoagulation included subcutaneous enoxaparin 20 mg bid starting 8 h post-surgery and was maintained until patient discharge (in the absence of thrombotic/hemorrhagic complications), and acetylsalicylic acid 50 mg/day starting at 12 h post-surgery until discharge, when it is increased up to 100 mg/day.

## Infections and *De Novo* Neoplasms

Infections were considered when requirement for hospital admission. Cytomegalovirus (CMV) prophylaxis with valganciclovir was administered to all patients for 1 month post-transplant or three if a donor/recipient mismatch for CMV was present. Infection was defined as any replication in CMV load post-transplant, regardless of the presence of CMV disease. BK nephropathy was defined an increase in BK viremia >10.000 UI/mL, regardless of the presence of biopsy proven BK nephropathy.

*De novo* neoplasms were considered as any neoplasia diagnosed during the post-transplant period, including solid tumours, post-transplant lymphoprolipherative disorders (PTLD) and excluding non-melanoma skin cancer.

## Outcomes

Primary outcomes were recipient survival and death-censored kidney or pancreas graft survival at 1, 5, 10, and 20 years after transplantation, and graft rejection during follow up. Our secondary outcomes were defined as number of infections requiring hospital admission, CMV infection, BK virus nephropathy, and new onset neoplasms.

Patient survival was calculated from the date of transplantation to the date of death from any cause. Patients alive at the last follow-up were censored at that date. Pancreas graft failure was defined as any of the following: a) graft removal, b) C-peptide <1 ng/mL or c) total daily insulin dose >0.5 U/Kg.

Kidney graft failure was defined as return to dialysis or re-transplantation. Kidney DGF was defined as the need for at least one session of hemodialysis during the first week following SPKT.

Graft survival was analyzed using death-censored estimates to evaluate the effect of induction therapy on graft failure, particularly from immunological causes, independent of patient mortality. Nevertheless, to reduce the risk of bias derived from potential competing risks between recipient death and graft failure, a competing risk analysis was also performed.

## Rejection Diagnosis and Treatment

All rejection episodes (for both pancreas and kidney) were biopsy-proven. Diagnostic criteria were based on the Banff classification in use at the time of diagnosis for either pancreas or kidney grafts. In cases of pancreas T cell–mediated rejection (TCMR), patients received three doses of methylprednisolone (500 mg/day) followed by five doses of thymoglobulin (1.25 mg/kg/day). For pancreas antibody-mediated rejection (ABMR), treatment consisted of three doses of methylprednisolone (250 mg/day), two doses of rituximab (400 mg/day), and five sessions of plasma exchange. For kidney graft rejection, the same therapeutic protocols were applied, except in cases of TCMR grade I, in which thymoglobulin was not administered.

## Statistical Analysis

Data are presented as mean (standard deviation, SD) for continuous variables and median [interquartile range (IQR)] for the non-continuous ones. The corresponding tests used were t-test, Mann-Whitney test, Chi-square or Fisher's Exact test as appropriated. Competing risk analysis for graft survival was performed using the Fine–Gray subdistribution hazard model.

Inverse probability of treatment weighting (IPTW) was used to account for covariate imbalance between basiliximab and

**TABLE 1 |** Baseline characteristics of included recipients.

| | Thymoglobulin (n = 172) | Basiliximab (n = 133) | P value |
|---|---|---|---|
| Gender (Male) | 102 (59) | 88 (66) | 0.24 |
| Ethnicity | | | 0.59 |
| Caucasian | 162 (94) | 128 (95) | |
| Hispanic | 9 (5) | 5 (5) | |
| Asian | 1 (1) | 0 (0) | |
| Age at SPKT (years) | 40.56 ± 7.58 | 40.96 ± 7.19 | 0.64 |
| BMI (kg/m$^2$) | 23.60 ± 5.30 | 22.90 ± 5.40 | 0.26 |
| Diabetes Mellitus type | | | 1.00 |
| Type 1 | 171 (99) | 132 (99) | |
| Type 2 | 0 (0) | 0 (0) | |
| Other types | 1 (1) | 1 (1) | |
| Diabetes Mellitus duration at SPKT (years) | 25 [21–31] | 24 [20–31] | 0.11 |
| Dialysis before transplant | 146 (85) | 117 (88) | 0.50 |
| Dialysis duration (months) | 23 [13–34] | 31 [21–40] | <0.001 |
| Waiting list duration at SPKT (months) | 10.5 [4.75–18.25] | 17 [9–27] | 0.002 |
| Retinopathy | 168 (98) | 126 (95) | 0.22 |
| Neuropathy | 91 (53) | 60 (45) | 0.17 |
| Ischemic Heart Disease | 16 (10) | 22 (17) | 0.08 |
| Peripheral Artery Disease | 44 (26) | 41 (31) | 0.37 |
| Hypertension | 121 (70) | 86 (65) | 0.50 |
| Smoking habit | 57 (40) | 72 (54) | 0.04 |
| Transplant era (after 2008) | 120 (69) | 82 (62) | 0.15 |
| High risk of CMV infection | 13 (8) | 26 (20) | 0.003 |
| Total HLA mismatches | | | 0.38 |
| 0–2 | 2 (1) | 0 (0) | |
| 3–4 | 30 (17) | 20 (15) | |
| ≥5 | 140 (82) | 113 (85) | |
| cPRA pre-transplant >5% | 8 (5) | 8 (6) | 0.61 |
| Maintenance immunosuppression | | | 0.22 |
| FK + MMF | 164 (95) | 129 (97) | |
| FK + mTORi | 6 (4) | 1 (1) | |
| CsA + MMF | 2 (1) | 3 (2) | |
| Prednisone withdrawal | 46 (27) | 44 (33) | 0.31 |
| Tacrolimus trough levels (ng/mL) | | | |
| 1 month | 10.86 ± 2.82 | 11.82 ± 1.46 | 0.49 |
| 6 months | 9.66 ± 1.66 | 9.80 ± 0.92 | 0.44 |
| 12 months | 9.14 ± 1.02 | 8.32 ± 1.28 | 0.27 |
| 5 years | 7.52 ± 0.94 | 7.07 ± 0.53 | 0.37 |
| Kidney DGF | 13 (8) | 17 (13) | 0.17 |

*Data are means ± SD, n (%) or median [IQR] unless otherwise indicated. SPKT, simultaneous pancreas-kidney transplantation; BMI, body mass index; CMV, cytomegalovirus; HLA, human leukocyte antigen; cPRA, calculated Panel Reactive Antibody; PDN, prednisone; FK, tacrolimus; MPS, mycophenolate; mTORi, mTOR, inhibitors; CsA, cyclosporine; DGF, delayed graft function.*

thymoglobulin groups. IPTW was estimated from a propensity score from a logistic regression model to receive basiliximab as the induction agent. The model included factors associated with the donor and either of the outcomes: dialysis duration before transplant, diabetes duration before transplant, time on the waiting list, HLA mismatches between donor and recipient, type of maintenance immunosuppression, prednisone withdrawal, recipient age at transplantation, cold ischemia time for kidney graft, Pancreas Donor Risk Index (PDRI), pancreas transplantation era, recipient smoking habit, cPRA before transplant.

A stabilized weighting method was performed by multiplying the IPTW by the proportion of recipients treated with basiliximab and thymoglobulin. Check for adequate balance of covariates after IPTW analyses was performed by calculation of standardized differences and an absolute difference greater than 0.2 represented a meaningful imbalance. All subsequent analyses were performed on the weighted, covariate-balanced population. Kaplan-Meier was used to estimate patient and graft survival and compared using a log-rank test. Logistic regression was used to calculate odds ratio for graft rejection, infections and neoplasms, and Cox proportional regression was performed to estimate patient and graft hazards.

All variables analyzed presented less than 10% of missing values. Given the low percentage, imputation methods were not applied, and analyses were conducted with the available data.

Statistical analysis was performed using IBM SPSS Statistics 30.0 (SPSS, Inc; Chicago, Illinois) software for MacOS and Python programming language (Python Software Foundation, 2024) in MacOS. All tests were two-tailed and a significance of 0.05 was used. Graphs were generated using the Python programming language in MacOS.

**TABLE 2** | Donor characteristics.

| | Thymoglobulin (n = 172) | Basiliximab (n = 133) | P value |
|---|---|---|---|
| Gender (Male) | 100 (60) | 81 (61) | 0.91 |
| Age (years) | 32.85 ± 12.22 | 32.43 ± 10.55 | 0.76 |
| BMI (kg/m$^2$) | 23.72 ± 3.28 | 23.47 ± 2.94 | 0.54 |
| Hypertension history | 12 (8) | 4 (3) | 0.07 |
| Smoking habit | 40 (28) | 34 (28) | 1.00 |
| Alcohol consumption | 15 (10) | 6 (5) | 0.11 |
| PDRI risk | 1.35 ± 0.61 | 1.30 ± 0.38 | 0.55 |
| ICU Length of Stay (days) | 2 [1–4] | 2 [1–5] | 0.64 |
| Donation after Circulatory Death | 22 (15) | 2 (2) | <0.001 |
| Pancreas CIT (hours) | 8.77 ± 2.54 | 11.19 ± 3.11 | <0.001 |
| Kidney CIT (hours) | 10.93 ± 2.82 | 13.36 ± 3.23 | <0.001 |

*Data are means ± SD, n (%) or median [IQR] unless otherwise indicated. BMI, body mass index; PDRI, pancreas donor risk index; ICU, intensive care unit; CIT, cold ischemia time.*

# RESULTS

## Recipient and Donor Characteristics

A total number of 305 SPKT recipients were included in the study (**Table 1**). In 172 (56%), thymoglobulin was used as the induction agent, while in 133 (44%) basiliximab was administered as the induction therapy. The mean follow up time for the whole cohort was 12.08 ± 6.84 years. Recipient age at SPKT was similar between both groups. Type 1 Diabetes (T1D) was predominant in both groups, in which diabetes duration was also similar. Most of the patients were on dialysis at SPKT in both groups, although time on dialysis before transplant was higher in the basiliximab one, as well as time on the waiting list. Smoking habit was more frequent in the basiliximab group, as well as patients at high risk of CMV infection.

**Table 2** summarizes donor characteristics. Age at donation was similar between both groups. No differences were observed for PDRI score among the studied groups. Donors after Circulatory Death (DCD) were more frequent in the thymoglobulin group. Pancreas and kidney Cold Ischemia Time (CIT) were longer in the basiliximab one.

After IPTW adjustment, no significant differences were observed between both groups neither for recipient nor for donor characteristics. **Supplementary Table S1** shows standardized differences for donor and recipient characteristics before and after IPTW adjustment.

## Recipient Survival

Patient survival at 1, 5, 10, and 20 years after SPKT was 98.8%, 98.1%, 94% and 86.2% in the thymoglobulin group, respectively. For basiliximab group, survival was not significantly different, being 99.2%, 94.6%, 92.2% and 80.5% for the same time periods, respectively (Log Rank P = 0.31) (**Figure 1A**). Unadjusted Cox regression analysis showed that basiliximab was not associated with an increased risk of patient death compared to thymoglobulin [HR 1.47 (0.69–3.14), P = 0.32]. A similar scenario was observed after IPTW adjustment, with no difference for patient death comparing both groups [HR 1.01 (0.43–2.34) for basiliximab group, P = 0.99] (**Table 3**). The main cause of recipient death in both groups were infections (50% vs. 37% for thymoglobulin and basiliximab groups, respectively), followed by neoplasms (34% vs. 26% for thymoglobulin and basiliximab, respectively), with no differences between groups (P = 0.55) (**Supplementary Table S2**).

## Pancreas Graft Survival

In the thymoglobulin group, pancreas death-censored graft survival at 1, 5, 10, and 20 years after SPKT was 95.9%, 93.1%, 88%, 86.1%, respectively. For basiliximab one, pancreas graft survival was similar, being 95.5%, 90.7%, 88.2%, 83.3% for the same time periods, respectively (Log Rank P = 0.83) (**Figure 1B**). No differences were observed for overall pancreas graft survival (Log Rank P = 0.57, **Supplementary Figure S1A**). Unadjusted Cox regression analysis showed no increased risk of pancreas failure with basiliximab compared to thymoglobulin as induction [HR 1.08 (0.55–2.10), P = 0.83]. These results were maintained after IPTW weighting [HR 1.57 (0.75–3.28) for basiliximab group, P = 0.24] (**Table 3**). A similar scenario was observed when performing a competing risk analysis, with a HR 0.93 [0.48–1.83], P = 0.84 for the basiliximab group.

## Kidney Graft Survival

Kidney graft survival rates in the thymoglobulin group at 1, 5, 10, and 20 years post-SPKT were 97.1%, 95.5%, 89%, and 84.7%, respectively. Similarly, kidney graft survival in the basiliximab group was 98.5%, 94.6%, 90.4%, and 89.6% at the corresponding time points (Log Rank P = 0.56) (**Figure 1C**). No differences were observed for overall kidney graft survival (Log Rank P = 0.99, **Supplementary Figure S1B**). According to unadjusted Cox regression analysis, there was no significant increase in the risk of kidney graft failure with basiliximab when compared to thymoglobulin [HR 0.80 (0.38–1.70), P = 0.56]. This finding remained consistent following IPTW adjustment [HR 1.49 (0.84–2.63) for the basiliximab group, P = 0.17] (**Table 3**). A similar scenario was observed when performing a competing risk analysis, with a HR 1.24 [0.57–2.70], P = 0.58 for the basiliximab group.

## Graft Rejection

Throughout the entire follow-up period, 34 pancreas rejection episodes (20%) occurred in the thymoglobulin group and 32 episodes (24%) in the basiliximab group, with no

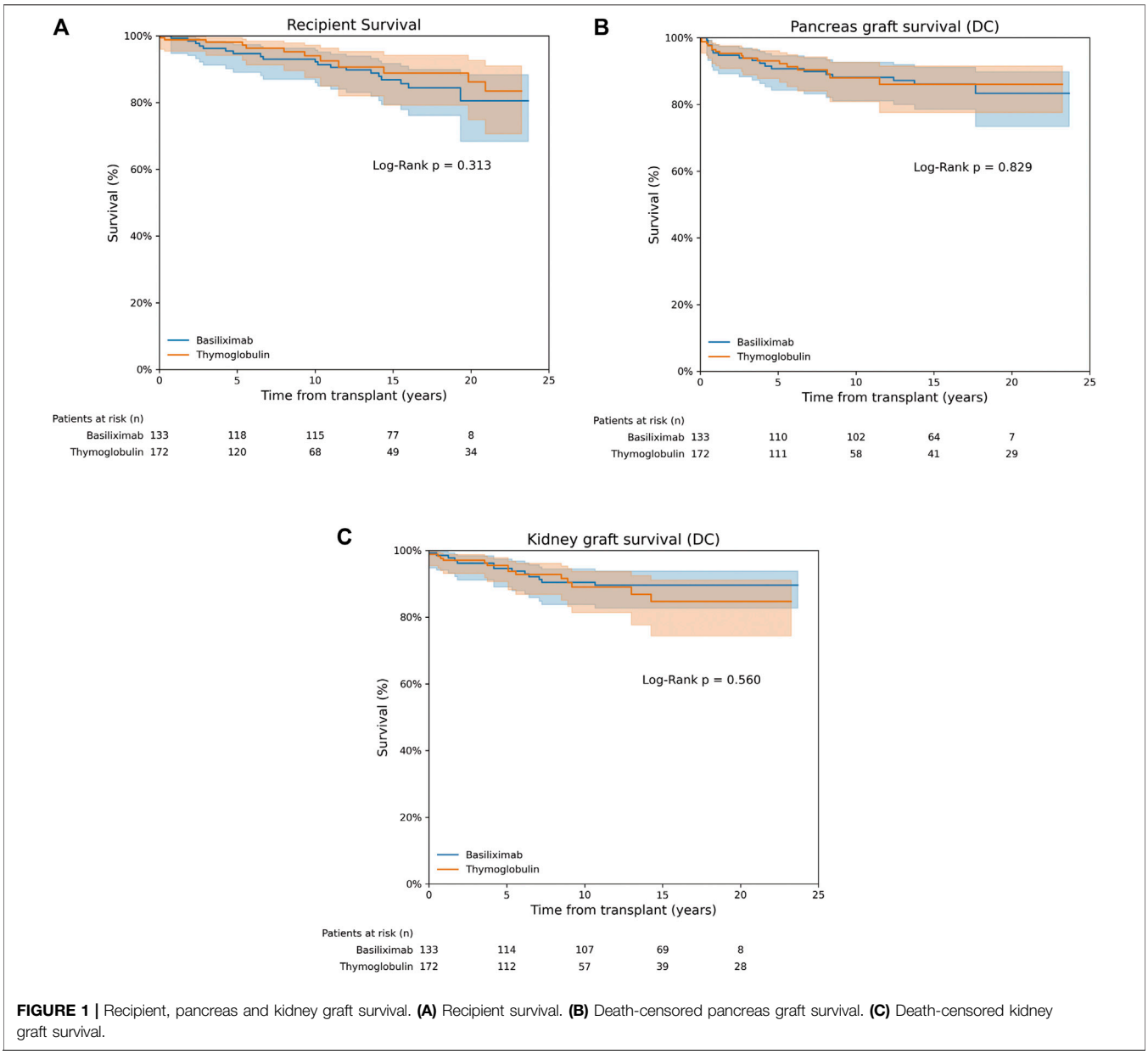



**FIGURE 1 |** Recipient, pancreas and kidney graft survival. **(A)** Recipient survival. **(B)** Death-censored pancreas graft survival. **(C)** Death-censored kidney graft survival.

**TABLE 3 |** Non-adjusted and IPTW-weighted Cox regression for patient, pancreas and kidney graft survival.

| | HR [95% CI][a] | P value |
|---|---|---|
| Non-adjusted | | |
| Patient death | 1.47 [0.69–3.14] | 0.32 |
| Pancreas graft failure | 1.08 [0.55–2.10] | 0.83 |
| Kidney graft failure | 0.80 [0.38–1.70] | 0.56 |
| IPTW-weighted | | |
| Patient death | 1.01 [0.43–2.34] | 0.99 |
| Pancreas graft failure | 1.57 [0.75–3.28] | 0.24 |
| Kidney graft failure | 1.49 [0.84–2.63] | 0.17 |

[a]*The Thymoglobulin group was considered the reference group.*

statistically significant difference between them (P = 0.40) (**Table 4**). Rejection occurred after a median time of 4 [1–23] and 6 [1–13] months for the thymoglobulin and basiliximab groups, respectively (P = 0.69).

In both groups, the most frequent type of pancreas rejection was TCMR, with 29 (17%) and 32 (24%) cases for thymoglobulin and basiliximab, groups, respectively. There was no statistical support for an association between rejection type and treatment group (P = 0.06). However, a tendency toward a different rejection pattern was observed for the pancreas graft: in the basiliximab group, all cases were TCMR, whereas in the thymoglobulin group, 3% of cases were ABMR. Pancreas graft

**TABLE 4 |** Pancreas and kidney graft rejection during follow up.

| | Thymoglobulin (n = 172) | Basiliximab (n = 133) | P value |
|---|---|---|---|
| Pancreas rejection | 34 (20) | 32 (24) | 0.40 |
| ABMR | 5 (3) | 0 (0) | 0.06 |
| TCMR | 29 (17) | 32 (24) | |
| Kidney rejection | 11 (7) | 14 (11) | 0.19 |
| ABMR | 5 (3) | 2 (2) | 0.10 |
| TCMR | 6 (4) | 12 (9) | |

*Data are n (%). ABMR, Antibody-Mediated Rejection. TCMR, T Cell-Mediated Rejection.*

**TABLE 5 |** Non-adjusted and IPTW-weighted logistic regression for pancreas and kidney graft rejection.

| | OR [95% CI][a] | P value |
|---|---|---|
| Non-adjusted | | |
| Pancreas graft rejection | 1.28 [0.74–2.22] | 0.37 |
| Kidney graft rejection | 1.72 [0.76–3.93] | 0.20 |
| IPTW-weighted | | |
| Pancreas graft rejection | 1.49 [0.84–2.63] | 0.17 |
| Kidney graft rejection | 1.31 [0.54–3.15] | 0.55 |

[a]*The Thymoglobulin group was considered the reference group.*

rejection was the cause of graft loss in 14 cases in the thymoglobulin group and 11 cases in the basiliximab group, representing 82% and 61% of all graft losses (41% and 34% of treatment failure), respectively (P = 0.16).

Kidney graft rejection rate was similar between thymoglobulin and basiliximab groups, observing 11 (7%) and 14 (11%) kidney graft rejection episodes (P = 0.19). Rejection occurred after a median time of 8 [1–53] and 8 [2–44] months for the thymoglobulin and basiliximab groups, respectively (P = 0.93).

The most frequent type in both groups was TCMR (4% and 9% in the thymoglobulin and basiliximab groups, respectively). No statistically significant association was found between rejection type and treatment group (P = 0.10) (**Table 4**). Kidney graft rejection was the cause of graft loss in 2 cases in the thymoglobulin group and 1 case in the basiliximab group, representing 13% and 8% of all graft losses (18% and 7% of treatment failure), respectively (P = 0.63).

When assessing the risk of graft rejection, basiliximab was not associated with a higher risk of rejection, either for pancreas [OR 1.28 (0.74–2.22), P = 0.37] or kidney graft [OR 1.72 (0.76–3.93), P = 0.20] compared to thymoglobulin. These results were consistent after IPTW weighting, with an OR of 1.49 [0.84–2.63] (P = 0.17) for pancreas and 1.31 [0.54–3.15] (P = 0.55) for kidney graft and basiliximab compared to thymoglobulin (**Table 5**).

## Infections and New Onset Neoplasms

The rate of post-transplant infections (except for CMV and BKV) that required patient admission was similar between thymoglobulin and basiliximab (35% vs. 38%, respectively. P = 0.55). Infections occurred after a median time of 35 [1–109] and 34 [22–80] days for thymoglobulin and basiliximab, respectively (P = 0.57) Nevertheless, when specifically considering CMV infection and BK nephropathy, thymoglobulin group exhibited a significantly higher rate of CMV infection (29% vs. 14% for thymoglobulin vs. basiliximab, respectively. P = 0.002) and BK nephropathy (12% vs. 4%, P = 0.01) (**Table 6**). CMV infection occurred after a median time of 3 [2–5] and 3 [1–5] months (P = 0.42), while BK infection occurred after a median time of 9 [6–30] and 28 [23–30] months for thymoglobulin and basiliximab, respectively (P = 0.40). Induction with basiliximab was significantly associated with a reduced risk of CMV infection [OR 0.39 (0.21–0.70), P = 0.002] and BK nephropathy (OR 0.28 [0.10–0.77], P = 0.01) compared to thymoglobulin (**Table 7**). This association was maintained after IPTW adjustment (OR 0.41 [0.23–0.72], P = 0.002 for basiliximab and CMV infection; OR 0.31 [0.12–0.80], P = 0.02 for basiliximab and BK infection).

The incidence of new onset neoplasms was similar between both groups (6% vs. 10% for thymoglobulin and basiliximab groups, respectively, P = 0.21). Median time to neoplasm diagnosis was 73 [39–90] and 170 [95–201] months for thymoglobulin and basiliximab groups, respectively (P = 0.08). In this case, no significant association was identified between basiliximab and neoplasm development compared to thymoglobulin, either in the unadjusted [OR 1.72 (0.76–3.93), P = 0.20] or IPTW-weighted analysis [OR 1.24 (0.48–3.21), P = 0.66] (**Tables 6**, **7**).

# DISCUSSION

T-cell depleting agents (as thymoglobulin or alemtuzumab) and the IL2R blocker basiliximab have become the most frequently used induction agents in SPKT [5, 8, 13]. Nevertheless, information regarding post-transplant outcomes for each treatment remains controversial. Thus, in the present study we retrospectively compared post-transplant outcomes in a cohort of low immunological risk SPKT recipients after using either thymoglobulin or basiliximab as induction agents, focusing on long-term patient and grafts survival, as well as the incidence of post-transplant infections and neoplasms. Recipient, pancreas and kidney graft survival were similar among the two studied groups, as well as the incidence of graft rejection. Remarkably, basiliximab was not associated with a higher risk of pancreas and kidney graft rejection but significantly reduced the risk of CMV and BKV infection compared to thymoglobulin.

**TABLE 6 |** Infections and new onset neoplasms during follow up.

| | Thymoglobulin (n = 172) | Basiliximab (n = 133) |
|---|---|---|
| Infections that require hospitalization | 59 (35) | 51 (38) |
| CMV infection | 48 (29) | 18 (14) |
| BK infection | 21 (12) | 5 (4) |
| New onset neoplasms | 11 (6) | 14 (10) |
| PTLD | 2 (1) | 3 (2) |
| Breast | 2 (1) | 0 (0) |
| Melanoma | 2 (1) | 2 (1) |
| Colon | 3 (2) | 3 (2) |
| Kidney | 0 (0) | 2 (1) |
| Other | 2 (1) | 4 (3) |

*Data are expressed as n (%). CMV, cytomegalovirus; PTLD, Post-Transplant Lymphoproliferative Disease.*

**TABLE 7 |** Non-adjusted and IPTW-weighted logistic regression for infection and new-onset neoplasms.

| | OR [95% CI] | P value |
|---|---|---|
| Non-adjusted | | |
| Infections that require hospitalization | 1.18 [0.74–1.89] | 0.49 |
| CMV infection | 0.39 [0.21–0.70] | 0.002 |
| BK infection | 0.28 [0.10–0.77] | 0.01 |
| New onset neoplasms | 1.72 [0.76–3.93] | 0.20 |
| IPTW adjusted | | |
| Infections that require hospitalization | 1.45 [0.92–2.33] | 0.11 |
| CMV infection | 0.41 [0.23–0.72] | 0.002 |
| BK infection | 0.31 [0.12–0.80] | 0.02 |
| New onset neoplasms | 1.24 [0.48–3.21] | 0.66 |

[a]*The Thymoglobulin group was considered the reference group. CMV, cytomegalovirus.*

A multicenter randomized clinical trial demonstrating the benefit of induction therapy in SPKT was published in 2003 and ever since the use of induction therapy in pancreas transplantation has become almost ubiquitous [13, 14]. In this study, no differences were observed on 12-month graft survival between T-cell depleting agents or IL2R blockers. Nevertheless, in 2015, Kopp et al [15] reported a higher rate of pancreas rejection with IL2R blockers compared to Thymoglobulin in a long-term follow up study over 30 years in pancreas transplantation, although no differences in graft survival were observed. Since then, T-cell depleting agents have progressively gained relevance over IL2R blockers in the last decade, representing up to 80% of induction agent used in the USA [14]. Some studies have previously compared T-cell depleting agents and IL2R blockers as induction therapy in SPKT [9, 10, 16]. In 2011, Bazerbachi et al [9]. reported no differences for recipient and pancreas graft survival after 5 years, although basiliximab increased by 7-times the risk of pancreas TCMR at 1 year. Similar results were reported by Aziz et al [10] with a larger cohort of pancreas recipients. Remarkably, no increased risk of pancreas rejection was observed when only low immunological risk patients were considered (defined as cPRA <10%), although no information about pre-transplant DSAs was available. Our results are in line to those reported by Aziz et al, although with a longer follow up. Furthermore, in our study we considered as low immunological risk patients those with a cPRA <20% and no pre-transplant DSAs. The gap between cPRA cut off may be explained by the possibility to measure DSAs before transplant, which would allow to consider patients with a cPRA between 10% and 20% as low risk cases. Nevertheless, although no statistically significant differences in kidney or pancreas rejection rates were observed between thymoglobulin and basiliximab, our data showed a numerical trend toward higher rejection in the basiliximab group, as previously reported in other studies [17, 18]. Nevertheless, this tendency did not translate into inferior long-term graft or patient survival. This observation highlights the importance of careful recipient selection when considering basiliximab to avoid clinically relevant increases in rejection.

Thymoglobulin has also become the preferred induction agent in DCD pancreas transplantation due to a theoretically increased risk of rejection because higher severity of ischemia-reperfusion injury compared to Donors After Brain Death (DBD) [19]. Different studies have demonstrated that pancreas graft survival, patient survival, and rates of acute rejection are equivalent between DCD and DBD pancreas transplants, although thymoglobulin is the most frequent induction agent [19–21]. In our study, the proportion of DCD donors was higher in the thymoglobulin group, although CIT for both pancreas and kidney grafts were slightly longer in the basiliximab group. These findings suggest that basiliximab may also be effective in settings involving prolonged CIT. However, the higher incidence of DCD donors in the thymoglobulin group limits the ability to draw definitive conclusions regarding the efficacy of basiliximab in DCD transplants. This underscores the need for individualized selection of induction therapy based on the specific donor–recipient profile.

Post-transplant infections and neoplasms are two of the most important complications associated with T-cell depleting agents because their profound immunosuppressant effect [11, 12]. In our cohort, thymoglobulin was associated with an increased risk of CMV infection (up to 60%) compared to basiliximab. Noticeably, this observation persisted even when adjusting for confounding factors and considering that a higher number of recipients with CMV mismatch were present in the basiliximab group. These data are in line to those reported previously [10]. Similar to CMV, thymoglobulin increased the risk of BK virus infection up to 70% compared to basiliximab, a finding that has been previously suggested but no solidly demonstrated in those studies comparing thymoglobulin and basiliximab in SPKT [9, 10, 16]. No differences in the risk of post-transplant neoplasms were observed between the two study groups; however, neoplasms tended to occur earlier in the thymoglobulin group, suggesting a potential adverse effect associated with thymoglobulin use. Moreover, it has to be considered that, according to our center policy, induction with basiliximab was changed to thymoglobulin after 2013, thus conferring to the thymoglobulin cohort a shorter follow up that can falsely reduce the incidence of new onset neoplasms.

The results of our study reinforce some of the recommendations from the First World Consensus Conference on Pancreas Transplantation, particularly those regarding the impact of non-depleting agents on patient and graft outcomes [5]. In addition, the observed tendency toward earlier neoplasm development may support concerns about a higher risk of oncologic complications with depleting agents, an issue also highlighted in that Consensus.

Although our study focused on thymoglobulin and basiliximab as induction agents, these findings may also be relevant when evaluating alemtuzumab, another T-cell depleting agent used in pancreas transplantation. Previous studies in pancreas transplantation have suggested that alemtuzumab achieve comparable graft and recipient outcomes to thymoglobulin [22–24]. In this context, our findings indicating that basiliximab provides equivalent long-term outcomes to thymoglobulin, with a lower incidence of CMV and BK virus infection, raise the possibility that non-depleting IL2R blockers might offer a safer alternative even in comparison to alemtuzumab, at least for carefully selected low-risk recipients. This hypothesis has been recently addressed in a retrospective study performed by Swaab et al. [25]. They reported similar short-term graft outcomes between IL2R blockers and alemtuzumab induction in a small cohort of SPKT recipients, in line with previous studies [10]. Future studies directly comparing basiliximab, thymoglobulin, and alemtuzumab could therefore provide valuable guidance for tailoring induction therapy according to individual immunologic risk and infection susceptibility.

An additional consideration arising from our results is the potential role of "no induction" protocols in selected SPK recipients. Our cohort included exclusively SPK recipients, a population known to have lower immunologic risk compared with other pancreas transplant modalities (pancreas transplant alone and pancreas after kidney), and further restricted to low-risk immunologic profiles [17, 18, 26]. Given this context and the outcomes observed, it is conceivable that similar results could be achieved in carefully selected low-risk SPK recipients even without induction therapy, as has been suggested in prior studies [27, 28]. Therefore, future prospective studies are warranted to evaluate this strategy and to identify the patient characteristics that may allow safe omission of induction therapy.

Our study has the inherent limitations of a single-center, retrospective design. In addition, the two induction treatments were administered during different time periods, so a potential year effect cannot be entirely excluded. The choice of induction therapy followed institutional policy, with a transition from basiliximab to thymoglobulin after 2013. Nevertheless, the single-center setting ensured a homogeneous cohort, particularly in terms of SPKT management and treatment protocols, thereby reducing the risk of bias. Furthermore, our analysis accounted for improvements in pancreas transplantation observed since 2008, which also helps minimize bias related to the different administration periods of thymoglobulin and basiliximab.

With a follow-up period spanning 20 years, our findings add valuable long-term data on induction therapy in SPKT. Specifically, our results indicate that in recipients with low immunological risk, basiliximab offers comparable patient and graft survival outcomes to thymoglobulin, while being associated with a lower incidence of opportunistic infections post-transplantation. Randomized controlled trials are necessary to draw definitive conclusions about the optimal induction therapy for SPKT.

## 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 humans were approved by Comité d'Ètica per a la Investigació amb Medicaments (HCB/2025/0613). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

EM-M: Conceptualization, Methodology, Formal Analysis, Investigation, Writing – original draft. AR-G: Investigation, Writing – original draft. JV-M: Investigation, Writing – original draft. BMÁ: Investigation, Writing – original draft. IR: Investigation, Writing – original draft, AB: Investigation, Writing – original draft. JF-F: Conceptualization, Methodology, Writing – review and editing. AA: Conceptualization, Methodology, Writing – review and editing. MR-B: Methodology, Writing – review and editing. MM: Conceptualization, Methodology, Writing – review and editing, FD: Conceptualization, Writing – review and editing. PV-A: Conceptualization, Methodology, Investigation, Supervision, Writing – review and editing.

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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.2025.15263/full#supplementary-material

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ORIGINAL RESEARCH
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# Cognitive Performance in Patients With Alcohol-Associated Liver Disease Undergoing Liver Transplantation

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Cognitive impairment (CI) in alcohol-related liver cirrhosis (ALD) is often underestimated, primarily attributed to hepatic encephalopathy (HE), despite evidence suggesting that deficits may persist after liver transplantation (LT). This study assessed CI both before and after LT through a structured psychiatric evaluation. A total of 101 ALD patients listed for LT were assessed; 61 underwent transplantation. Three patients died pre-LT, and six post-LT, leaving 55 for longitudinal cognitive evaluation. The Addenbrooke's Cognitive Examination III (ACE III) was administered at LT listing and 7.1 months post-LT. Pre-LT CI was prevalent, with 86% scoring below the ACE III threshold. Mild cognitive impairment (MCI) was observed in 33%, and 52% had a high probability of dementia. Post-LT, ACE III scores improved ($\Delta$ +7.07 ± 8.47, $P < 0.01$), with the greatest gains in memory (+1.46, $P = 0.01$) and verbal fluency (+1.43, $P = 0.02$), while attention remained largely unchanged. Despite overall cognitive recovery, persistent deficits were observed, particularly in executive function and fluency. LT improves cognition, but persistent deficits suggest CI in ALD is not entirely reversible. These findings underscore the need for targeted cognitive interventions before and after LT.

**Keywords: alcohol-related liver disease, cognitive impairment, liver transplantation, blood ammonia level, hepatic encephalopathy**



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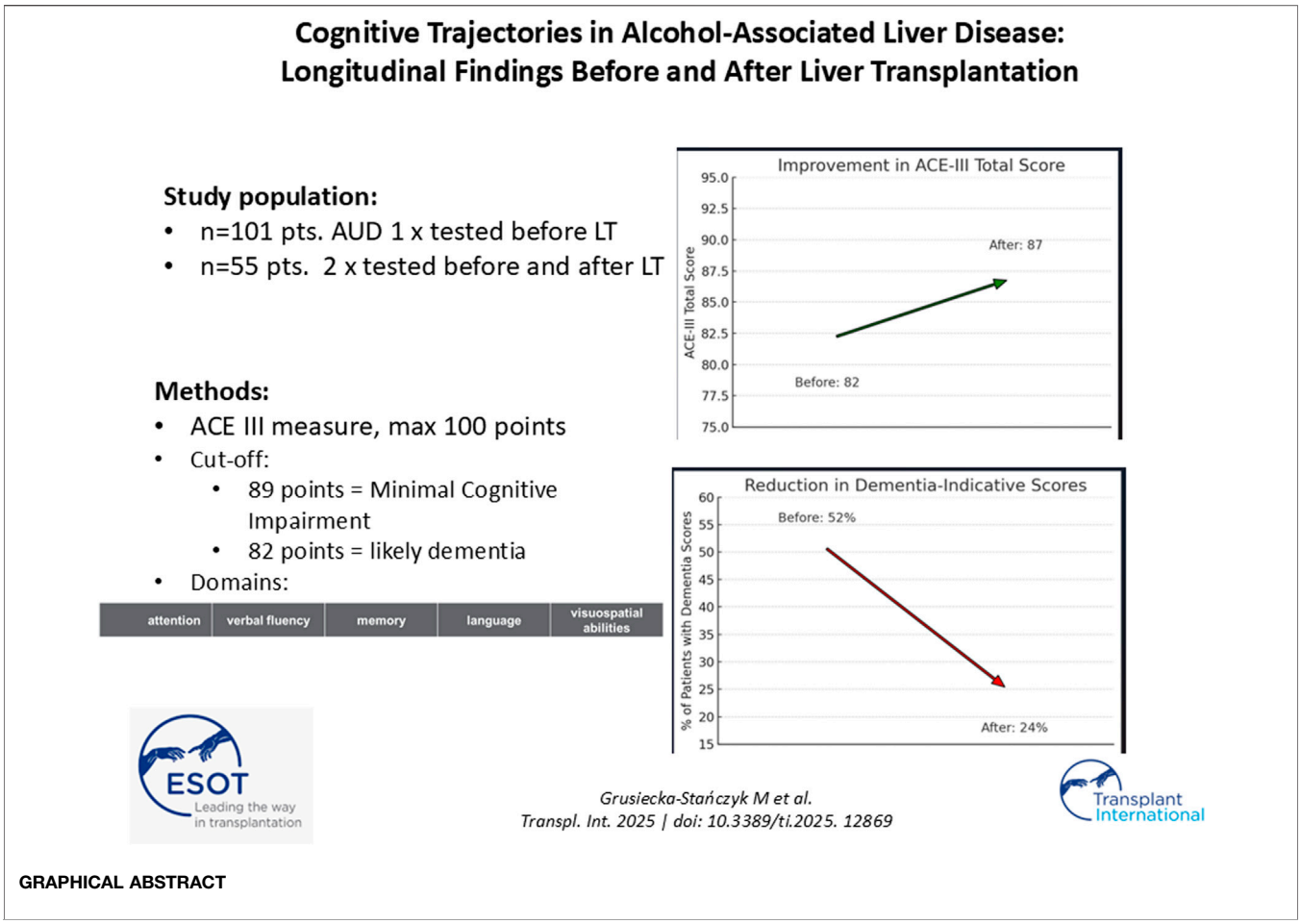
## INTRODUCTION

Alcohol is the most widely abused psychoactive substance globally, with high-risk drinking reported in up to 30% of Western populations, contributing substantially to morbidity and mortality [1]. Alcohol use disorder (AUD), as defined by the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V), encompasses a spectrum of maladaptive drinking behaviors that result in clinically significant physical, psychological, or social dysfunction [2]. Excessive alcohol intake is a well-established cause of liver cirrhosis, which remains a leading indication for liver transplantation (LT), particularly in cases of severe alcoholic hepatitis and end-stage liver disease (ESLD) [3, 4].

Cirrhosis-related neurocognitive decline is commonly attributed to hepatic encephalopathy (HE), a complication of advanced liver dysfunction associated with hyperammonemia and disruption of the liver-brain axis [5, 6]. While blood ammonia levels are a recognized biomarker of HE, they




**GRAPHICAL ABSTRACT**

primarily have a high negative predictive value and do not reliably correlate with cognitive impairment (CI) [5, 6]. Although HE is considered reversible after LT, studies indicate that some patients with overt HE pre-transplantation exhibit persistent or even worsening cognitive deficits post-LT, suggesting that CI may result from more complex and multifactorial mechanisms [7, 8].

In patients with AUD, cognitive deficits extend beyond HE. Chronic alcohol consumption leads to widespread and potentially irreversible neurotoxic effects, including oxidative stress, mitochondrial dysfunction, and neuroinflammation. Acetaldehyde, the primary metabolite of ethanol, induces cellular damage by forming protein adducts and generating reactive oxygen species, which impair mitochondrial DNA and neuronal integrity [9]. Concurrently, elevated levels of pro-inflammatory cytokines—such as TNF-α, IL-6, IL-1β, and MCP1—are known to disrupt neuroplasticity and contribute to structural and functional changes in key brain regions, including the hippocampus and prefrontal cortex [10, 11].

As a result, individuals with AUD are particularly susceptible to a broad spectrum of cognitive deficits, including impairments in executive function, attention, abstract reasoning, psychomotor speed, visuospatial skills, language, and both verbal and visual memory [12–14]. These deficits are often linked to alcohol-induced reductions in hippocampal white matter volume and damage to the prefrontal cortex—areas crucial for memory, decision-making, and behavioral regulation. Structural brain abnormalities, minimal and overt HE, chronic alcohol use, diabetes, and gut microbiome dysbiosis may further contribute to pre-transplant CI and could influence its persistence after LT [15, 16].

Despite these known associations, cognitive impairment in patients with alcohol-related liver cirrhosis awaiting LT remains under-investigated. Existing studies on CI in LT recipients with cirrhosis of mixed etiologies report a post-transplant prevalence of CI ranging from 0% to 36% [17], but they are limited by methodological inconsistencies, variable definitions of HE and CI, and heterogeneous patient populations. Additionally, most prior research originates from neurology or psychiatry domains, often employing diverse and non-standardized diagnostic tools, which complicates comparisons and limits clinical applicability [18–20].

To address these limitations, the present pilot study aimed to perform a structured, longitudinal evaluation of cognitive function in a homogeneous cohort of patients with AUD-related ESLD. By assessing cognition both at the time of listing and after liver transplantation in a single-center setting,

this study seeks to provide new insight into the nature, evolution, and clinical relevance of cognitive impairment in this vulnerable patient population.

Given the limited availability of long-term, prospective data on the trajectory of cognitive impairment (CI) in patients before and after liver transplantation (LT) - particularly in clinically homogeneous populations - two primary research objectives were formulated:

1. To conduct a quantitative and qualitative assessment of cognitive impairment prior to liver transplantation in patients with alcohol-related liver disease (ALD) qualified for transplantation within a single transplant center.
2. To analyze changes in cognitive function following liver transplantation, with particular emphasis on the dynamics and potential improvement of cognitive performance in this specific patient population.

# PATIENTS AND METHODS

Between November 2022 and January 2024, a total of 101 consecutive adult patients with alcohol use disorder (AUD) were enrolled in the study (78% male; mean age: 53 ± 11 years; mean MELD score: 16 ± 7), all of whom were evaluated as potential candidates for liver transplantation (LT). Among them, 17% had hepatocellular carcinoma (HCC), while the remaining 83% were assessed for LT due to chronic liver failure.

Exclusion criteria included regular use of sedative medications, severe overt hepatic encephalopathy, and psychiatric or neurodegenerative disorders precluding cognitive assessment. No patients with acute alcoholic hepatitis were included in the study.

Plasma ammonia concentration was measured using an enzymatic method with glutamate dehydrogenase (GLDH) on the Dimension EXL analyzer (Siemens Healthineers, Forchheim, Germany), with a reference range of 19–55 μg/dL.

Of the 101 patients who underwent baseline cognitive assessment, 61 successfully received liver transplants. Three patients died before LT and an additional six died postoperatively. Ultimately, 55 individuals (including 14 women, 25%) underwent repeated cognitive evaluation (**Figure 1**). The mean age in this subgroup was 56 years.

All patients were routinely assessed during hospitalization by a psychiatrist dedicated to the transplant program, as part of the standard pre-transplant evaluation protocol. This included a comprehensive psychiatric consultation and administration of the Addenbrooke's Cognitive Examination III (ACE-III). In patients who completed both assessments, psychiatric evaluation was conducted at listing for liver transplantation and again at an average of 7.1 months post-transplantation (SD = 1.45; range: 6–11 months; median: 7 months), ensuring methodological consistency and enabling reliable longitudinal analysis.

## Cognitive Functions Assessment

The Addenbrooke Cognitive Test III (ACE III) with a cut-off of 89 points for Mild Cognitive Impairment (MCI), and <82 points for a high probability of dementia was used to evaluate cognitive functions. The ACE III test covers five main domains of cognition, i.e., attention, verbal fluency, memory, language and visuospatial abilities, and is widely used, due to its specificity and sensitivity as well as simplicity and feasibility for administration, not only for physicians. The Polish version is available free of charge [21]. The ACE III was previously validated using standard neuropsychological tests [22]. Beside this, the center already has own experience in using this diagnostic tool, in the form of projects already published [23, 24].

## Statistical Analyses

Statistical analyses were performed using SPSS (SPSS Statistics, version 28.0. IBM Corp., USA). Continuous variables are shown as mean ± standard deviation (SD) and categorical variables are expressed as absolute and relative (in per cent) frequencies. The Kolmogorov-Smirnov test was applied to determine whether continuous variables were normally distributed. The Wilcoxon Mann-Whitney U test or Student´s t-test were used for analyzing continuous variables. $Chi^2$ test and Fisher´s exact test were used for group comparisons of categorical variables. The correlations between ACE III results and clinical variables were evaluated by Spearman's rank correlation coefficient. The associations between ACE III result suggesting probability of dementia (ACE III <82 points) and clinical data were assessed by univariate and multivariate logistic regression analyses. Statistical procedures were performed two-sided and p-values <0.05 reflected to be statistically significant.

## Ethics

Appropriate informed consent was obtained from each patient included in the study. The study protocol was approved by the Bioethics Committee of the Medical University of Warsaw (approval number KB/81/2022) and conforms with the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

# RESULTS

## Preliminary Assessment of Cognitive Function

At the initial stage of the study, cognitive function was assessed in 101 individuals. Overall, cognitive impairment, as evaluated using the Addenbrooke's Cognitive Examination III (ACE-III), was observed in 86% of participants: 33% met the criteria for mild cognitive impairment (MCI), while 52% met the criteria indicating a high likelihood of dementia.

The mean total ACE-III score was 78.32 ± 11.99, ranging from 50 to 96, with a median of 79.0 (interquartile range [IQR]: 70.0–87.0).

Among the five cognitive domains assessed, the greatest deficits were observed in verbal fluency, followed by visuospatial abilities. The mean scores for attention, memory, language, and visuospatial skills were 16.11, 17.34, 22.43, and 11.84 points, respectively, with median scores of 17.0, 18.0, 23.0, and 12.0. Details are presented in **Table 1**. As variables followed

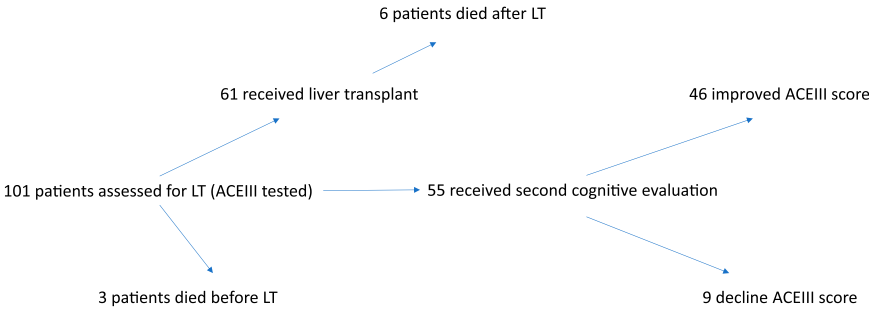



**FIGURE 1 |** Flow chart of patient inclusion, liver transplantation outcomes, and cognitive assessment results before and after transplantation.

**TABLE 1 |** ACE-III cognitive performance among liver transplant candidates with ALD.

| N = 101 | Age | Child-Pugh class | MELD | ACEIII | Attention | Memory | Fluency | Langugage | Visuo-spatial abilities | Years of education |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean ± SD | 52.54 ± 10.52 | 8.28 ± 1.94 | 16.0 ± 6.56 | 78.32 ± 11.99 | 16.11 ± 1.96 | 17.34 ± 4.66 | 10.21 ± 2.48 | 22.43 ± 3.98 | 11.84 ± 3.29 | 12.35 ± 2.91 |
| median | 53.0 | 8.0 | 14.0 | 81.0 | 17.0 | 18.0 | 10.0 | 24.0 | 12.0 | 12.0 |
| range | 24–73 | 5–14 | 6–41 | 42–100 | 8–18 | 3–26 | 3–14 | 4–26 | 1–17 | 8–20 |
| IQR | 45–61 | 7–10 | 11–20 | 71–86 | 15–18 | 15–20 | 9–12 | 21–25 | 10–15 | 10–13 |

non-normal distributions (as per Kolmogorov–Smirnov test), both means (±SD) and medians (IQR) are reported for all ACE III subdomains to enhance interpretability.

The mean venous blood ammonia concentration was 86 ± 57 μg/dL, with hyperammonemia (>55 μg/dL) observed in 45% of patients. As shown in **Table 1**, patients exhibiting signs of dementia had significantly shorter education duration ($P < 0.001$) and higher Child-Pugh scores ($P = 0.04$). However, no other clinical differences were identified—such as ammonia level or MELD score—when compared to patients with ACE-III scores >82.

The likelihood of dementia was significantly higher in patients with alcohol-related liver disease (ALD) alone compared to those with ALD and concomitant hepatocellular carcinoma (HCC) (P = 0.015). Among the 17 patients with ALD + HCC, 4 (23.5%) had ACE-III scores below 82, indicating a high probability of dementia. In contrast, 52% of patients in the ALD-only group fell below this threshold.

A total of ten deaths were recorded in the cohort, with a trend toward higher mortality among patients scoring <82 on the ACE-III (15%) versus those scoring ≥82 (4%), though this did not reach statistical significance ($P = 0.066$), as shown in **Table 2**. Moreover, 76 individuals (75%) were listed for liver transplantation, but no significant differences were observed in ACE-III total scores or individual cognitive domains based on transplant listing status.

The mean total ACE-III score in the entire cohort was 78 ± 12 points, with the lowest scores observed in the domains of verbal fluency and visuospatial abilities. The ACE-III score was significantly correlated with years of education and the Child-Pugh score, as shown in **Table 3**.

Notably, the overall MELD score was not associated with the total ACE-III score; however, it showed a significant correlation with the attention and language subdomains of the ACE-III (**Table 3**). Additionally, age was negatively correlated with the verbal fluency subscale and showed a negative trend in relation to the overall ACE-III score (**Table 3**).

Multivariable analysis demonstrated that both the Child-Pugh score (OR 1.51; 95% CI: 1.14–2.00) and years of education (OR 0.65; 95% CI: 0.53–0.79) were independently associated with ACE-III scores below 82 points, indicating a high likelihood of dementia (**Table 4**). In contrast, age, sex, blood ammonia level, presence of hyperammonemia, and MELD score showed no significant association with ACE-III scores <82.

## Results of Repeated Cognitive Assessment (N = 55 Patients)

### Cognitive Function Before Liver Transplantation

Before transplantation, the mean ACE-III score was 80.62 ± 10.40, with a median of 82.0 (range: 51–97; IQR: 75.0–87.0). Among the assessed cognitive domains, the language domain was best preserved (mean: 23.19 ± 3.44), while the greatest deficits were observed in verbal fluency (mean: 10.21 ± 2.48). Memory and visuospatial abilities showed moderate performance (memory: 18.15 ± 4.16; visuospatial: 12.22 ± 3.15). The mean attention score was 16.38 ± 1.39.

The average number of years of education in this group was 12.19 ± 3.24.

Data are presented in **Table 5**.

### Cognitive Function After Liver Transplantation (n = 55 Patients)

An overall improvement in cognitive function was observed following liver transplantation. The mean ACE-III score

**TABLE 2 |** Clinical characteristics of the study group and subgroups with positive and negative screening results for suspected dementia based on the ACE-III test (i.e., <82 points).

| | Entire cohort | ACE III positive for dementia suspicion | ACE III negative for dementia suspicion | P – value |
|---|---|---|---|---|
| N, % | 101 (100%) | 53 (52.5%) | 48 (47.5%) | - |
| Age, years | 52.5 ± 10.5 | 53.3 ± 10.0 | 51.7 ± 11.1 | 0.304 |
| Females, n (%) | 22 (21.8%) | 11 (20.8%) | 11 (22.9%) | 0.814 |
| MELD (points) | 16.0 ± 6.6 | 16.9 ± 7.0 | 15.0 ± 6.0 | 0.114 |
| Child-Pugh (points) | 8.3 ± 1.9 | 8.7 ± 1.9 | 7.9 ± 2.0 | 0.044 |
| Blood ammonia, ng/mL | 85.5 ± 56.8 | 82.8 ± 45.8 | 88.6 ± 67.5 | 0.961 |
| Years of education | 12.4 ± 2.9 | 11.2 ± 2.2 | 13.7 ± 3.1 | <0.001 |
| ACE III, points | 78.3 ± 12.0 | 70.1 ± 10.8 | 87.4 ± 4.2 | <0.001 |
| Death, n (%) | 10 (10%) | 8 (15%) | 2 (4%) | 0.066 |

*Abbreviations: ACE-III, Addenbrooke's Cognitive Examination III; MELD, Model for End-Stage Liver Disease score.*

**TABLE 3 |** Correlations between variables and ACE-III total and domain scores, presented as rho values.

| | Total ACE III | Attention ACEIII | Verbal fluency ACEIII | Memory ACEIII | Language ACE III | Visuospatial abilities ACEIII |
|---|---|---|---|---|---|---|
| Age | –0.190 | –0.098 | –0.262** | –0.136 | –0.146 | –0.129 |
| Female | 0.016 | –0.022 | 0.008 | –0.044 | –0.030 | 0.118 |
| Education period | 0.390** | 0.225* | 0.077 | 0.417** | 0.139 | 0.266** |
| Child-Pugh score | –0.262** | –0.320** | –0.253* | –0.121 | –0.270** | –0.200* |
| MELD score | –0.161 | –0.240* | –0.177 | –0.098 | –0.251* | –0.076 |
| Blood ammonia level | –0.075 | –0.063 | –0.091 | –0.063 | –0.056 | –0.132 |
| Total ACE III | 1 | 0.522** | 0.714** | 0.717** | 0.601** | 0.746** |

*Abbreviations: ACE-III, Addenbrooke's Cognitive Examination III; MELD, Model for End-Stage Liver Disease score. Rho values were calculated using Spearman's rank correlation coefficient, and p values <0.05 were considered statistically significant.*
** –p < 0.05; ** –p < 0.001.*

**TABLE 4 |** Logistic regression analysis for ACE-III scores <82 in patients with alcohol-related liver cirrhosis.

| | Univeriable | | Multivariable | |
|---|---|---|---|---|
| | ***P* value** | **OR (95% CI)** | ***P* value** | **OR (95% CI)** |
| Years of education | <0.001 | 0.70 (0.59–0.84) | <0.001 | 0.65 (0.53–0.79) |
| Child-Pugh score | 0.039 | 1.25 (1.01–1.55) | 0.004 | 1.51 (1.14–2.00) |
| MELD score | 0.133 | 1.05 (0.99–1.12) | - | |
| Age | 0.413 | 1.02 (0.98–1.05) | - | |
| Blood ammonia | 0.612 | 1.00 (0.99–1.01) | - | |
| Hiperammonemia | 0.732 | 0.87 (0.40–1.92) | - | |
| Females | 0.793 | 0.88 (0.34–2.27) | - | |

*Abbreviations: ACE-III, Addenbrooke's Cognitive Examination III; MELD, Model for End-Stage Liver Disease score; OR, odds ratio.*

increased to 87.0 ± 6.7 points, with a median of 86.0 (range: 73–98; interquartile range [IQR]: 83.0–91.0).

The most notable improvements were seen in memory (mean: 20.83 ± 3.88; median: 21.0) and verbal fluency (mean: 12.08 ± 1.56; median: 12.0). Improvement was also evident in attention (mean: 17.0 ± 1.18), language (mean: 23.85 ± 2.23), and visuospatial abilities (mean: 13.93 ± 2.14). Data are presented in **Table 6**.

## Changes in Cognitive Function Following Liver Transplantation (Δ Scores)

Following liver transplantation, patients exhibited a mean increase of 7.07 ± 8.47 points in total ACE-III scores (median: +5.0; range: –10 to 34; interquartile range [IQR]: 2.0–9.0). The most pronounced improvements were observed in the following cognitive domains:

- Memory: +1.46 ± 2.91 (median: +1.0; range: –7 to 10; IQR: 0.0–3.0)
- Verbal Fluency: +1.43 ± 1.89 (median: +2.0; range: –3 to 6; IQR: 0.0–2.0)
- Visuospatial Abilities: +1.52 ± 2.57 (median: +2.0; range: –5 to 7; IQR: 0.0–3.0)
- Attention: +0.71 ± 1.23 (median: +1.0; range: –3 to 4; IQR: 0.0–1.0)
- Language: +0.32 ± 2.54 (median: 0.0; range: –4 to 14; IQR: –1.0–1.0)

A Wilcoxon signed-rank test confirmed that the observed post-transplant improvement in total ACE-III scores was statistically significant ($Z \approx 420.0$, $p < 0.0001$), indicating that the changes were unlikely to have occurred by chance. Moreover, statistically significant gains were also observed across most individual subdomains (*all* $p < 0.01$), further supporting the robustness of the cognitive recovery pattern.

Among the 55 patients assessed, 42 (76%) demonstrated cognitive improvement, 9 (16%) experienced decline, and 4 (8%) remained stable. This distribution was also statistically significant (Wilcoxon signed-rank test, $p < 0.01$) and is depicted in **Figure 2**.

| Test | Z/W-statistic | p-value |
|---|---|---|
| Wilcoxon | ≈ **420.0** | **< 0.0001** |

### Cognitive Decline After Liver Transplantation

Although the overall trajectory pointed toward cognitive improvement, a decline in total ACE-III scores was observed in 16.4% of patients (9 out of 55). Specific cognitive domains most frequently affected by post-transplant deterioration included:

- Language–decline in 29.1% of patients (16/55)
- Memory–decline in 20.0% of patients (11/55)
- Attention–decline in 16.4% of patients (9/55)
- Verbal Fluency–decline in 14.5% of patients (8/55)
- Visuospatial Abilities–decline in 10.9% of patients (6/55)

The overall distribution of cognitive change categories is illustrated in **Figure 3**, showing that the majority of patients (n = 42; 76%) demonstrated post-transplant cognitive improvement, while 16% (n = 9) experienced decline and 7% (n = 4) remained unchanged (**Figure 3**).

### Exploratory Comparison: Improved vs. Declined Cognitive Trajectory

To further explore factors associated with cognitive outcomes following liver transplantation, we performed an exploratory subgroup analysis comparing patients who demonstrated cognitive improvement with those who experienced decline, based on changes in total ACE-III scores 6 months post-transplant. On average, patients in the improved group were younger (55.4 ± 8.8 vs. 59.3 ± 7.4 years) and had more years of education (12.7 ± 2.9 vs. 11.4 ± 3.1 years). The difference in educational attainment between the groups reached statistical significance (Mann–Whitney $U$, $p = 0.045$), while the difference in age did not ($p = 0.165$).

In a broader comparison including patients with no change in cognitive performance (n = 4), Kruskal–Wallis testing revealed a trend toward significance for years of education ($p = 0.072$), with age differences remaining non-significant ($p = 0.148$). These findings suggest that educational background may play a role in cognitive recovery after liver transplantation, although results should be interpreted cautiously due to the limited sample size. A summary of this exploratory analysis is presented in **Table 7**.

## DISCUSSION

This study provides a comprehensive evaluation of cognitive function at the time of liver transplant listing and in the early post-transplant period in a homogeneous cohort of patients with alcohol use disorder (AUD)-related liver cirrhosis. Cognitive impairment (CI) is increasingly recognized in patients with end-stage liver disease (ESLD), but available data remain heterogeneous, encompassing mixed etiologies, various diagnostic tools, and differing disease severity. Thus, a critical gap persists in understanding the true prevalence and profile of CI in well-defined liver transplant candidate populations.

To the best of our knowledge, this is the first study to examine cognitive performance both before and after liver transplantation (LT) in a cohort exclusively composed of patients with AUD-related ESLD. It delivers new insights into a crucial yet underexplored dimension of peri-transplant care. A recent review by Siddiqui et al. [17] included 24 studies with a median of 30 patients per study and follow-up ranging from 1 month to 1.8 years post-LT. The prevalence of CI varied from 4% to 36% within the first 8 months post-transplant and from 0% to 16% thereafter. Due to methodological variability, CI was grouped into six cognitive domains: attention, executive functions, working memory, long-term memory, language, and visuospatial abilities. However, no prior study has provided precise pre-LT CI data specifically in AUD candidates.

**TABLE 5 |** ACE-III results among liver transplant recipients with alcohol-related liver disease (ALD).

| N = 55 | Age | ACEIII 1 | Attention 1 | Memory 1 | Fluency 1 | Language 1 | Visuo-spatial abilities 1 | Year of education |
|---|---|---|---|---|---|---|---|---|
| Mean ±SD | 53.19 ± 9.72 | 80.62 ± 10.40 | 16.38 ± 1.39 | 18.15 ± 4.16 | 10.21 ± 2.48 | 23.19 ± 3.44 | 12.22 ± 3.15 | 12.19 ± 3.24 |
| median | 53.0 | 82.0 | 17.0 | 19.0 | 10.0 | 24.0 | 13.0 | 12.0 |
| range | 32–74 | 51–97 | 12–18 | 8–25 | 3–14 | 10–26 | 3–16 | 8–20 |
| IQR | 46.0–60.0 | 75.0–87.0 | 16.0–18.0 | 16.0–22.0 | 9–12 | 21.0–25.0 | 10.0–14.0 | 10.0–14.0 |

**TABLE 6 |** Second ACE-III assessment in liver transplant recipients with alcohol-related liver disease (ALD).

| N = 55 | ACEIII 2 | Attention 2 | Memory 2 | Fluency 2 | Language 2 | Visuo-spatial abilities 2 | Time form LT (months) |
|---|---|---|---|---|---|---|---|
| Mean ±SD | 87.0 ± 6.7 | 17.0 ± 1.18 | 20.83 ± 3.88 | 12.08 ± 1.56 | 23.85 ± 2.23 | 13.93 ± 2.14 | 7.13 ± 1.45 |
| median | 86.0 | 17.0 | 21.0 | 12.0 | 25.0 | 14.0 | 7.0 |
| range | 73–98 | 13–18 | 10–25 | 9–14 | 15–26 | 7–16 | 6–11 |
| IQR | 83.0–91.0 | 16.0–18.0 | 18.0–24.0 | 11.0–13.0 | 22.0–25.0 | 13.0–15.0 | 6.0–8.0 |

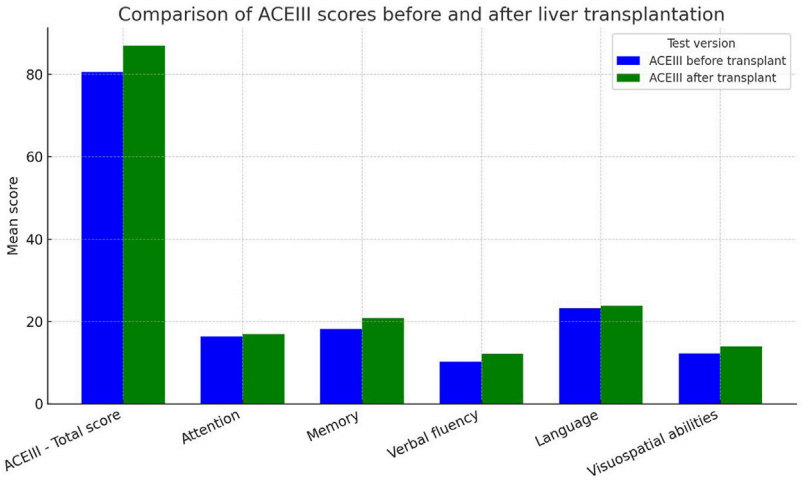



**FIGURE 2 |** ACE-III scores before and after liver transplantation in recipients with alcohol-related end-stage liver disease.

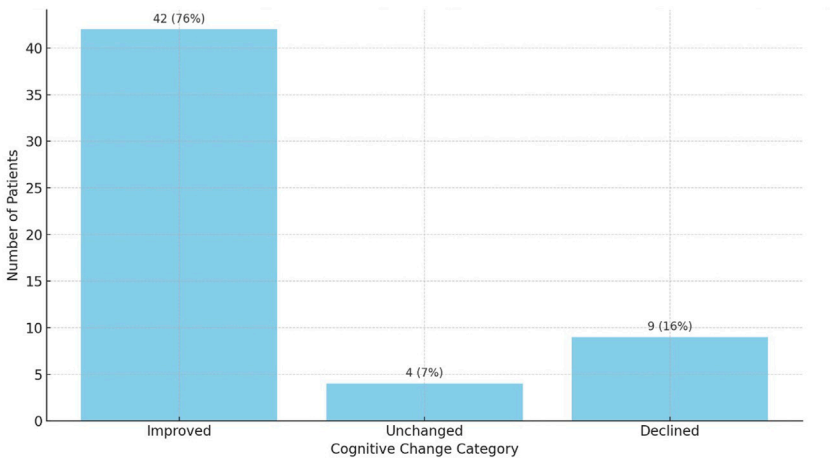



**FIGURE 3 |** Distribution of cognitive change categories after liver transplantation based on ACE-III scores (n = 55). Cognitive improvement was observed in 42 patients (76%), decline in 12 (22%), and no change in 1 patient (2%).

Our single-center analysis demonstrated a strikingly high prevalence of significant CI suggestive of dementia, with only 14% of patients scoring within the normal range on the ACE-III. The lowest performance was noted in verbal fluency and visuospatial domains, echoing findings by Lee et al. [25] and Sorrell et al. [26], who reported deficits in memory, visuospatial construction, attention, and immediate memory in patients with alcohol-related cirrhosis. Prior work by our group also showed significantly higher ACE-III scores and lower CI prevalence in patients with end-stage kidney disease (ESKD) compared to those with ESLD, with memory and visuospatial function being particularly impaired in the ESLD cohort [27].

Alcohol-induced brain damage is influenced by the quantity, age of onset, and duration of drinking, along with age, education, genetics, and prenatal exposure [14, 20, 28]. Stavro et al. [29] and Crowe et al. [28] described diffuse cognitive deficits in AUD, consistent with our cohort's mean ACE-III score of 78, with dysfunction across all domains. Verbal fluency and visuospatial impairments were particularly prominent, aligning with findings from Crowe et al. [28] and others [30–34]. Post-transplant studies by Campagna et al. [15] and Siddiqui et al. [17] also reported persistent deficits in attention, executive function, memory, and visuospatial processing.

Although the pathophysiological mechanisms linking ESLD and CI remain complex, factors such as neuroinflammation, hippocampal atrophy, and oxidative damage to the prefrontal cortex have been implicated [13, 35, 36]. MRI studies have shown reduced hippocampal volume in AUD patients compared to healthy controls [37]. Our data revealed a significant negative correlation between age and verbal fluency, with a trend toward

**TABLE 7 |** Comparison of patients with improved vs. declined cognitive performance post-transplant.

| Group | N | Age (mean ± SD) | Education years (mean ± SD) |
|---|---|---|---|
| Improved | 12 | 55.4 ± 8.8 | 12.7 ± 2.9 |
| Declined | 9 | 59.3 ± 7.4 | 11.4 ± 3.1 |
| No change | 4 | 52.0 | 10.5 |
| p-value (Kruskal-Wallis) | | 0.148 | 0.072 |
| p-value (Mann–Whitney U) | | 0.165 | 0.045 |

*Due to non-normal distribution and small sample sizes, non-parametric tests were used: Kruskal–Wallis for three-group comparisons and Mann–Whitney U for pairwise testing.*

lower total ACE-III scores in older patients. This supports findings by Campagna et al. [15], who suggested a critical vulnerability window at ages 50–60, possibly reflecting cumulative alcohol-related neurotoxicity.

Educational attainment emerged as a protective factor against CI, with ACE-III scores positively correlated with years of education, in line with Schneeweis et al. [38]. Conversely, a higher Child-Pugh score was associated with poorer cognitive outcomes and an increased likelihood of dementia-range ACE-III results. The MELD score did not correlate with total ACE-III performance but did show associations with attention and language subdomains. This divergence may relate to MELD's omission of nutritional parameters like serum albumin. Chronic malnutrition and sarcopenia, common in advanced liver disease, are known contributors to cognitive dysfunction [39–41].

Our findings align with reports showing greater post-LT CI prevalence in patients with pre-transplant MELD scores of 22–26 (21%–36%) compared to those with scores of 11–19 (8%–13%) [16, 17]. These trends highlight the multifactorial burden of CI in advanced liver disease.

In our cohort of 55 patients who completed both pre- and post-transplant cognitive evaluations, we observed a significant overall improvement in ACE-III scores, with the most notable gains in memory, verbal fluency, and visuospatial domains. Language function showed only modest improvement and remained one of the most frequently impaired areas. These findings align with prior reports suggesting that LT may reverse cognitive deficits, particularly those associated with hepatic encephalopathy and systemic inflammation [15, 17]. The Wilcoxon signed-rank test confirmed the statistical significance of this improvement ($p < 0.0001$), further supporting the role of LT in promoting cognitive recovery in the majority of patients (**Figure 2**).

However, as illustrated in **Figure 3**, recovery was not universal - approximately 16% of patients experienced cognitive decline, most often in language and memory domains, while 8% remained unchanged. This variation highlights the need for long-term neurocognitive surveillance and for identifying risk factors associated with suboptimal outcomes.

Preliminary findings (**Table 7**) suggest that younger age and higher educational attainment may offer some protection against cognitive decline following liver transplantation. Given the high prevalence and clinical consequences of cognitive impairment in patients with alcohol-related end-stage liver disease, routine cognitive assessment during pre-transplant evaluation appears warranted. Early detection of individuals at increased risk could enable personalized cognitive support strategies and potentially enhance long-term clinical outcomes. CI has been shown to negatively impact treatment adherence, decision-making capacity, and overall prognosis in transplant recipients [42].

The association between higher educational attainment and cognitive improvement post-transplant is consistent with the cognitive reserve hypothesis, which posits that individuals with greater lifelong cognitive engagement - often reflected by formal education - may be more resilient to the effects of brain injury, including those related to hepatic encephalopathy. Although the current sample size is limited, the statistically significant difference in education between improved and declined patients lends further support to this concept.

Notably, age did not significantly differentiate outcome groups, suggesting that within this cohort, cognitive reserve may have been a more relevant determinant of cognitive recovery than chronological age.

Future studies should aim to incorporate neuroimaging and biomarkers to elucidate the mechanisms underlying CI. Clinical trials of cognitive training and pharmacologic interventions in this population are also needed to guide individualized care strategies.

## Limitations

This study has several limitations. First, data on the duration and quantity of alcohol consumption were not collected. However, all patients had abstained from alcohol for at least 6 months, verified by psychiatric assessment. Second, DSM-5 criteria were not applied to diagnose major or minor neurocognitive disorders. Nonetheless, ACE-III testing was performed by a psychiatrist during standard pre-transplant evaluation, following exclusion of major neurological conditions via MRI. Third, minimal hepatic encephalopathy was not assessed using tools such as the MMSE due to the pilot nature of the study.

Additionally, comparing our findings with previous research is challenging due to variability in study populations, methodologies, and sample sizes. Despite these limitations, our study provides important data from a relatively large, etiologically homogeneous cohort of AUD-related ESLD patients, showing a disturbingly high prevalence of CI. Routine cognitive screening may facilitate earlier interventions and improved clinical management.

## CONCLUSION

This study demonstrates a concerning prevalence of severe cognitive impairment, potentially indicative of dementia, in patients with alcohol-related ESLD both before and after liver transplantation. Although most patients showed post-transplant improvement, persistent deficits in memory and language highlight the need for ongoing monitoring. Our findings support routine cognitive screening in this population and underscore the importance of further research into predictive markers and therapeutic interventions aimed at preserving and enhancing cognitive function after LT.

## 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 humans were approved by the BioethicsCommittee of the Medical University of Warsaw (approval number KB/81/2022). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MG-S and PO - data collection. MJ - statistical analysis, manuscript revision. JR-W, AG, JM - concept, data processing. MG-S, MJ, JR-W - manuscript preparation. MG-S, MJ, PO, AG, JM, JR-W - revision and approval of the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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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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# The Individual Impact of Machine Perfusion on Liver and Kidney on Donor Expansion in Simultaneous Liver and Kidney Transplantation

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Machine perfusion (MP) use for both organs can increase organ usage in simultaneous liver and kidney transplantation (SLKT). We analyzed 6,956 SLKT performed between 2015 and 2024 using the United Network for Organ Sharing database. The primary outcomes were the 1-year graft survival for kidney and liver. Donor types and MP use for liver and/or kidney were captured and associations with outcomes were evaluated. SLKT from Donation after circulatory death donors (DCD) increased from 4.5% in 2015 to 16% in 2023. The median Kidney Donor Profile Index (KDPI) has increased from 23% in 2015 to 28% in 2023. MP use for kidney and liver also increased from 21% to 51% and 0%–17%, respectively. KDPI >85% was an independent risk factor of 1-year kidney graft failure in the no kidney MP group [HR 2.03, 95% CI 1.20–3.44, p = 0.009], but not in the kidney MP group. DCD was found to be an independent risk factor of 1-year liver graft failure in the no liver MP group [HR 1.56, 95% CI 1.19–2.03, p = 0.001], but not in the liver MP group. MP for both organs may contribute to expanding the donor pool for SLKT without compromising post-transplant outcomes.

**Keywords: donation after circulatory death, donor expansion, kidney donor profile index, machine perfusion, simultaneous liver and kidney transplantation**

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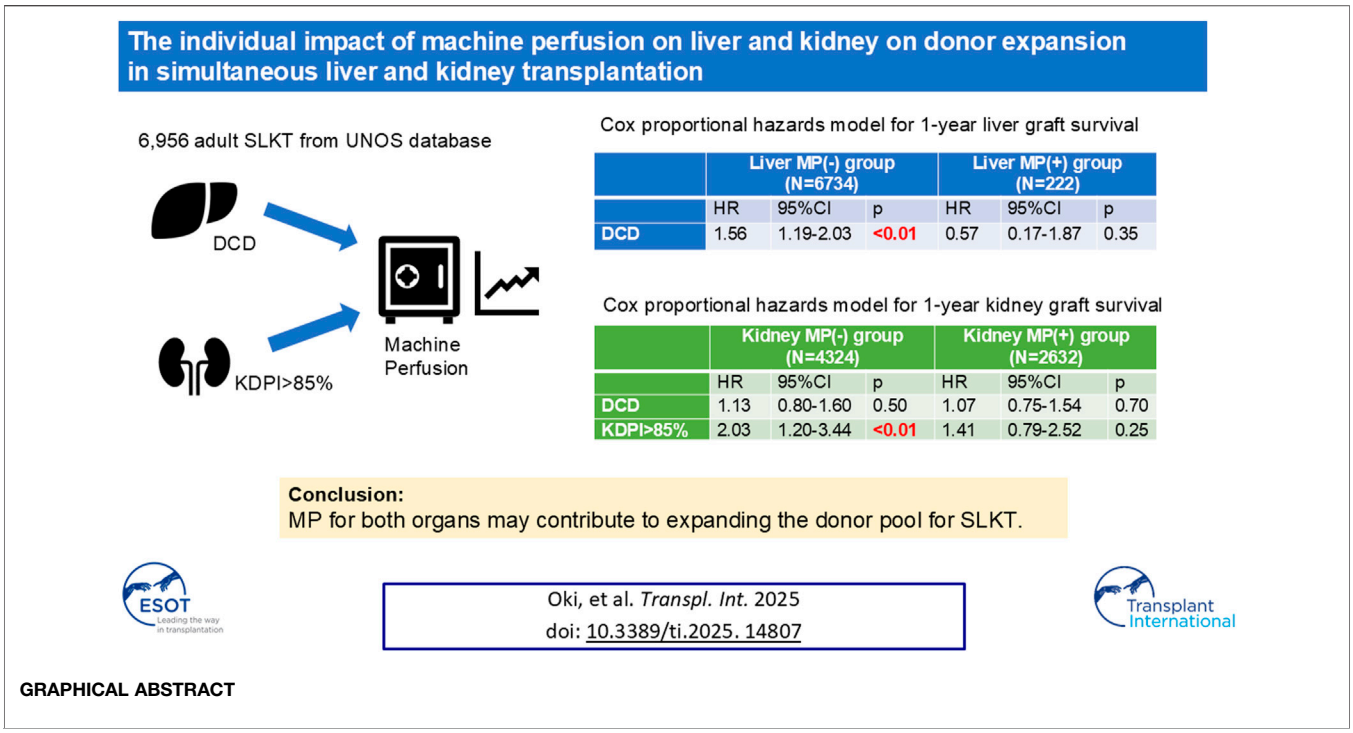
## INTRODUCTION

The high demand for organs in both kidney and liver transplantation along with efforts to expand the donor pool has resulted in the increased adoption of machine perfusion (MP) technologies in recent years. Several MP technologies to optimize organ preservation, such as hypothermic machine perfusion (HMP) or normothermic machine perfusion (NMP) have been developed [1].

In liver transplantation, MP could potentially enable the use of lower-quality livers that were not suitable for transplantation before, including donation after circulatory death donors (DCD), older donors, organs with longer cold and warm ischemia time and livers with macrosteatosis [2, 3]. In kidney transplantation, a meta-analysis of 16 studies demonstrated significantly lower DGF and PNF rates in the HMP group compared to those of static cold storage despite having a longer cold ischemia time (CIT) [4]. Furthermore, it has been reported that HMP improved DGF compared with standard cold storage in DCD, particularly in kidneys with a Kidney Donor Profile Index (KDPI) greater than 85%, which are at a higher risk for graft failure [5, 6].




**GRAPHICAL ABSTRACT**

The increase in nationally performed DCD simultaneous liver-kidney transplantations (SLKT) has been observed [7]. Nunez-Nateras et al compared outcomes of donation after brain death (DBD) and DCD in SLKT, and reported similar kidney DGF rates, similar 1-year patient survival (96.7% vs. 95.4% in DCD and DBD), similar 1-year liver allograft survival (93.3% vs. 93.1%) and similar 1-year kidney allograft survival (93.3% vs. 93.1%) [7]. However, MP was not incorporated in this study [7]. The use of MP is also increasing in SLKT, accounting for 1 in 4 kidney allografts since 2017 [8]. Chang et al reported that MP was associated with a reduction in DGF (adjusted Odds Ratio 0.74), but it did not significantly affect PNF. MP were used more often in DCD organs (7.9% vs. 4.5%, $p < 0.01$) [8]. Given these findings, MP use for both organs in SLKT can potentially increase organ usage from medically complex donors such as those with KDPI >85%, or DCD, without compromising outcomes, but there are few reports which have investigated the individual impact of MP for each organ on donor expansion in SLKT. Thus, the aim of this study was to evaluate 1) the temporal change of donor type and MP use for kidney or liver and 2) compare whether medically complex donors, such as those with KDPI >85% or DCD, pose a risk factor for 1-year graft survival between the use of MP and without MP for each organ in SLKT.

## MATERIALS AND METHODS

### Patients and Data Collection

This was a retrospective cohort study using the United Network for Organ Sharing (UNOS) database. We identified all adult (≥18 years) recipients of deceased-donor SLKT performed between January 2015, and March 2024. Recipient characteristics (age, gender, race, kidney/liver disease etiology, history of diabetes, body mass index (BMI), Model for End-Stage Liver Disease (MELD) score), severity of liver disease at transplantation (ascites, hepatic encephalopathy, serum albumin (Alb), bilirubin (Bil), international normalized ratio (INR), sodium (Na) at transplantation), severity of renal disease at transplantation (on dialysis or not, creatinine (Cre), estimated glomerular filtration fate (eGFR) at transplantation), and donor characteristics (age, gender, BMI, terminal serum creatinine, donor type, Kidney Donor Profile Index (KDPI)) were obtained from UNOS data. Primary liver disease etiology was reviewed and divided into five groups, alcohol-related liver disease (ALD), nonalcoholic steatohepatitis (NASH), hepatitis C virus infection (HCV), biliary diseases, and others. Primary kidney disease etiology was reviewed and divided into six groups, hepatorenal syndrome, diabetic nephropathy, nephrosclerosis, glomerular nephritis (GN), polycystic kidney disease (PKD) and others. Since the detailed information regarding MP protocols used was not available, it should be noted that MP includes HMP and NMP, which cannot be distinguished in this study. Normothermic regional perfusion (NRP) might have been used but there is not data for it in UNOS. In addition, back-to-base MP might not be captured in UNOS data. Postoperative variable included DGF for kidney, primary non-function (PNF), and re-transplantation. DGF was defined as the requirement for dialysis during the first 7 days following SLKT [9], while PNF for kidney was defined as graft failure of the kidney occurring within 90 days post-transplant [10]. PNF for liver was defined as liver function incompatible with life, requiring retransplantation or resulting in death within 7 days of surgery [11].

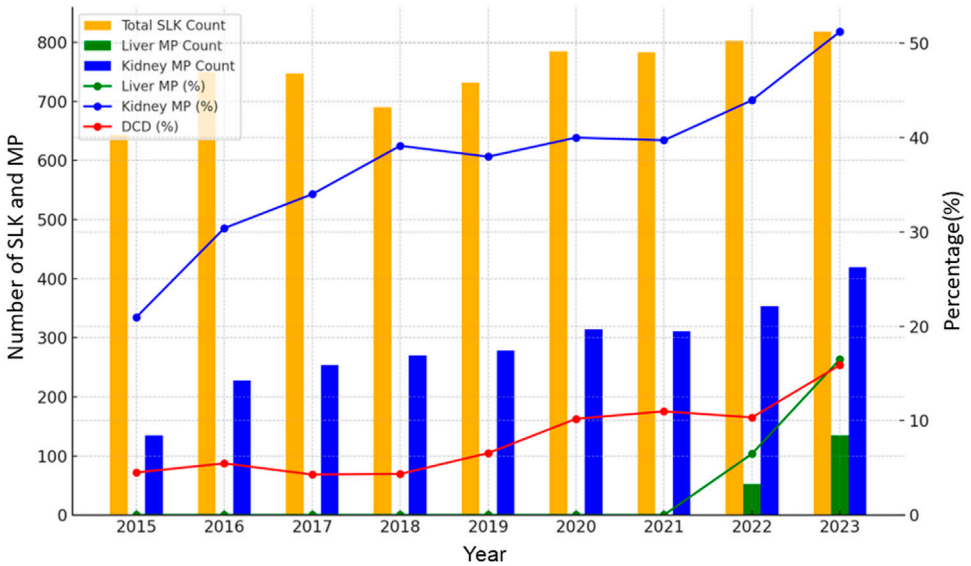



**FIGURE 1 |** Trends in DCD rate and MP use in SLKT from 2015 to 2023. The number of SLKT transplants showed a steady increasing trend with a slight fluctuation between 2015 and 2023. MP use for kidney and liver also increased from 21% to 51% and 0%–17%, respectively.

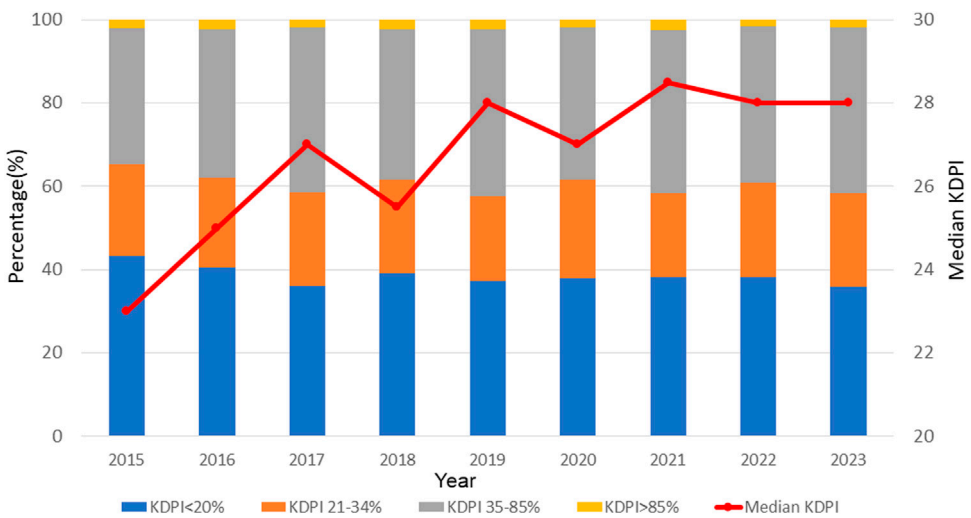



**FIGURE 2 |** Trends in KDPI in SLKT from 2015 to 2023. Although the ratio of KDPI >85% did not significantly change from 2015 to 2023, the median KDPI has shown an increasing trend from 23 in 2015 to 28 in 2023.

This study used the Standard Transplant Analysis and Research file provided by the Organ Procurement and Transplantation Network (OPTN)/UNOS in which all individually identifiable information is encrypted. Henry Ford Institutional Review Board (IRB) exempted IRB approval to conduct this study using this database.

## Outcomes

The primary outcomes included the 1-year graft survival for kidney and liver. "Graft failure" refers to graft failure from any cause, including death and retransplant. For kidney failure, this also includes return to maintenance dialysis. "Graft survival" similarly refers to the absence of all-cause graft failure by the definition of OPTN[1]. First, patients were classified into two groups; kidney MP group (those who used MP for kidney) and no kidney MP group (those who did not use MP for kidney). We evaluated whether KDPI >85% or DCD were identified as a risk factor for 1-year kidney graft failure in kidney MP group and no kidney MP group, respectively. The risks were adjusted for recipient factors such as age [12], gender

[1]https://srtr.transplant.hrsa.gov/ADR/Chapter?name=Preface&year=2023

**TABLE 1 |** Comparison of background characteristics according to the presence of machine perfusion for kidney or liver allograft.

| Recipient Variables | Kidney | | | Liver | | |
|---|---|---|---|---|---|---|
| | No Machine Perfusion (N = 4,324) | Machine Perfusion (N = 2,632) | *p*-value | No Machine Perfusion (N = 6,734) | Machine Perfusion (N = 222) | *p*-value |
| Age (y.o.) | 59.0 (51.0, 64.0) | 58.0 (50.0, 64.0) | 0.886 | 59.0 (50.0, 64.0) | 60.0 (53.0, 65.0) | 0.008 |
| Male (%) | 2,578 (59.6) | 1,555 (59.1) | 0.669 | 3,996 (59.3) | 137 (61.7) | 0.488 |
| Race/ethnicity (%) | | | <0.001 | | | 0.131 |
| White | 2,647 (61.2) | 1743 (66.2) | | 4,246 (63.1) | 144 (64.9) | |
| Black | 553 (12.8) | 287 (10.9) | | 824 (12.2) | 16 (7.2) | |
| Hispanic | 840 (19.4) | 454 (17.2) | | 1,248 (18.5) | 46 (20.7) | |
| Asian | 207 (4.8) | 96 (3.6) | | 290 (4.3) | 13 (5.9) | |
| Other | 77 (1.8) | 52 (2.0) | | 126 (1.9) | 3 (1.4) | |
| BMI (kg/m$^2$) | 27.4 (23.7, 32.0) | 27.6 (24.2, 32.2) | 0.014 | 27.5 (23.9, 32.1) | 27.6 (23.8, 32.2) | 0.729 |
| Diabetes (%) | 1900 (44.0) | 1,086 (41.3) | 0.028 | 2,884 (42.9) | 102 (45.9) | 0.371 |
| Etiology of liver disease (%) | | | 0.013 | | | <0.001 |
| NASH | 978 (23.9) | 659 (25.2) | | 1,575 (24.2) | 62 (27.9) | |
| Alcohol | 983 (24.0) | 615 (23.5) | | 1,576 (24.3) | 22 (9.9) | |
| HCV | 551 (13.4) | 281 (10.7) | | 816 (12.6) | 16 (7.2) | |
| Biliary diseases | 152 (3.7) | 97 (3.7) | | 241 (3.7) | 8 (3.6) | |
| Other | 1,433 (35.0) | 968 (36.9) | | 2,287 (35.2) | 114 (51.4) | |
| MELD score | 29.0 (23.0, 35.0) | 28.0 (23.0, 34.0) | 0.369 | 29.0 (23.0, 35.0) | 27.0 (21.3, 32.0) | <0.001 |
| Encephalopathy (%) | | | 0.018 | | | 0.037 |
| Grade I | 1,376 (33.4) | 798 (30.4) | | 2,119 (32.5) | 55 (24.8) | |
| Grade II | 2,176 (52.8) | 1,422 (54.2) | | 3,470 (53.2) | 128 (57.7) | |
| Grade III | 567 (13.8) | 404 (15.4) | | 932 (14.3) | 39 (17.6) | |
| Ascites (%) | 1820 (44.2) | 1,236 (47.1) | 0.018 | 2,953 (45.3) | 103 (46.4) | 0.784 |
| Albumin (g/dL) | 3.2 (2.7, 3.7) | 3.1 (2.6, 3.6) | 0.001 | 3.2 (2.7, 3.7) | 3.2 (2.6, 3.6) | 0.399 |
| Sodium (mmol/L) | 136.0 (133.0, 139.0) | 136.0 (133.0, 139.0) | 0.103 | 136.0 (133.0, 139.0) | 136.0 (133.0, 139.0) | 0.450 |
| INR | 1.6 (1.2, 2.1) | 1.5 (1.2, 2.0) | 0.135 | 1.6 (1.2, 2.0) | 1.5 (1.2, 1.9) | 0.007 |
| Total bilirubin (g/dL) | 2.5 (0.9, 7.1) | 2.3 (1.0, 6.4) | 0.184 | 2.4 (0.9, 7.0) | 1.8 (0.8, 4.8) | 0.005 |
| Etiology of kidney disease (%) | | | <0.001 | | | 0.006 |
| Hepatorenal | 1701 (39.3) | 1,131 (43.0) | | 2,714 (40.3) | 118 (53.2) | |
| Diabetes | 869 (20.1) | 492 (18.7) | | 1,328 (19.7) | 33 (14.9) | |
| PKD | 226 (5.2) | 175 (6.6) | | 390 (5.8) | 11 (5.0) | |
| Hypertension | 242 (5.6) | 106 (4.0) | | 342 (5.1) | 6 (2.7) | |
| Glomerulonephritis | 168 (3.9) | 100 (3.8) | | 259 (3.8) | 9 (4.1) | |
| Other | 1,118 (25.9) | 628 (23.9) | | 1701 (25.3) | 45 (20.3) | |
| Hemodialysis (%) | 2,810 (68.4) | 1792 (68.6) | 0.914 | 4,462 (68.6) | 140 (63.3) | 0.105 |
| Creatinine (mg/dL) | 3.5 (2.3, 5.1) | 3.4 (2.3, 5.0) | 0.604 | 3.4 (2.3, 5.1) | 3.2 (2.0, 4.7) | 0.064 |
| eGFR (mL/min/1.73 m$^2$) | 17.0 (11.0, 27.3) | 17.0 (11.0, 27.4) | 0.804 | 17.0 (11.0, 27.3) | 17.6 (11.7, 30.7) | 0.126 |
| Donor Variables | | | | | | |
| Age (y.o.) | 33.0 (24.0, 44.0) | 36.0 (27.0, 46.0) | <0.001 | 34.0 (25.0, 45.0) | 38.5 (29.0, 47.0) | <0.001 |
| Male (%) | 1,692 (39.1) | 1,002 (38.1) | 0.388 | 2,613 (38.8) | 81 (36.5) | 0.529 |
| Race/ethnicity (%) | | | 0.002 | | | 0.052 |
| White | 2,737 (63.3) | 1795 (68.2) | | 4,370 (64.9) | 162 (73.0) | |
| Black | 663 (15.3) | 355 (13.5) | | 999 (14.8) | 19 (8.6) | |
| Hispanic | 754 (17.4) | 390 (14.8) | | 1,113 (16.5) | 31 (14.0) | |
| Asian | 108 (2.5) | 57 (2.2) | | 158 (2.3) | 7 (3.2) | |
| Other | 62 (1.4) | 35 (1.3) | | 94 (1.4) | 3 (1.4) | |
| BMI(kg/m$^2$) | 26.2 (23.0, 30.4) | 26.7 (23.4, 30.7) | 0.001 | 26.3 (23.1, 30.4) | 28.6 (24.7, 32.4) | <0.001 |
| Cause of death (%) | | | 0.438 | | | 0.091 |
| anoxia | 1868 (43.2) | 1,162 (44.1) | | 2,919 (43.3) | 111 (50.0) | |
| cerebrovascular accident | 880 (20.4) | 498 (18.9) | | 1,338 (19.9) | 40 (18.0) | |
| trauma | 1,447 (33.5) | 884 (33.6) | | 2,270 (33.7) | 61 (27.5) | |
| Creatinine (mg/dL) | 0.90 (0.70, 1.20) | 0.88 (0.67, 1.19) | 0.003 | 0.90 (0.70, 1.20) | 0.73 (0.60, 1.04) | <0.001 |
| Distance of donation to transplantation hospital (km) | 110.0 (24.0, 261.0) | 94.0 (16.0, 213.3) | <0.001 | 102.0 (20.0, 240.0) | 166.0 (63.8, 339.3) | <0.001 |
| DCD (%) | 296 (7.2) | 321 (12.2) | <0.001 | 501 (7.7) | 116 (52.3) | <0.001 |
| KDPI category (%) | | | 0.140 | | | 0.060 |
| <20% | 1,602 (38.8) | 979 (37.2) | | 2,514 (38.4) | 67 (30.2) | |
| 20%–34% | 911 (22.1) | 570 (21.7) | | 1,432 (21.9) | 49 (22.1) | |
| 35%–85% | 1,542 (37.3) | 1,018 (38.7) | | 2,459 (37.6) | 101 (45.5) | |
| >85% | 74 (1.8) | 65 (2.5) | | 134 (2.0) | 5 (2.3) | |
| CIT for kidney (hour) | 9.77 (7.60,12.3) | 19.3 (11.0, 26.7) | [a] | 11.0 (8.20,18.2) | 19.6 (15.5, 26.1) | [a] |
| CIT for liver (hour) | 6.08 (5.00, 7.47) | 5.90 (4.73,7.50) | [a] | 5.98 (4.83,7.30) | 13.0 (10.1,16.2) | [a] |
| Machine perfusion for liver (%) | 57 (1.3) | 165 (6.3) | <0.001 | - | - | - |

(Continued on following page)

**TABLE 1 |** (*Continued*) Comparison of background characteristics according to the presence of machine perfusion for kidney or liver allograft.

| Recipient Variables | Kidney | | | Liver | | |
|---|---|---|---|---|---|---|
| | No Machine Perfusion (N = 4,324) | Machine Perfusion (N = 2,632) | *p*-value | No Machine Perfusion (N = 6,734) | Machine Perfusion (N = 222) | *p*-value |
| Machine perfusion for kidney (%) | - | - | - | 2,467 (36.6) | 165 (74.3) | <0.001 |
| Outcomes | | | | | | |
| DGF for kidney | 1,256 (31.0) | 662 (25.3) | <0.001 | 1857 (28.8) | 61 (28.4) | 0.898 |
| PNF for kidney | 285 (6.6) | 194 (7.4) | 0.213 | 461 (6.8) | 18 (8.1) | 0.465 |
| PNF for liver | 78 (1.8) | 37 (1.4) | 0.207 | 114 (1.6) | 1 (0.5) | 0.153 |
| Re-transplantation (%) | | | 0.576 | | | 0.235 |
| Re-transplantation for kidney | 15 (0.3) | 11 (0.4) | | 26 (0.4) | 0 (0.0) | |
| Re-transplantation for liver | 44 (1.0) | 29 (1.1) | | 68 (1.0) | 5 (2.3) | |
| Re-transplantation for both organs | 6 (0.1) | 1 (0.0) | | 7 (0.1) | 0 (0.0) | |

*Continuous data are presented as median (IQR): y.o., year old; %, percent; BMI, body mass index; NASH, nonalcoholic steatohepatitis; HCV, hepatitis C virus; MELD, model for end-stage liver disease; INR, international normalized ratio; PKD, polycystic kidney disease; DGF, delayed graft function; PNF, primary non-function; DCD, donation after circulatory death; KDPI, kidney donor profile index; CIT, cold ischemic time.*

[a]*The comparison of CIT between MP and non-MP was not possible or did not reflect actual impact of CIT, since in MP cases, CIT recorded in the UNOS data included MP time.*

**TABLE 2 |** Multivariable Cox proportional hazards model for 1-year kidney graft failure according to the presence of machine perfusion for kidney allograft.

| Variables | No Kidney MP group | | Kidney MP group | |
|---|---|---|---|---|
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| Recipient variables | | | | |
| Age | 1.02 (1.01–1.03) | 0.002 | 1.01 (0.995–1.02) | 0.238 |
| Male | 1.17 (0.96–1.42) | 0.113 | 0.997 (0.80–1.25) | 0.977 |
| BMI | 1.01 (0.99–1.02) | 0.371 | 1.03 (1.01–1.05) | 0.002 |
| Race (ref: White) | | | | |
| Black | 0.80 (0.59–1.08) | 0.143 | 1.28 (0.93–1.78) | 0.132 |
| Hispanic | 0.84 (0.66–1.08) | 0.173 | 0.89 (0.66–1.21) | 0.461 |
| Asian | 0.90 (0.57–1.41) | 0.635 | 1.00 (0.56–1.81) | 0.990 |
| Other | 0.61 (0.25–1.49) | 0.281 | 0.59 (0.22–1.60) | 0.302 |
| Diabetes | 1.10 (0.90–1.33) | 0.352 | 1.37 (1.09–1.73) | 0.008 |
| Donor variables | | | | |
| Male | 0.80 (0.66–0.96) | 0.017 | 0.88 (0.70–1.10) | 0.268 |
| KDPI >85% | 2.03 (1.20–3.44) | 0.009 | 1.41 (0.79–2.52) | 0.250 |
| Race (ref: White) | | | | |
| Black | 0.82 (0.62–1.09) | 0.169 | 1.20 (0.88–1.64) | 0.255 |
| Hispanic | 0.99 (0.77–1.28) | 0.933 | 1.15 (0.84–1.56) | 0.383 |
| Asian | 1.65 (1.02–2.66) | 0.042 | 1.17 (0.55–2.50) | 0.684 |
| Other | 0.92 (0.41–2.07) | 0.844 | 1.28 (0.53–3.13) | 0.583 |
| DCD | 1.13 (0.80–1.60) | 0.500 | 1.07 (0.75–1.54) | 0.703 |
| Machine perfusion for Liver | 1.10 (0.90–1.33) | 0.352 | 1.16 (0.69–1.94) | 0.579 |

*DCD, donation after circulatory death; KDPI, kidney donor profile index.*

[13], BMI [14], race [15], diabetes mellitus [16] and race of donor [15] which have been reported to be associated with kidney graft failure in SLKT or kidney only transplantation. Donor factors such as age, BMI, and cause of death which were part of KDPI were not included as adjustment covariables.

Second, patients were classified into two groups; liver MP group (those who used MP for liver) and no liver MP group (those who did not use MP for liver). Similarly, we investigated whether DCD was related to 1-year liver graft failure in liver MP group and no liver MP group, respectively. The risks were adjusted for recipient factors such as age, gender [17], BMI [18], diabetes mellitus [19], race [20], MELD score [21] and donor factors such as age [22], gender [23], BMI [24], race [25], cause of death [25] which have been reported to be associated with liver graft failure/mortality in liver only transplantation.

Third, we also examined the impact of MP on DGF in higher KDPI groups, using KDPI cutoffs of 35, 60, and 85. The risks were adjusted for recipient's age, gender, BMI, race, diabetes mellitus, donor's age, gender, BMI, race, and DCD.

Finally, we divided the patients into four group; group1: patients who did not use MP for both kidney and liver, group2: patients who used MP for only kidney, group3: patients who used MP for only liver, group4: patients who used MP for both kidney and liver, and compared the graft survival between the four groups.

## Statistical Analysis

All statistical analyses were conducted using software (SPSS®, Version <27.0.1>; IBM Corp., Armonk, NY, United States). Continuous data were expressed as median (interquartile

**TABLE 3 |** Multivariable Cox proportional hazards model for 1-year liver graft failure according to the presence of machine perfusion for liver allograft.

| Variables | No liver MP group | | Liver MP group | |
|---|---|---|---|---|
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| Recipient variable | | | | |
| Age | 1.02 (1.01–1.03) | <0.001 | 0.98 (0.93–1.04) | 0.544 |
| Male | 0.88 (0.75–1.04) | 0.130 | 0.52 (0.16–1.66) | 0.267 |
| BMI | 1.01 (0.99–1.02) | 0.158 | 0.97 (0.88–1.07) | 0.531 |
| Race (ref: White) | | | | |
| Black | 1.14 (0.90–1.44) | 0.278 | 1.21 (0.19–7.85) | 0.843 |
| Hispanic | 0.85 (0.69–1.05) | 0.136 | 0.42 (0.08–2.22) | 0.306 |
| Asian | 0.97 (0.66–1.44) | 0.894 | - | 0.987 |
| Other | 0.49 (0.22–1.11) | 0.087 | - | 0.994 |
| Diabetes | 1.16 (0.98–1.36) | 0.083 | 1.23 (0.44–3.47) | 0.694 |
| MELD score | 1.02 (1.01–1.03) | <0.001 | 1.12 (1.02–1.23) | 0.016 |
| Donor variable | | | | |
| Age | 1.01 (0.99–1.01) | 0.110 | 1.07 (1.01–1.14) | 0.033 |
| Male | 0.88 (0.75–1.04) | 0.130 | 0.75 (0.26–2.16) | 0.595 |
| BMI | 1.00 (0.99–1.02) | 0.509 | 0.99 (0.89–1.10) | 0.803 |
| Race (ref: White) | | | | |
| Black | 0.998 (0.79–1.25) | 0.986 | 3.24 (0.57–18.4) | 0.184 |
| Hispanic | 1.05 (0.84–1.30) | 0.691 | 3.34 (0.66–17.0) | 0.147 |
| Asian | 1.46 (0.94–2.28) | 0.096 | 45.4 (3.41–602) | 0.004 |
| Other | 0.81 (0.38–1.71) | 0.579 | 2.91 (0.25–33.7) | 0.392 |
| Cause of death (ref: anoxia) | | | | |
| cerebrovascular accident | 1.16 (0.93–1.44) | 0.184 | 0.08 (0.01–0.69) | 0.022 |
| trauma | 1.06 (0.88–1.28) | 0.565 | 0.45 (0.10–2.00) | 0.291 |
| DCD | 1.56 (1.19–2.03) | 0.001 | 0.57 (0.17–1.87) | 0.353 |
| Machine perfusion for kidney | 1.12 (0.96–1.31) | 0.159 | 1.07 (0.26–4.29) | 0.928 |

*MELD, model for end-stage liver disease; DCD, donation after circulatory death.*

range). Student t-tests or Mann–Whitney U-tests were used to compare continuous variables. The chi-square test or Fisher's exact test was used to compare the categorical variables. Univariable and multivariable Cox regression analyses were performed to examine significant factors associated with 1-year graft failure. Multivariable logistic regression analyses were performed to examine significant factors associated with DGF for kidney. The Kaplan-Meier method and log-rank test were used to compare differences in 1-year graft survival between four groups divided based on the presence of MP for kidney or liver. *P-values* less than 0.05 were inferred as significant.

# RESULTS

## Trend of SLKT

In total, 6,956 adult SLKT were performed during the study period. Between 2015 and 2023, the number of SLKT transplants showed a steady increasing trend with a slight fluctuation, reaching 800 cases in 2022 (**Figure 1**). SLKT from DCD increased from 4.5% in 2015 to 16% in 2023. MP use for kidney and liver also increased from 21% to 51% and 0%–17%, respectively between 2015 and 2023. **Figure 2** demonstrates the changes in KDPI, represented by KDPI categories and the median KDPI. Although the ratio of KDPI >85% did not significantly change from 2015 to 2023, the median KDPI has shown an increasing trend from 23 in 2015 to 28 in 2023 (**Figure 2**).

## Characteristics of Study Participants

**Supplementary Table S1** presents the background characteristics. The average patient age was 59.0 years, of which 59.4% were male. The average donor age was 34.0 years. SLKT from DCD donors accounted for 9.2% of the total. Donor kidneys with a KDPI <20% were the most frequently used, while kidneys with a KDPI >85% accounted for 2.0% of the total. Overall, 37.8% of kidney allografts were placed on MP (N = 2,632) and 3.2% of liver allografts were placed on MP (N = 222).

## Comparison of Patient' Characteristics According to MP for Kidney or Liver

The comparison of patients' characteristics between kidney MP group and no kidney MP group is shown in **Table 1**. Kidney allografts subjected to MP were more frequently DCD (12.2% vs. 7.2%, $p < 0.001$). Although no statistical difference was found in KDPI category, donors were older in kidney MP group. (median 36.0 vs. 33.0 years, $p < 0.001$). The severity of kidney disease, as indicated by hemodialysis status, serum creatinine level, and eGFR at the time of transplantation, showed no statistically significant differences (**Table 1**).

**Table 1** also shows the comparison of patients' characteristics between liver MP group and no liver MP group. More than half of liver allografts treated with MP were DCD organs (52.3% vs. 7.7%, $p < 0.001$). Although the MELD score was lower in the liver MP group (median 27.0 vs. 29.0, $p < 0.001$), there were no statistically significant differences in the presence of dialysis or serum creatinine levels at the time of transplantation between two

**TABLE 4 |** Multivariable logistic regression model for DGF.

| Variables | KDPI >35 | | KDPI >60 | | KDPI >85 | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| Machine perfusion for kidney | 0.72 (0.61–0.86) | <0.001 | 0.74 (0.56–0.98) | 0.038 | 0.59 (0.27–1.28) | 0.182 |
| Machine perfusion for liver | 0.71 (0.44–1.14) | 0.154 | 0.52 (0.22–1.25) | 0.144 | 1.05 (0.08–13.3) | 0.968 |

*Adjusted for recipient's age, gender, BMI, race, diabetes mellitus, donor's age, gender, BMI, race and DCD.*

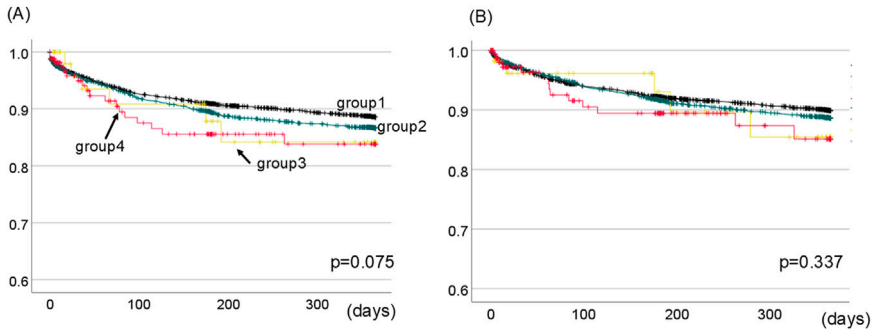



**FIGURE 3 |** 1-Year kidney **(A)** and liver **(B)** graft survival between four groups divided based on the presence of MP for kidney or liver. The black line shows group1 (patients who did not use MP for both kidney and liver). The green line shows group2 (patients who used MP for only kidney). The yellow line shows group3 (patients who used MP for only liver). The red line shows group4 (patients who used MP for both kidney and liver). There was no statistically difference in 1-year kidney or liver graft survival between four groups. [p = 0.075 **(A)**, p = 0.337 **(B)**, respectively.].

groups. The incidence of DGF was significantly lower in the kidney MP group compared to no kidney MP group. In contrast, there were no significant differences between MP and non-MP groups for either organ regarding the incidence of PNF (for kidney or liver) or the rate of re-transplantation.

## Kidney Machine Perfusion and 1-Year Kidney Graft Survival/1-Year Patient Survival

Cox hazard models were used to evaluate the factors related to 1-year kidney graft failure in kidney MP group and no kidney MP group (**Supplementary Table S2**; **Table 2**). KDPI >85% was an independent risk factor of 1-year kidney graft failure in the no kidney MP group [HR 2.03, 95% CI 1.20–3.44, p = 0.009]. However, when MP for kidney was used, KDPI >85% was not found to be the risk factor related to 1-year kidney graft failure [HR 1.41, 95% CI 0.79–2.52, p = 0.250]. There was no significant relationship between MP for the liver and 1-year kidney graft failure in either group. DCD was not identified as a risk factor for 1-year kidney graft failure in both groups (**Table 2**).

## Liver Machine Perfusion and 1-Year Liver Graft Survival/1-Year Patient Survival

We compared the risk factors related to 1-year liver graft failure in the liver MP group and the no liver MP group by using Cox hazard model (**Supplementary Table S3**; **Table 3**). DCD was found to be an independent risk factor of 1-year liver graft failure in the no liver MP group [HR 1.56, 95% CI 1.19–2.03, p = 0.001], but not in the liver MP group [HR 0.57, 95% CI 0.17–1.87, p = 0.353]. The MP for kidney was not related to 1-year liver graft survival in both groups (**Table 3**).

## The Influence of MP on DGF

Kidney MP was associated with decreased a risk of DGF among the recipients with KDPI >35%, as well as among the patients with KDPI >60%. [(OR 0.72, 95% CI 0.61–0.86, p < 0.001), (OR 0.74, 95% CI 0.56–0.98, p = 0.038), respectively] However, there was no significant association between kidney MP and DGF in the group with KDPI >85% (p = 0.182). Liver MP was not associated with DGF in all KDPI groups (**Table 4**).

## The Influence of MP on 1-Year Graft Survival

To investigate the influence of MP for 1-year graft survival, we compared the 1-year graft survival between four groups; group 1: patients who did not use MP for both kidney and liver (N = 4,267), group 2: patients who used MP for only kidney (N = 2,467), group 3: patients who used MP for only liver (N = 57), group 4: patients who used MP for both kidney and liver (N = 165).

There was no statistically significant difference in 1-year kidney graft survival between the four groups (p = 0.075, **Figure 3A**). Similarly, we examined the influence of MP

on 1-year liver graft survival. The 1-year liver graft survival was comparable between four groups (p = 0.337, **Figure 3B**).

## DISCUSSION

The utilization of MP in SLKT has significantly increased in the U.S. The use of MP for kidney and liver increased from 21% to 51% and from 0% to 17%, respectively, between 2015 and 2023. Additionally, the proportion of SLKT from DCD rose from 4.5% in 2015 to 16% in 2023. Although MP has demonstrated many benefits in kidney-only and liver-only transplants [4, 26, 27], there are limited studies addressing the role of MP in SLKT. Of note, there are few studies that have examined the significance of MP in SLKT, particularly in the context of expanding donor pool. While DCD was a risk factor for 1-year liver graft failure in the absence of liver MP, it was not a risk factor when liver MP was used. Similarly, while KDPI >85% was associated with an increased risk of 1-year kidney graft failure without kidney MP, kidney MP might mitigate this risk. There was no difference in 1-year kidney or liver graft survival based on the use of MP for each organ individually or both, compared to no MP. Thus, MP for both organs might contribute to expanding the donor pool for SLKT without compromising post-transplant outcomes even with more use of marginal donor grafts.

The introduction of the MELD score into OPTN deceased donor liver allocation policy in 2002 has resulted in a substantial rise in SLKT in the US. [28]. Of the total number of SLKT from 2002 to 2012, 49% of donor kidneys had a KDPI <35% and were prioritized for pediatric candidates in kidney-alone allocation [28]. In 2017, an UNOS allocation policy for SLKT was established in the US, setting the eligibility criteria for SLKT. As a result, there was a temporary decline from 2016 to 2019 [29]. However, along with the increase of use of MP preservation in SLKT, the number of SLKT has shown a rising trend again since 2020. It should be noted that MP for kidney was used in approximately half of total SLKT in 2023, and MP for liver began being used in 16% from 2022, although MP for liver was rarely used until 2021. From these findings, it is indicated that MP has contributed to the increase in SLKT.

There are many studies which compared outcomes of DBD and DCD in SLKT. Croome et al compared outcomes of DCD SLKT performed between 2000 and 2010 and 2011–2018 by using the UNOS data. Improvement in patient, liver graft, and kidney graft survival rates in DCD-SLKT was seen between these two eras [30]. They concluded that patients who underwent DCD-SLKT achieved comparable outcomes to those of matched patients who underwent DBD-SLKT in recent periods. The effect of MP was not captured in their study. Vinson et al performed the study which compared the overall outcomes of accepting a DCD SLKT now vs. waiting for a DBD SLKT in patients waitlisted for SLKT, stratified by MELD score (≤20, 21–30, >30) [31]. DCD SLKT could be a preferred option for the patients with MELD score >30 (incremental value of 0.31 quality-adjusted life years for DCD vs. DBD) [31]. Our study, using more recent UNOS data, demonstrated that DCD was identified as the risk factor related to 1-year liver graft failure. Meanwhile, DCD was not associated with the outcome, even after adjusting for the MELD score, when MP for liver was available. Although the type of MP in this study cannot be distinguished, the MP for liver in SLKT has been increasing since 2018, and it is possible that NMP was predominantly used. NMP techniques focus on keeping the liver in a condition which is similar to physiological metabolism and supports its synthetic functions [3]. A randomized clinical trial in liver transplantation conducted in the United States demonstrated that NMP preservation of deceased donor livers significantly reduced the incidence of early allograft dysfunction and ischemic biliary complications [32]. The use of NMP also contributed to a significant increase in the utilization of DCD donors [32]. Therefore, it is indicated that the introduction of MP increased the feasibility of using DCD donors in SLKT as well. In this analysis, MP was not found to be a factor that improved liver graft survival, but further accumulation of cases may lead to positive expectations in the future.

As mentioned above, high-quality donor kidneys with lower KDPI have been often used for SLKT, which otherwise would have been allocated to the prioritized groups on the kidney transplant alone waiting list [33]. The KDPI of kidney grafts used for SLKT remains relatively low; however, the proportion of KDPI <20% decreased from 43.4% in 2015 to 36.0% in 2023, while the use of KDPI 35%–85% increased from 32.7% in 2015 to 39.9% in 2023. At present, though kidneys with KDPI >85% are infrequently used for SLK, to expand the donor pool, the use of higher KDPI organs is unavoidable. Montenovo et al investigated the effects of MP on development of DGF according to KDPI in kidney only transplantation. They found that MP was associated with significantly decreased development of DGF in donors with KDPI >60% [34]. In our study, MP for kidney was also associated with decreased a risk of DGF among the recipients with KDPI >60% as well as among the recipients with KDPI >35%. However, there was no significant association between MP for kidney and DGF in the group with KDPI >85%. The number of patients with KDPI >85% was 283, and 55 of them developed DGF. This relatively small sample size may have influenced the observed result. DGF has historically been associated with inferior graft survival [35]. Further research incorporating longer observation period could offer deeper insights into the impact of MP on long-term kidney graft survival.

Then, in which cases and how should MP techniques be applied? NMP offers preserving the organ by supplying oxygen under near-physiological conditions, which is associated with an increase in proteins that mediate the key metabolic processes, including fatty acid ß-oxidation, the tricarboxylic acid cycle, and acid phosphorylation [4]. NMP also enhances specific cellular defense mechanisms, producing an effect similar to ischemic preconditioning [4]. It has been reported that MP techniques were associated with lower rates of ischemic cholangiopathy in DCD liver transplantation due to the potential to reduce ischemic-reperfusion injury [32]. The use of MP for liver in DCD-SLKT may be recommended not only for expanding donor eligibility but also for reducing complications associated with DCD. As for the use of MP on the kidney, it might be better to use MP in cases with a higher KDPI. Bachmann et al demonstrated that KDPI correlated with glomerulosclerosis (r = 0.30), arteriosclerosis (r = 0.33), interstitial fibrosis, and tubular atrophy (r = 0.28) as well as the

extent of acute tubular injury (r = 0.20) [36]. Acute tubular injury caused by longer ischemia time is the main cause of DGF [37]. In higher KDPI kidneys with significant pre-existing tubular atrophy or tubular injury, MP may be helpful in minimizing the additional impact of ischemia. Therefore, the indication of MP for both kidney and liver should be particularly considered in SLKT with DCD and/or higher KDPI.

There are some limitations in our study. First, the OPTN/UNOS registry lacks the detail of available MP devices. The pumping duration and additional MP characteristics such as HMP and NMP, as well as use of back-to-base MPs, were not available. NRP might have been used but there is not data for it in UNOS. Thus, we did not incorporate the CIT for each organ into the analysis, considering the CIT in cases with MP may not accurately reflect the actual time. It should be noted that comparison of CIT between MP and non-MP was not possible or did not reflect actual impact of CIT, since in MP cases, CIT recorded in the UNOS data included MP time. Additionally, there is no detailed data regarding organ procurement techniques in UNOS data. Second, this was a retrospective study using the OPTN/UNOS registry, which lacks donor and recipient clinical detail. We may not have sufficient data on the unknown factors or unmeasured confounding variables affecting graft survival.

There has been a rapid rise in the use of MP for both kidney and liver allograft in the US. Although DCD was a risk factor for liver graft failure without MP for liver, it ceased to be a risk factor when MP for liver was applied. Likewise, although KDPI >85% was linked to a higher risk of kidney graft failure without MP for kidney, MP for kidney might help reduce this risk. MP might also enable the use of lower-quality organs that are currently unsuitable for transplantation, thereby further expanding the donor pool in SLKT. Further investigations would be warranted to confirm these findings and the assessments of optimal candidates for maximizing the effectiveness of this valuable technology should be explored.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

RO and SN conceived the idea of the study. RO developed the statistical analysis plan and conducted statistical analyses. RO, LR, EK, AM, AA-K, AN, DK, AY, MA, and SN contributed to the interpretation of the results. RO, IR, and SA-J drafted the original manuscript. SN supervised the conduct of this study. All authors reviewed the manuscript draft and revised it critically on intellectual content. All authors contributed to the article and approved the submitted version.

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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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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14807/full#supplementary-material

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LETTER TO THE EDITOR
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# Rejection after BKPyV DNAemia—Are We Treating Too Cautiously?

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**Keywords: kidney transplantation, biopsy proven acute rejection, BK polyomavirus, rejection treatment, graft function**

Dear Editors,

After kidney transplantation, BK polyomavirus (BKPyV) DNAemia affects approximately 10%–30% of recipients. In a subset of these cases (1%–10%), BKPyV DNAemia progresses to BK polyomavirus-associated nephropathy (BKPyVAN), marked by renal impairment and the risk of graft failure [1–4]. Treatment primarily involves the reduction of immunosuppression since effective antiviral therapy against BKPyV is lacking [5–8].

However, a clinical dilemma arises when patients develop biopsy-proven acute rejection (BPAR) following an episode of BKPyV DNAemia. In our experience, clinicians are often hesitant to initiate full immunosuppressive therapy due to concerns about reactivating BKPyV infection. This creates uncertainty about whether a more restrained approach to rejection treatment is warranted in this setting.

To explore this, we conducted a single-center, retrospective cohort study including adult kidney transplant recipients (KTRs) transplanted at the Leiden University Medical Center (LUMC) between 2011 and 2020. Patients with primary non-function, multi-organ transplants, missing follow-up data, or participation in investigational immunosuppressive trials were excluded.

Patients were categorized into three groups [1]: those with biopsy-proven acute rejection (BPAR) preceded by BKPyV DNAemia (BKPyV-BPAR) [2], those with BPAR occurring ≥6weeks post-transplantation without prior BKPyV DNAemia (BPAR-only), and [3] those without BPAR during follow-up (No-BPAR). The ≥6-week threshold for the BPAR-only group was chosen because BKPyV DNAemia is typically not present before this period. Consequently, rejection episodes occurring from week 6 are more likely to be pathophysiological and clinically comparable to those preceded by BKPyV DNAemia.

Graft outcomes, including eGFR over time and graft loss at 5 years, were compared between groups. To assess rejection management, we evaluated adherence to our center's treatment protocol. Therapy was considered "less than protocol" if prescribed doses were reduced or agents were withheld entirely. In addition, to gain insight into potential delays in treatment of rejection, we calculated the time interval between a ≥20% rise in serum creatinine and the initiation of rejection treatment.

Data were extracted from the transplantation database, which is directly linked to structured data of electronic health record and pathology department. Data were analyzed using Chi-square test, One-way ANOVA, and linear Mixed-Effects Model (LMM) to assess longitudinal eGFR trends at 6 weeks, 6 months, 1 year, 3 years and 5 years after transplantation.

At our center, BKPyV DNAemia is screened at 1.5, 3, 6, and 12 months after kidney transplantation using quantitative real-time PCR on serum samples. If BKPyV DNAemia is detected, monitoring frequency increases to every 2 weeks. For viral loads <10E4 copies/mL,

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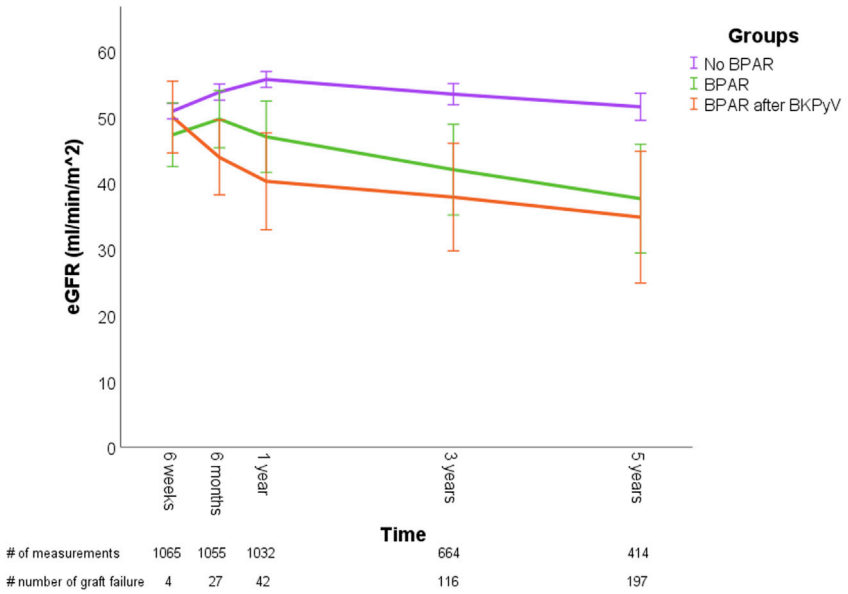


**FIGURE 1 |** Graft outcomes in kidney transplant patients without BPAR, with BPAR, and BPAR preceded by BKPyV DNAemia. Graphs shows the mean eGFR (ml/min/1.73 m2) at 6 weeks, 6 months, 1, 3, and 5 years after transplantation. Error bars display 95% confidence interval for the mean. The number of measurements at each time point is shown below the x-axis, additionally, the number of graft failures, defined as return to dialysis or death is indicated separately.

calcineurin inhibitor (CNI) trough levels are checked and, if on target, prednisone is tapered to 5 mg/day and mycophenolate mofetil (MMF) dose is halved. For loads >10E4 copies/mL, the CNI dose is halved and MMF is discontinued. After two consecutive undetectable serum BKPyV loads, immunosuppression is increased to standard protocol trough levels.

BPAR was defined by histopathological assessment according to the Banff classification of the time of biopsy. In cases with suspected BKPyVAN—typically BKPyV DNAemia >4 log copies/mL with rising creatinine—a kidney biopsy was performed. The distinction between TCMR Banff 1A and BKPyVAN can be challenging, as both can present with interstitial inflammation and tubulitis. To reduce misclassification, SV40-staining was systematically applied in biopsies with tubulointerstitial inflammation. A positive SV40-staining in combination with detectable BKPyV DNAemia is considered as BKPyVAN. Whereas negative SV40-staining in combination of undetectable BKPyV DNAemia in the setting of tubilitis favors TCMR.

This study was reviewed by the LUMC ethics committee, which concluded that formal approval was not required (ID:131830).

A total of 968 KTRs were included in this study. Of these, 870 (89.9%) were classified as No-BPAR, 66 (6.8%) as BPAR-only, and 32 (3.3%) as BKPyV-BPAR. Patients in both rejection groups (BPAR-only and BK-BPAR) were younger, more frequently re-transplanted, and more often immunized (PRA >5%) compared to those in the No-BPAR group. When examining rejection within the groups, mixed rejection was more common in the BKPyV-BPAR group compared to the BPAR-only group (31.3% vs. 10.6%), whereas T-cell mediated rejection (TCMR) was more common in the BPAR-only group (74.2% vs. 59.4%), $p = 0.038$. In the BKPyV-BPAR group, BPAR occurred on average 210 ± 429 days after BKPyV DNAemia.

With regard to rejection treatment, patients in the BKPyV-BPAR group more often received less immunosuppressive treatment than recommended by the local rejection management protocol (details in **Supplementary Table S1a**), compared to those in the BPAR-only group (19.7% vs. 56.3%, $p < 0.001$). Moreover, the initiation of rejection treatment — measured from the time of a ≥20% rise in serum creatinine — was significantly delayed in the BKPyV-BPAR group compared to the BPAR-only group (average of 16.5 ± 23.1 vs. 7.8 ± 10.5 days, $p = 0.012$). **Supplementary Table S1b** further details how maintenance immunosuppression was adjusted before and after rejection in the BKPyV-BPAR group. When further stratifying the BKPyV-BPAR group based on the timing of rejection treatment, patients who received therapy within ≤7 days after the 20% increase in serum creatinine showed a mean eGFR improvement of 6.6 ± 6.7 mL/min/1.73 $m^2$ at day 7 after rejection treatment. In contrast, in patients where treatment was initiated >7 days after the creatinine rise, the mean eGFR increase was only 0.4 ± 7.6 mL/min/1.73 $m^2$ ($p = 0.03$).

When analyzing graft function, graft loss at 5-year occurred in 3.9% of No-BPAR patients, 27.7% in the BPAR-only group, and 45.0% in the BKPyV-BPAR group ($p < 0.001$). In univariate LMM analysis, patients in the BKPyV-BPAR group showed a non-significant trend towards lower eGFR compared to the BPAR-only group (−1.6 mL/min; 95%CI: −8.8 to 5.6; $p = 0.659$), which was similar in multivariate analysis (−1.0 mL/min; 95%CI: −7.3 to

5.3; p = 0.750). The eGFR trajectories over time for the three groups are displayed and illustrated in **Supplementary Table S2**; **Figure 1**.

When examining the cause of graft loss, rejection was the predominant reason (91.7%) in the BKPyV-BPAR group. Importantly, no cases of graft loss were attributed to BKPyVAN (**Supplementary Table S2**).

In addition, the occurrence of BKPyV DNAemia after rejection therapy was examined in both groups. In the BPAR group, 4 patients (6.2%) experienced a recurrence of BKPyV DNAemia following rejection treatment, including 1 patient who developed BKPyVAN. The average peak viral load was 4.1 ± 1.9 log copies/mL, with an average duration of 278 ± 86 days. Recurrence occurred on average 193 ± 130 days after initiation of rejection therapy. In the BPAR preceded by BKPyV DNAemia group, 3 patients (9.7%) developed recurrent BKPyV DNAemia, with an average peak load of 2.8 ± 0.6 log copies/mL and a mean duration of 90 ± 22 days. Recurrence was observed on average 240 ± 380 days after rejection treatment.

An explanation for the limited recurrence of BKPyV DNAemia may be that primary BKPyV episodes do not merely reflect a state of over-immunosuppression, but rather a first-time infection with a novel, previously unencountered BKPyV subtype. Once this initial infection has resolved, protective immunity against that specific strain may limit the risk of recurrence. Supporting this hypothesis, Schmitt et al. demonstrated that VP1 gene sequences in urine samples from 20 matched donor-recipient pairs were completely identical after transplantation, underscoring the role of donor-derived BKPyV and the possible transmission of a new subtype to the recipient [9].

Our study has some limitations, including its retrospective design, a relatively small subgroup size, and limited BKPyV screening up to 1 year post-transplant, potentially missing later DNAemia. In most cases, the cause of graft failure was confirmed by recent biopsy or nephrectomy findings; however, in a few cases it was inferred from older biopsies (>2 months prior). As BKPyV DNAemia was largely absent in the interim, graft loss due to BKPyVAN appears unlikely.

In conclusion, this study shows that patients in whom BPAR was preceded by BKPyV DNAemia experienced both delayed initiation and less intensive rejection treatment, compared to patients with BPAR without prior BKPyV DNAemia. Despite a higher rate of graft loss in this group, the majority of which was attributed to rejection, while no grafts were lost due to BKPyVAN. These findings suggest that conservative rejection management in the context of prior BKPyV needs to be re-evaluated. Further prospective studies are needed to define the optimal management of rejection in the setting of prior BKPyV DNAemia and to better understand the interplay between BKPyV and alloimmunity.

## 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 humans were approved by Scientific committee of the department of internal medicine (HAIG) at Leiden University Medical Center (LUMC), in Leiden. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because This retrospective study was conducted using existing clinical data from a large cohort of kidney transplant recipients. Due to the extensive number of included patients and the fact that a significant proportion of them had deceased by the time of the study, obtaining written informed consent from all individuals was not feasible. The study was approved by the local institutional review board, which waived the requirement for informed consent based on these considerations and in accordance with national ethical guidelines.

## AUTHOR CONTRIBUTIONS

WM, Participated in research design, writing of the paper, participated in the performance of the research, Participated in data analysis. AV, Participated in research design, writing of the paper, AR Participated in research design, writing of the paper, DH Participated in research design, participated in the performance of the research, writing of the paper, JK Participated in research design, writing of the paper, MF Participated in research design, writing of the paper, JR Participated in research design, writing of the paper, participated in the performance of the research, Participated in data analysis. All authors contributed to the article and approved the submitted version.

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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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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15122/full#supplementary-material

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LETTER TO THE EDITOR
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# Beyond Antibodies in Post-Transplant FSGS: New Answers or Recurrent Questions?

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**Keywords: FSGS recurrence, glomerulonephritis, immunosuppression, genetics, complement**

Dear Editors,

More than fifty years have passed since Hoyer et al. first described recurrent focal segmental glomerulosclerosis (rFSGS) in kidney transplant recipients. Yet, despite significant advances in understanding this disease, graft survival remains alarmingly poor [1, 2]. Circulating permeability factors, particularly anti-nephrin antibodies, have long been implicated in primary rFSGS. A recent study by Batal et al. marked a significant breakthrough, demonstrating 100% specificity of this marker in recurrent cases. However, they also found that 62% of patients who developed rFSGS, did not have anti-nephrin antibody titers and lacked IgG deposits colocalizing with nephrin. This low sensitivity is a reminder that other, yet unidentified, pathogenic factors may contribute to recurrence, and the search for additional culprits is far from over. However, implementing large-scale studies is difficult due to the rarity of rFSGS cases. Moreover, the absence of standardized pathophysiology-based criteria leads to the grouping of different FSGS subtypes — each with distinct clinical, management, and prognostic features - contributing to methodological variability and inconsistent classification across studies. Addressing this heterogeneity rigorously is crucial for improving the accuracy of clinical assessments and the effectiveness of therapeutic interventions.

We have retrospectively reviewed primary rFSGS cases in our institution from January 2010 to December 2020. We included only native kidney biopsy-confirmed cases of FSGS or minimal change disease (MCD), that developed rapid-onset nephrotic syndrome post-transplant. Of the 1,372 kidney transplants performed, six allograft recipients (five males and one female) met these inclusion criteria (**Supplementary Figure S1**). The baseline and clinical characteristics of these patients are detailed in **Table 1**. Three had childhood-onset native kidney disease and only one was initially steroid-sensitive. Genetic testing was performed in four patients, and a TRPC6 gene variant was identified in one. Time from the initial diagnosis to the initiation of renal replacement therapy varied widely, ranging from 2 to 19 years. All six patients experienced recurrence within the first month post-KTx with a mean proteinuria of 11 g. Three patients received induction therapy with Basiliximab, three received thymoglobulin, and all were maintained on a triple regimen with steroids, tacrolimus, and anti-metabolite. Upon recurrence, plasmapheresis was promptly initiated in four patients. Three patients achieved complete remission after a median of 81 days. The remaining three patients, unresponsive to initial therapies, also showed a limited response to alternative modalities - additional dose of rituximab (N = 1) and adrenocorticotropic hormone (N = 1) - and progressed to graft loss.

We retrospectively assessed anti-nephrin antibodies in pre-transplant serum samples of two patients (supplementary methods). Patient #2 had elevated anti-nephrin antibody titers (466 UI/mL), while Patient #6 had negative titers.

It is unwise to draw broad conclusions from our small cohort, but we can reflect on the observed heterogeneity. As seen with some entities, such as membranoproliferative glomerulonephritis, we

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**TABLE 1 |** Baseline and clinical characteristics of the study group.

| Patient number id | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Gender | Male | Male | Male | Male | Female | Male |
| Age at disease presentation (years) | 2 | 1 | 5 | 30 | 18 | 45 |
| Native kidney histological findings | FSGS | FSGS | FSGS | FSGS | FSGS | MCD |
| Initial steroid sensitivity | Steroid-resistant | Steroid-sensitive | Steroid-resistant | Steroid-resistant | Steroid-resistant | Steroid-resistant |
| Genetic testing | Positive TRPC6 gene | Unknown | Unknown | Negative | Negative | Negative |
| Time from diagnosis to RRT (years) | 19 | 18 | 18 | 6 | 2 | 2 |
| Age at KTx (years) | 26 | 29 | 26 | 39 | 24 | 53 |
| KTx type | DD | DD | DD | DD | LD | DD |
| Induction agent | Basiliximab | Thymoglobulin | Thymoglobulin | Basiliximab | Thymoglobulin | Basiliximab |
| Delayed graft function | No | Yes | No | Yes | No | No |
| Recurrence timing | 1st week | 1st week | 3 weeks | 1st month | 1st month | 1st week |
| Post-KTx biopsy IF findings | Unknown | No IF deposits | No IF deposits | C3+ | IgM + | IgM 2+ C3+ |
| Initial treatment scheme | PP + CS | PP + RTX | PP + CS | CYC + CS | PP + CS + Ivlg | PP + CS + Ivlg + RTX + ACTH |
| Remission | No remission | Complete | Complete | No remission | Complete | No remission |
| Time to complete remission | - | 1 week | 2 weeks | - | 1 week | - |
| Outcome | Graft loss 2 months post-KTx | Death with functioning graft (Sudden cardiac death) | Graft loss 58 months post-KTx | Graft loss 2 months post-KTx | Functioning graft (end of follow-up 63 months post-KTx) | Graft loss 7 months post-KTx |
| Anti-nephrin antibodies in pre-KTx serum measurement | Not measured | Positive (466 UI/mL) | Not measured | Not measured | Not measured | Negative |

*FSGS: focal segmental glomerulosclerosis; MCD: minimal change disease; RRT: renal replacement therapy; KTx: Kidney Transplant; DD: deceased donor; LD: living donor; PP: plasmapheresis; CS: corticosteroids; RTX: rituximab; CYC: cyclophosphamide; Ivlg: Intravenous Immunoglobulin; ACTH: adrenocorticotropic hormone; IF: Immunofluorescence.*

believe shifting toward a pathophysiology-based approach could improve stratification, management, and improve future clinical trials. Based on our cohort, we present the following observations:

1. Detection of anti-nephrin antibodies pre-KTx appears to be a key biomarker for predicting the risk of immediate rFSGS. While not universally present, when detected, these antibodies seem to respond well to B-cell depletion agents (and possibly plasma exchange), though the effectiveness in preventing recurrence remains unknown. This was demonstrated in three prospectively followed native-kidney FSGS patients, where rituximab appeared to deplete anti-nephrin autoantibodies and was associated with clinical remission. [3] A recent case report by Habbig et al. also documents successful pre-KTx depletion of anti-nephrin antibodies with rituximab and plasma exchange in a pediatric FSGS patient, resulting in excellent graft function without post-KTx proteinuria. [4] Although not yet confirmed in larger cohorts, in this high-risk patients, preemptive management strategies could be considered to help monitor antibody titers and guide the timing of interventions, potentially preventing or delaying recurrence. The need for standardized, commercially available assays for anti-nephrin antibodies is clear. Such assays would confirm the exact role of anti-nephrin antibodies in prognostication or as therapeutic monitoring tools.
2. Genetic variants do not exclude recurrence. Thousands of genetic variants have been identified across more than 50 podocytopathy-associated genes implicated in FSGS, and most pathogenic variants are associated with a low risk of post-transplant recurrence. As highlighted by Mason et al., many reported cases lack confirmed pathogenicity and true recurrence in monogenic disease appears to be rare [5]. An exception is NPHS1 variants due to alloimmunization against donor nephrin, now considered a distinct entity rather than classical recurrence [5, 6]. Patient #4 carried a heterozygous missense variant in exon 13 of TRPC6 [NM_004621.6: c.2750G>C, p.(Gly917Ala)], classified as a variant of uncertain significance by ACMG guidelines. No familial segregation testing was performed, leaving inheritance undetermined. The variant is not present in databases such as ClinVar or gnomAD. This case illustrates the need for cautious interpretation of variants, ideally with the support of nephrogenetics experts, as variants of uncertain significance with unlikely causality do not rule out FSGS recurrence, and other immune or environmental mechanisms may influence disease expression. Molecular genetic testing remains thus essential in FSGS for predicting post-transplant recurrence risk. In line with this, novel agents are also being developed: a selective TRPC6 inhibitor (BI 764198) is currently undergoing phase 2 clinical trials and may represent a breakthrough in this subset of patients [7]. Gene therapy is also promising, with studies exploring adeno-associated virus-mediated gene delivery, particularly for NPHS2 variants, showing promising results for more targeted treatments [6].
3. There is growing evidence that complement activation plays a role in the pathogenesis of primary FSGS in the native kidney, with studies correlating worse prognosis to low plasma C3 levels,

complement deposits in biopsies, and elevated urinary complement byproducts [8]. In our cohort, all patients had histologic features of FSGS in post-transplant biopsies, and C3 deposits were observed in two cases. This suggests that complement activation may reflect more than nonspecific trapping in sclerotic lesions and, despite sample size, still raises the question of whether this early deposition suggests complement-mediated injury or represents an epiphenomenon without a direct correlation to disease activity. Interestingly, Shirai et al. found no complement components colocalizing with nephrin in rFSGS biopsies, despite strong IgG-nephrin interaction [9]. This suggests that anti-nephrin antibody–mediated injury may occur via complement-independent mechanisms, possibly involving non-complement-fixing IgG subclasses. In contrast, detection of C3 deposits in anti-nephrin-negative cases raises the possibility that complement activation may play a larger role in antibody-negative or alternative pathogenic pathways. Of note, in our cohort, the patient with positive anti-nephrin antibodies had no complement deposits, while the seronegative patient did, supporting the idea of distinct pathogenic pathways. Given the growing availability of anti-complement therapies, further research is necessary to determine if complement activation contributes to disease recurrence or works alongside other pathogenic mechanisms, allowing for a more personalized mechanistic-based use of the expanding therapeutic armamentarium.

The multifactorial nature of rFSGS appears to be clear, potentially influenced by genetic variants, circulating permeability factors, and immune-mediated mechanisms. This interaction could explain the variable recurrence rates and outcomes. Further research is necessary to decipher these factors' precise roles and interactions in disease development and progression. Future studies should aim to identify additional circulating permeability factors, explore the role of environmental factors in disease recurrence, and refine genetic testing to predict post-transplant outcomes. Moreover, targeted therapies addressing specific mechanisms—such as B-cell depletion for antibody-related cases, complement inhibition for complement-driven pathology, and genetic therapies for variant-driven cases—may revolutionize treatment and improve long-term graft survival.

## DATA AVAILABILITY STATEMENT

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

## ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

Conceptualization: JV, AH, and PF. Investigation: JV, AH, and PF. Methodology: JV, AH, CP, and RL. Writing – original draft: JV, AH, and RL. Writing – review and editing: CP, RL, and CR. Final review and editing: MM, LR, LS, CR, AF, and RA.

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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.

## GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15032/full#supplementary-material

**SUPPLEMENTARY FIGURE S1 |** Flowchart showing patient selection for inclusion. A total of 1,372 kidney transplant recipients were retrospectively reviewed. Stepwise exclusions were applied based on native biopsy availability, post-transplant manifestations, and recurrence confirmation by allograft biopsy.

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LETTER TO THE EDITOR
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# The Impact of Ischaemic Type Biliary Lesions on Healthcare Costs After Liver Transplantation With Grafts From Donors After Circulatory Death

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**Keywords: donation after circulatory death (DCD), biliary stricture, machine perfusion, costs and benefits, liver transplant**

Dear Editors,

Ischaemic type biliary lesions (ITBL) are characterised by diffuse, nonanastomotic intrahepatic biliary strictures with upstream dilatation, in the absence of other complications such as hepatic artery stenosis or ductopenic rejection [1, 2]. They are identified most commonly through magnetic resonance cholangiopancreatography (MRCP) [3, 4]. The resulting cholestasis leads to recurrent infections, the need for repeated biliary drainage procedures and eventually a large proportion will suffer graft loss [3,4].

The exact aetiology of ITBL is still not known, however a greater rate of ITBL has been reported grafts from donors after circulatory death (DCD) [5–8], likely due to ischaemia/reperfusion injury, microvascular thromboses and/or cytotoxic injury [3, 4].

Efforts to reduce waiting lists for liver transplantation have led to increasing use of marginal grafts, including an increased use of those from DCD donors. Furthermore, a marked increase in the number of DCD donors has been observed [9], with recent national figures showing that DCD donors now make up close to half of all donors [10]. This brings the need for optimisation of these grafts to the forefront.

There is evidence to suggest that novel perfusion and preservation strategies such as normothermic regional perfusion (NRP) and hypothermic oxygenated machine perfusion (HOPE) can reduce rates of biliary complications in DCD donors [11, 12]. However, these incur extra costs to health service providers who will understandably seek reassurance that the extra expenditure required to fund these technologies is justified.

We aimed to investigate the long-term impact of ITBL on health service utility at our institution after liver transplantation with DCD grafts and in so doing propose potential savings with new technology.

To ensure long-term follow-up consecutive whole static cold storage (SCS) DCD liver transplants between 2016 and 2018 were reviewed from our prospectively maintained institutional database. To be classified as having ITBL, patients required a diagnostic magnetic resonance cholangiopancreatography (MRCP) scan and the absence of anastomotic stricture. Those who underwent liver transplantation for primary sclerosing cholangitis (PSC) were excluded due to difficulties in distinguishing recurrent PSC from ITBL on MRCP. Those with concurrent hepatic artery stenosis or thrombosis were also excluded. To compare healthcare costs between patients who developed ITBL and the standard DCD cohort, ITBL patients were matched to patients who received a DCD SCS graft during the same period and did not develop ITBL. Matching was based on age (+/− 5 years), indication for transplant and UKELD (+/- 5) at the time of listing. For ITBL and matched

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**TABLE 1 |** Comparison of healthcare costs after index liver transplantation between ITBL and no ITBL controls.

| Tertiary centre hospital episode | | Unit cost | NHS tariff code | No ITBL (n = 19) | | ITBL (n = 19) | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | | | N | Mean cost per pt | N | Mean cost per pt | |
| Subsequent Operative Procedures | | | | | | | | |
| | Retransplant | £80,000 | N/A | 0 | £0 | 10 | £42,105.26 | **0.012** |
| | Incisional hernia repair | £6,760 | FF60A | 3 | £1,067 | 0 | £0.00 | 0.418 |
| | Hepaticojejunostomy | £21,495 | GA03C | 0 | £0 | 1 | £1,131.32 | 0.795 |
| | Laparotomy and washout | £21,495 | GA03C | 1 | £1,131 | 3 | £3,393.95 | 0.583 |
| Interventional Radiology | | | | | | | | |
| | ERCP | £9,653 | GB09D | 4 | £2,032 | 20 | £10,161.05 | 0.234 |
| | PTC drainage/imaging | £1,830 | YG06Z | 5 | £482 | 4 | £385.26 | 1 |
| | TIPPS with stent | £5,274 | YA10Z | 0 | £0 | 1 | £277.58 | 0.795 |
| | Angiogram+/-stenting | £5,274 | YA10Z | 2 | £555 | 1 | £277.58 | 1 |
| | Hepatic venogram | £5,274 | YA10Z | 1 | £278 | 6 | £1,665.47 | 1 |
| | CT guided drain | £10,005 | YF04A | 2 | £1,053 | 4 | £2,106.32 | 0.603 |
| | US guided drain | £10,005 | YF04A | 4 | £2,106 | 19 | £10,005.00 | 0.402 |
| | Fluoroscopic guided drain | £10,005 | YF04A | 0 | £0 | 2 | £1,053.16 | 0.795 |
| | CT liver ablation | £7,563 | YG01A | 1 | £398 | 0 | £0.00 | 0.795 |
| Diagnostic Radiology | | | | | | | | |
| | CT | £95 | RD24Z | 28 | £140 | 73 | £365.00 | 0.146 |
| | MRI Liver | £178 | RD03Z | 3 | £28 | 8 | £74.95 | 0.37 |
| | MRCP | £116 | RD01A | 11 | £67 | 46 | £280.84 | **<0.001** |
| | US abdomen | £55 | RD42Z | 105 | £304 | 205 | £593.42 | **0.006** |
| | US guided biopsy | £907 | YF05Z | 5 | £239 | 22 | £1,050.21 | **0.043** |
| | NM | £1,045 | YG12Z | 0 | £0 | 2 | £110.00 | 0.583 |
| | CXR | £28 | N/A | 74 | £109 | 164 | £241.68 | 0.085 |
| | AXR | £28 | N/A | 0 | £0 | 9 | £13.26 | 0.172 |
| | PICC | £1,729 | YR42A | 2 | £182 | 13 | £1,183.00 | 0.37 |
| | Transjugular biopsy | £1,676 | YG10Z | 1 | £88 | 4 | £352.84 | 1 |
| | Tubogram | £1,045 | YG12Z | 0 | £0 | 2 | £110.00 | 0.795 |
| Follow-up | | | | | | | | |
| | OPA | £206 | 306 | 622 | £7,114 | 650 | £7,417.37 | 1 |
| | Readmission ITU Bed Days | £2,737 | N/A | 5 | £720.26 | 67 | £9,651.53 | |
| | Readmission Ward Bed Days | £397 | N/A | 61 | £1,274.58 | 939 | £19,620.16 | **0.002** |
| Total cost | | | | | | | | |
| | | - | - | - | £17,817.11 | - | £111,675.80 | **0.007** |

*Bold values indicates the significant at the p < 0.05 level.*

patients, all hospital episodes after discharge from index transplant were retrieved using the electronic hospital record. Cost codes for each procedure or episode were obtained from the latest available NHS tariffs (2022/23). Graft and patient survival was calculated from the date of transplant to the date of death, retransplantation or last follow-up.

Of 115 DCD liver transplants during the study period, 19 developed ITBL (16.5%). Graft survival was significantly lower in the ITBL group (23.4 months vs. 72.8 months; p = 0.001), with 10 (53%) of the patients requiring retransplantation.

The total hospital costs were significantly higher amongst the ITBL group, with an average cost per patient of £111,675.80 (Range: £3,116-£271,278) compared to £17,817.11 (Range: £3,982 - £93,171) in the matched "No ITBL" control group (**Table 1**). A large contributor to the increased cost was retransplantation, however significantly increased costs due to increased use of diagnostic imaging and procedures, such as biopsies, were also observed. In addition, the ITBL group had a markedly increased number of readmission bed days (1006 days vs. 66 days; p = 0.002) (**Table 1**).

This cost-utility analysis demonstrates that the development of ITBL after DCD liver transplantation leads to significantly increased healthcare costs compared to matched "No ITBL" controls. Whilst decisions to fund novel perfusion and preservation technologies are complex, these findings show that ITBL represent a significant cost burden to the health service after liver transplantation and should be considered in future funding decisions.

The evidence for the efficacy of novel perfusion and preservation strategies in reducing non-anastomotic strictures (NAS) in DCD grafts is growing. NRP, which involves restoring circulation of warm, oxygenated blood in a controlled DCD setting [13, 14], has been shown to reduce ischaemic cholangiopathy [13], with two recent meta-analysis showing that NRP significantly reduces the NAS rate in DCD grafts compared to SCS [12, 15]. Recent evidence, including a randomised controlled trial, has shown that HOPE also reduces the risk of NAS after DCD donation compared to static cold storage [16–18]. Given that this study demonstrates a significant increase in follow-up and treatment costs for patients that develop ITBL, it follows that any novel perfusion and preservation strategies that reduce ITBL after DCD liver transplantation will significantly reduce follow-up costs.

These technologies will also have an impact upon waiting lists, which continue to grow [19]. From the aspect of donation, the latest figures from the United Kingdom (UK) show a decrease of

2% in the number donors after brain death (DBD) whilst the number of DCD donors has increased by 7% [19]. It is therefore incumbent upon the liver transplantation community to expand the use of DCD grafts in a safe manner. From the recipient side, through a reduction in ITBL, fewer grafts will be required for retransplantation.

Decisions of whether to fund new technologies to optimise DCD grafts, such as NRP and HOPE, are complex and this study only looks at one aspect. For example, in establishing a new service there are training, staff and consumable costs that must be accounted for. However, evidence that novel perfusion and strategies such as NRP and HOPE can both improve outcomes of DCD grafts and increase the number of DCD grafts that can be used safely continues to grow. Health service providers must therefore now weigh up the costs of growing waiting lists and complications, which are more prevalent with DCD SCS grafts, and compare these to the costs of introducing novel perfusion and preservation strategies for DCD grafts more widely.

## 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

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

## AUTHOR CONTRIBUTIONS

Manuscript writing: JH-S, GC, AH, AS, and KR. Data collection: JH-S and MB. All authors contributed to the article and approved the submitted version.

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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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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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LETTER TO THE EDITOR
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# Benefit of BK Polyomavirus Screening in the First Year After Lung Transplantation

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**Keywords: BK polyomavirus, routine BK polyomavirus DNAemia, lung transplantation, renal failure, early screening**

Dear Editors,

Other than case reports of BK polyomavirus-associated nephropathy (BKPyVAN) with end stage kidney disease [1] or BK polyomavirus (BKPyV)-related urothelial carcinoma [2], little is known about the incidence and effects of BKPyV replication in lung transplant recipients (LTR). Most of BKPyV infection data have been obtained from kidney transplant recipients (KTR) [2]. BKPyVAN, a cause of early renal graft failure, is positively correlated with high plasma BKPyV replication [2]. In KTR, early screening for active BKPyV infection together with reduction of immunosuppression have been shown to be effective for preservation of allograft function [2]. However, the effect of BKPyV replication in LTR has been understudied.

We performed a prospective analysis to compare the incidence and kinetics of BKPyV replication between LTR and KTR during the first year after transplantation and to evaluate the course of viral load in LTR.

Of the 310 adult patients who underwent kidney or lung transplantation in Montreal University hospitals (Canada) between January 2018 and January 2020, 195 KTR and 102 LTR had a functioning graft at least 3 months after transplantation and were used as the study population. BKPyV replication monitoring was performed using BKPyV QNAT analysis of plasma samples, as part of routine clinical care, at 1, 2, 3, 6, 9 and 12 months after transplantation.

In LTR, detection of BKPyV replication was not followed by a specific therapeutic intervention but a renal biopsy was scheduled in cases of unexplained kidney dysfunction. In KTR, BKPyV infection was managed by tapering off of the immunosuppressive drugs and regular monitoring for BKPyV-DNAaemia [2].

We examined the incidence, timing and kinetics of BKPyV replication in the LTR cohort and compared the results with those of the KTR cohort in the same period. Occurrence of biopsy proven BKPyVAN, acute rejection, and deaths as well as analysis of the estimated glomerular function rates (eGFR) at 12 and 24 months were evaluated in the LTR group (where BKPyV replication was not managed by reduction of immunosuppression).

The main characteristics and the outcomes of the patients are detailed in **Table 1**. The incidences of BKPyV replication during the first year after transplantation in LTR and KTR was 17% and 30.25% respectively. BKPyV replication occurred within the first 3 months after transplantation in 94% of LTR + and 64% of KTR +. The median peak viral load was 422 copies/mL (range 23–79683 copies/mL) in LTR with BKPyV replication (LTR +) and 4770 copies/mL (range 26–954,000 copies/mL) in KTR with BKPyV replication (KTR +) ($p = 0.0258$). Viral load reached 10,000 copies/mL or more, in 23% of LTR+ and 38.9% of KTR+ during the first 12 months.

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**TABLE 1 |** Characteristics of patients who received a lung transplant or a kidney transplant between January 2018 and January 2020[a], incidences of BKPyV-DNAemia in the first year after transplantation and outcomes after transplantation.

| Variables | Transplant received | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lungs | | | | Kidney | | | |
| | All (n = 102) | BKV - (n = 85) | BKV + (n = 17) | P value | All (n = 195) | BKV – (n = 136) | BKV + (n = 59) | P value[b] |
| Recipient characteristics | | | | | | | | |
| Age (y) median (IQR) | 58 (18–73) | 56 (18–73) | 61 (18–73) | 0. 30 | 55 (22–76) | 54.5 (22–76) | 55 (24–73) | 0.14 |
| Sex, male (%) | 70.5 | 71.76 | 64.7 | 0.56 | 66 | 66.9 | 66.1 | |
| Donor characteristics | | | | | | | | |
| Age (y) median (IQR) | 45 (14–84) | 45 (14–84) | 51 (24–68) | 0.43 | 52 (10–77) | 52 (10–77) | 51 (24–68) | 0.56 |
| Sex, male (%) | 56.8 | 60 | 41.1 | 0.18 | 64.6 | 59.5 | 76 | 0.03 |
| Living (%) | 0 | 0 | 0 | | 19.4 | 20.5 | 16.9 | 0.69 |
| Induction IS | | | | | | | | |
| with ATG (n) | 0 | 0 | 0 | | 50 | 36 | 14 | 0.72 |
| with Basilixumab (n) | 46 | 31 | 15 | 0.0001 | 145 | 100 | 36 | 0.72 |
| Maintenance IS | | | | | | | | |
| with TAC/myc.a/cort (n) | 95 | 79 | 16 | 1 | 194 | 135 | 58 | 0.51 |
| CMV Rneg/Dpos[c] | 25 | 21 | 4 | 1 | 39 | 30 | 9 | 0.39 |
| Ureteral stent placed | 0 | | | | 195 | | | |
| Acute rejection in the 1st year | 6 | 6 | 0 | | 21 | 9 | 12 | 0.009 |
| eGFR (ml/min/1.73 $m^2$) | | | | | | | | |
| After 1 year | 68.44 ± 26.35 (n = 94) | 68.56 ± 26.40 (n = 77) | 67.88 ± 26.92 (n = 17) | 0.96 | 55.57 ± 21 | 56.18 ± 21 | 54.19 ± 20 | 0.55 |
| After 2 years | 63.92 ± 25.94 (n = 90) | 67.51 ± 25.06 (n = 74) | 56.56 ± 29.39 (n = 16) | 0.21 | | | | |
| Proven BKPyVAN | 0 | 0[d] | 0 | | | | | |

*Abbreviations: CMV,cytomegalovirus; ATG, antithymocyte globulin; R, recipient; D, donor; TAC, tacrolimus; myc.a, mycophenolic acid; cort, corticoids; neg, negative; pos, positive; eGFR, estimated glomerular filtration rate; BKV +, with plasma BKPyV replication; BKV -, without plasma BKPyV replication.*

*[a]This study was approved by the University of Montreal Institutional Review Board.*

*[b]Descriptive statistics were calculated with categorical data reported as counts and percentages, continuous data as means ± standard deviations if normally distributed and medians with ranges if non-normally distributed. P values between BKPyV negative (-BKV- -) and BKPyV positive (-BKV+-) groups of <0.05 were considered statistically significant.*

*[c]All cytomegalovirus (CMV) seronegative recipients transplanted with organs from CMV seropositive donors received prophylaxis for 3–6 months after transplantation with intravenous ganciclovir or valganciclovir.*

*[d]One lung recipient of the BKV- group was diagnosed with biopsy-proven BKPyVAN in the fifth year after transplantation, with unexplained sub acute renal failure (eGFR, of 42 mL/min) and a plasma BKPyV load of 302,000 copies/mL.*

One year after transplantation, BKPyV-DNAemia persisted in nine of the LTR+ (median 2290 copies/mL, range 23–126 240 copies/mL). No LTR developed unexplained kidney dysfunction. The eGFR did not differ between LTR+ and LTR- (without BKPyV replication) groups at 12 months and 24 months after transplantation. Four deaths occurred, in the LTR -. LTR+ did not differ from LTR- with respect to age, immunosuppression, and cytomegalovirus serostatus.

BKPyV replication is an important cause of kidney dysfunction in the first year after kidney transplantation [2]. Studies reporting the results of screening for BKPyV after lung transplantation are rare and often cross sectional [3–5]. Doucette et al [6] monitored BKPyV replication in urine and plasma samples from 28 LTR at 3, 6, and 9 months post-transplantation and reported no detectable BKPyV-DNAemia in any subject and BKPyV-DNAuria in 17.8% of LTR. In our larger prospective study performed within 102 LTR, at 1, 2, 3, 6, 9, and 12 months after transplantation, monitoring demonstrated BKPyV replication in 17%. This value is high, but less that of our kidney transplant group (30. 25%) studied with the same molecular assay, during the same period when LTR and KTR were under tacrolimus, mycophenolic acid and corticosteroids. BKPyV replication was detected early after transplantation, in both groups: the first positive case of BKPyV-DNAemia was observed in the first trimester in LTR and in KTR. The rate of replication was lower in LTR than in KTR.

While the diagnosis of BKPyV replication was not followed by changes in the immunosuppression regimen in LTR+, the viral load reached more than 10,000 copies/mL, a value that correlates strongly with presumptive BKPyVN in KTR [2]. A kidney biopsy was not systematically performed on LTR + patients. The renal functions of the LTR+ and LTR- patients were not different at 1 or even 2 years after transplantation. Interestingly, one LTR- subject developed renal dysfunction with biopsy-proven BKPyVAN 4 years after transplantation.

The conditions that lead to the development of BKPyVAN from BKPyV replication may differ between KTR and LTR. Some factors are specific to the kidney transplant: the graft (major reservoir of latent BKPyV) may transmit the virus [2]. Acute

tubular damage due to early intra-renal immunological and ischemic reperfusion lesions [7], and the placement of ureteral stent during kidney transplant surgery [8] may play a role in the development of BKPyVAN. BKPyV-DNAemia, a surrogate marker of BKPyVAN in kidney transplantation [2] has not be established as such in lung transplantation. Whether there is a lung transplant specific timeline of BKPyVAN remains unclear [9] Recently, Dube et al [1] reported 11 cases of biopsy proven BKPyVAN in LTR presenting with unexplained kidney dysfunction, at a median of 46 months after transplantation. In KTR, before screening protocols were available, most cases of BKPyVAN were diagnosed, in the first year after transplantation, in patients with kidney failure [10].

Based on the results of our prospective, observational study conducted on 102 LTR, routine BKPyV-DNAemia surveillance during the first year after lung transplantation is unlikely to be beneficial as it is in kidney transplantation [2]. To detect BKPyVAN early and preserve renal function, future investigations should focus on the benefit of routine screening (yearly?) after the first year of lung transplantation. Nonetheless BKPyV-DNAemia should be tested upon suspicion of kidney dysfunction (at any time after transplantation) for the diagnosis of BKPyVAN.

## 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

ER, DT, and CP developed the study design. ER, DT collected data. All authors contributed to the article and approved the submitted version.

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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.

## GENERATIVE AI STATEMENT

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