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TRANSPLANTATION LEARNING JOURNEY

ESOT Guidelines from the Transplantation Journey 3.0

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SPECIAL ISSUE EDITORIAL
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ESOT Guidelines From the Transplantation Learning Journey 3.0

Umberto Cillo[1], Ina Jochmans[2,3], Nuria Montserrat[4,5,6], Liset H. M. Pengel[7], Raj Thuraisingham[8], Nazia Selzner[9] and Annemarie Weissenbacher[10] on behalf of ESOT Guideline Taskforce*

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Keywords: transplantation, clinical practice, consensus platform, guidelines, personalized medicine

Editorial on the Special Issue

ESOT Guidelines From the Transplantation Learning Journey 3.0

We are experiencing an unprecedented transformational era where advancements in personalized medicine are substantially redefining the medical landscape. In this rapidly evolving environment, the future of scientific evidence development and interpretation, along with the effective transmission of clinical guidance, must undergo critical changes.

The scientific community must urgently and proactively facilitate the shift from conventional, "one-size-fits-all" clinical research to more personalized methodologies that thoroughly consider genetic, environmental, and lifestyle factors in a person-centered approach. As this trend accelerates, the methods for generating and interpreting scientific evidence must evolve to address the complexity and granularity of data produced by individualized treatments, ensuring continued relevance in clinical guidance.

In this new context, traditional randomized controlled trials (RCTs), while still valuable, often oversimplify clinical complexities, rendering them inadequate to capture the nuances of personalized interventions. Instead, n-of-1 trials, real-world data, and adaptive trial designs—where individual responses to treatments are closely monitored—are increasingly set to play a central role. This conceptual change is already occurring in areas such as cardiovascular care and oncology, with potentially transformative implications for organ transplantation. The move to individualized care is both essential and urgent in our field, where each patient's immune system, genetic background, response to immunosuppressive therapy, and multi-procedural history vary widely. Developing scientific evidence that accurately represents this diversity, and reshaping how we translate findings into actionable clinical recommendations, are top priorities. We strongly believe that prominent scientific organizations must embrace the responsibility to promote, guide, and monitor this paradigm shift within their communities.

In line with this, in 2021, the European Society of Organ Transplantation (ESOT) established a taskforce dedicated to guidelines and a platform to activate consensus processes and guideline production within a rigorous methodological environment. Beyond utilizing traditional frameworks for reviewing and evaluating scientific evidence, the ESOT guideline taskforce has prioritized areas within organ transplantation where evidence gaps and/or the transition to precision medicine require expert-driven analysis to inform current clinical guidelines and identify critical research

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needs for the future. The role of experts in interpreting scientific findings is crucial, as the development of this evidence increasingly incorporates data sources like genomic data, real-world evidence, and adaptive trials. By balancing the promise of personalized care with the rigorous standards of evidence-based medicine, experts serve as critical guides in integrating precision medicine into clinical practice.

To support this historical shift, ESOT has sponsored multiple consensus processes, ensuring robust methodological and logistical support, and created a dedicated platform to facilitate this transition (Cillo et al.).

In this Special Issue, Transplant International publishes the first peer-reviewed articles from this ESOT initiative, offering readers an in-depth exploration of clinical guidance across a range of organ transplantation domains. For example, a consensus led by Park et al. recommends adopting donor-derived cell-free DNA (dd-cfDNA) and urine chemokines (CXCL9 and CXCL10) to identify antibody-mediated rejection in patients experiencing both acute and stable graft dysfunction.

Consensus guidelines led by van den Broek et al. recommend routine, continuous monitoring of donor-specific antibodies (DSA) to optimize long-term kidney graft survival. Although DSA provides valuable insights into subclinical rejection, biopsy confirmation is still necessary for assessing the need for treatment.

Zaza et al. report the first attempt to redefine and standardize pre-implantation biopsy procedures for evaluating kidney grafts from expanded criteria donors (ECD), emphasizing the need for consistent protocols and shared evaluation parameters within the European transplant community.

For the first time, liver transplantation for patients with Primary Sclerosing Cholangitis (PSC) and Inflammatory Bowel Disease (IBD) was addressed in a consensus setting (Carbone et al.). Key challenges—such as the waitlisting process, cancer risks, and heightened perioperative and long-term risks—underline the need for a tailored approach to graft selection, intraoperative management, and postoperative immunosuppression.

Similarly, the first consensus on downstaging, bridging, and immunotherapy in liver transplantation for hepatocellular carcinoma (HCC) patients has been established. Claasen et al. strongly recommend adopting downstaging protocols in HCC patients, regardless of stage, noting that multimodal approaches can significantly improve both recurrence-free and overall survival.

While value-based healthcare and person-centered approaches are now widely recognized as essential to modern medicine, value-based endpoints have yet to be fully developed in organ transplantation. This Special Issue introduces a pioneering consensus on value-based endpoints in liver transplantation, identifying transplant benefit and quality-adjusted life years as the most relevant measures for person-centered outcomes (Carbone et al.). PROMS and PREMS have been identified as important research areas moving forward.

Berenguer et al. conclude that in liver transplantation, biomarkers are still limited in predicting the recurrence of certain liver diseases (e.g., MASH, alcohol relapse, autoimmune diseases). However, these biomarkers show promise in predicting post-transplant HCC recurrence and chronic kidney disease, helping guide clinicians in optimizing immunosuppressive therapies.

In the cardiothoracic setting, Nikolova et al. suggest that peripheral blood gene expression profiling (GEP) assays serve as reliable non-invasive tool to rule out acute cellular rejection in stable, low-risk heart transplant patients. They also indicate that dd-cfDNA measurements could be applied to detect both clinical and subclinical rejection in heart and lung transplants. Emerging biomarkers, including cfDNA epigenetic analysis, fragment omics, exosomes, and microRNA, are currently under investigation.

Ferrer-Fàbrega et al. present an important consensus statement on machine perfusion (MP) in whole pancreas or islet transplantation advocating for a collaborative approach to enhance knowledge evidence in this field.

Amarelli et al. reached broad agreement on the potential of MP technology to expand and improve cardiothoracic organ transplants, recommending the establishment of a pan-European MP registry to promote clinical and cost-effectiveness studies.

Finally, Annema et al. address the often-overlooked topic of prehabilitation for transplant candidates, advocating a multimodal strategy that emphasizes exercise, nutrition, and psychosocial support to improve outcomes. A coordinated effort and a core outcome set for future research are proposed to address the shortage of high-quality studies in this area.

In conclusion, this Special Issue compiles the outcomes of methodologically rigorous consensus processes, balancing existing evidence with expert insights to provide clinical guidance in several critical, previously unexplored areas of organ transplantation. We are confident that readers will find this Special Issue an innovative overview, presenting a broad perspective on precision medicine in organ transplantation and posing significant questions for future research.

AUTHOR CONTRIBUTIONS

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

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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EXECUTIVE SUMMARY
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ESOT Consensus Platform for Organ Transplantation: Setting the Stage for a Rigorous, Regularly Updated Development Process

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Abbreviations: AGREE, Appraisal of Guidelines for REsearch & Evaluation; CET, Centre for Evidence in Transplantation; ECTTA, European Cardio Thoracic Transplant Association; EKITA, European Kidney Transplant Association; ELITA, European Liver and Intestine Transplant Association; EPITA, European Pancreas and Islet Transplant Association; ESOT, European Society for Organ Transplantation; ETAHP, European Transplant Allied Healthcare Professionals; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; GT, Guideline Taskforce; ILTS, International Liver Transplantation Society; NIH, National Institute of Health; PICO, Population, Intervention, Comparator and Outcome; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SC, Steering committee; TJL, Transplant Learning Journey; VBHC, Value-based health care; YPT, Young Professionals in Transplantation.

The European Society for Organ Transplantation (ESOT) has created a platform for the development of rigorous and regularly updated evidence based guidelines for clinical practice in the transplantation field. A dedicated Guideline Taskforce, including ESOT-council members, a representative from the Centre for Evidence in Transplantation, editors of the journal Transplant International has developed transparent procedures to guide the development of guidelines, recommendations, and consensus statements. During ESOT's first Consensus Conference in November 2022, leading experts will present in-depth evidence based reviews of nine themes and will propose recommendations aimed at reaching a consensus after public discussion and assessment by an independent jury. All recommendations and consensus statements produced for the nine selected topics will be published including the entire evidence-based consensus-finding process. An extensive literature review of each topic was conducted to provide final evidence and/or expert opinion.

Keywords: organ transplantation, methodology, guidelines, consensus conference, platform

INTRODUCTION

High-quality, evidence-based clinical practice guidance documents to support best practice in solid organ transplantation along with improving the quality of life are increasingly needed. These are statements that include recommendations intended to optimize patient care, lead to better clinical outcomes, and improve cost effectiveness. Furthermore, they provide the opportunity to identify areas requiring further research and serve an educational scope. Clinical Practice Guideline statements are informed by a systematic review of evidence and an assessment of the benefits of alternative care options. The multidisciplinary and multiprocedural nature of organ transplantation, the intrinsic difficulty in designing and carrying out numerically and methodologically sound comparative studies, and the ever-changing landscape of knowledge and therapeutics, challenge the realization of a solid evidence framework in some crucial areas of the field. Solid organ transplants, therefore, more than other clinical areas, need implementation of a systematic, continuous expert work dedicated to guideline and consensus production to help clinicians with framing evidence and expert opinions into clinical practical approaches (1–3).

The European Society of Organ Transplantation (ESOT) is recently giving high priority to the development of clinical practice guidelines launching a structured and continuous dedicated action plan. In January 2022, ESOT created a guideline taskforce (GT) composed of ESOT leadership and Transplant International editorial board members. The GT has the fundamental commitment to promote methodologically homogeneous guideline and consensus activities and to warrant trustworthiness, transparency and continuity of the processes. Furthermore, the GT selects cutting edge topics, initiates and realizes consensus processes among experts, draws guidelines and promotes dissemination of the compiled products.

Guideline and consensus related material will undergo widespread dissemination within the transplant community through publications in Transplant International, ESOT congresses, and platforms as well as through networking *via* social media. Patients and their representatives will play an active role in the consensus development processes and will be targets of the dissemination activities according to the principles and concepts of value-based health care (VBHC). When appropriate, the GT will involve stakeholders including those in health care management and economics, organ sharing organizations, and health care policy makers.

Besides drafting a uniform methodology for ESOT guidance/guideline production and promoting topic selection, the GT created a platform for the development of methodologically solid and up-to-date evidence-based guidelines for clinical practice in the transplantation field. This platform guarantees procedural and logistical continuity to ESOT activities in the field of consensus processes and guideline production.

The first edition of the Transplant Learning Journey (TLJ) 3.0, after several months of preparatory work, is there to produce systematic reviews of evidence and to grade evidence followed by drafting and sharing recommendations. During TLJ 3.0 in Prague 13th–15th November 2022, the 3-day consensus conference, a series of consensus-based clinical guidance documents comprising research topics considered as cutting-edge will be established.

AIMS

The main purpose of the TLJ 3.0 ESOT GT and the consensus conference is to provide methodologically solid evidence-based and best-practice recommendations reflecting the latest knowledge.

While creating clinical guidance through expertise and knowledge from all stakeholders involved in organ transplantation within the ESOT community and beyond, a further goal is to provide resources in the form of reference databases on an available platform maintained and updated continuously to lead the way in organ transplantation.

The present report is intentionally submitted for publication and it will be freely available prior to TLJ 3.0 event, to make publicly available and report fully with trustworthiness and transparency (1, 2) the new course of ESOT guideline and consensus processes in organ transplantation. The aim is to disclose the methodology of the ESOT consensus platform from its conception to its development, in line with the principles of openness and transparency (1, 2), which are fundamental where relevant potential policy changes are expected. In that light, this report was submitted to Transplant International prior to the event.

METHODS

A dedicated ESOT GT established a methodologic action plan in January 2022 and elaborated a handbook formalizing the processes associated with the preparation of ESOT Clinical Practice Guidelines, including selection of topics for new guidelines, writing, reviewing, approval, dissemination, and update. The document also defines the governance of the process and the roles of the various committees. This handbook has been open to be consulted on the ESOT website since the end of September 2022.

In line with the established action plan, the ESOT GT launched the event "Transplant Learning Journey (TLJ) 3.0" as an in-person consensus conference, designed as a modified NIH (National Institute of Health) model consensus development conference (1–6). Such a consensus development process was organized in collaboration with ESOT sections ELITA, EKITA, EPITA, ECTTA, ETAHP, the Education Committee, and YPT. The ILTS collaborated as well for some specific topics.

The platform, and its future developments, will represent ESOT's permanent operative tool to regularly elaborate and deliver rigorous and homogenous consensus statements and publications. Due to the known limitations related to face-to-face consensus conferences, particular attention has been given to methods for topic selection, selection and number of steering committee members, and review of evidence.

The Delphi method will be applied to arrive at a group opinion by surveying the expert panels including SC, conference attendees and jury members. The final result will reflect a solid consensus of experts in the field (7, 8).

In the setting of the ESOT TLJ consensus conferences, the Delphi method is an appropriate technique as it can help to come to a conclusion under several circumstances which have been described in the late 1970s already (9). When a topic, or facing a challenge, in transplantation is not perfectly suitable for precise objective analytical techniques but benefit from subjective experts' opinions, Delphi rounds can be particularly useful to find consensus. This technique is also helpful and supportive to draw a conclusion when discussion participants cannot be brought together to have direct, face-to-face interactions and discussions for a variety of reasons (timing, costs, pandemic, etc.) and remote ± anonymous voting is needed (9). In the particular setting of TLJ 3.0, a public appraisal of the results the Delphi conducted study "ENGAGE" (European Giudelines for the Management of Graft Recipient Consensus Project) will be realized.

The Delphi method will also be applied to rediscuss and modify crucial recommendations if consensus will not be reached at TLJ 3.0.

Topic Selection for the 2022 European Society for Organ Transplantation Consensus Conference

An open call for topic proposals was issued to ESOT Sections and Committees in January 2022. Overall, 25 topic proposals were received and sent out to all members of the GT who rated them individually at a first step according to following criteria: 1) rating the proposal from 1 to 10; 2) recommending the topic yes/no; 3) marking the proposed group members 1) good proposition, 2) good but unbalanced, i3) needs to be discussed.

In a joint meeting, the GT reviewed and prioritized all submitted proposals and selected nine that met the following criteria: 1) cutting edge topics for which a consensus would have an impact on healthcare; 2) lack of similar guidelines or recommendations for this topic or an urgent need for an update of a previous version; 3) identification of barriers or data gaps requiring consensus recommendations to progress the field; 4) feasibility in the context of TLJ 3.0 meeting including minimal availability of published evidence; 5) completion of previous activated ESOT consensus processes; 6) collaborative forum of European and international leaders to exchange experience and knowledge.

Figure 1 shows the nine topics selected by the GT and validated by the ESOT Executive Committee for the ESOT consensus conference during the TLJ 3.0 in Prague on November 13th–15th (10).

Steering Committee Member Selection

For each of the selected topics, a specific steering committee (SC) was composed. The SC consists of a chair and co-chair, expert-members in the topic field, the Centre for Evidence in Transplantation (CET) (11), a YPT-representative working with the SC to collect and analyze the available topic-relevant literature, and a GT member to liaise with ESOT.

The GT had the final responsibility to nominate the SC members for each topic, though it did invite the topic proposers to suggest expert members. Depending on the balance of the proposed group representatives (expertise, gender, nationality etc., see below), the GT did either accept or request a modification of the member composition.

Each SC is led by a chair and a co-chair to warrant independency between topic proposers and guideline developers and to avoid bias and imbalances (12); selection of chair and co-chair followed a collaborative decision making process (GT and topic proposers) after exclusion of conflict of interests. The SC comprises of 8–14 members with a range of backgrounds to warrant a multidisciplinary expert discussion. In one case (Biomarker prediction in solid organ transplantation) the wide range of subtopics required a larger SC of 23 experts. When selecting

Topic	Subject
Machine perfusion in cardiothoracic transplantation	Cardiothoracic
Histopathological analysis of pre-implantation donor kidney biopsy: Redefining the role in the process of graft assessment	Kidney
The value of monitoring (subclinical) DSA for kidney transplant outcomes	Kidney
Liver transplantation in patients with primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD)	Liver
Clinical endpoints in liver transplantation according to value based care	Liver
Downstaging, bridging and immunotherapy in liver transplantation for hepatocellular carcinoma (HCC)	Liver
Role of pancreas machine perfusion to increase the donor pool for beta cell replacement	Pancreas
Prehabilitation for solid organ transplant candidates	Transversal
Molecular biology testing for non-invasive diagnosis of allograft rejection	Transversal

FIGURE 1 | Topics selected by ESOT Guideline Taskforce (GT) for consensus conference, TLJ 3.0, Prague November 2022.

TABLE 1 | Composition of the nine steering committees (SC).

Topic: Machine perfusion in cardiothoracic transplantation
Chairs: Arne Neyrinck, Cristiano Amarelli
Steering committee: Clemens Aigner, Irene Bello, Massimo Boffini, Stephan Clark, Marita Dalvindt, Julien de Wolf, Stephan Ensminger, David Gomez de Antonio, Martin Schweiger, Sandro Sponga, Bettina Wiegmann
Topic: Histopathological analysis of pre-implantation donor kidney biopsy: Redefining the role in the process of graft assessment (Part 1)
Chairs: Lucrezia Furian, Gianluigi Zaza
Steering committee: Jan Becker, David Cucchiari, Aiko de Vries, Albino Eccher, Sandrine Florquins, Jesper Kers, Lorna Marson, Marion Rabant, Michele Rossini
Topic: The value of monitoring (subclinical) donor specific antibodies (DSAs) for kidney transplant outcomes
Chair: Aiko de Vries
Steering committee: Dominique Bertrand, Klemens Budde, Emanuele Cozzi, Anthony Dorling, Marie Paule Emonds, Covadonga López del Moral, Soufian Meziyerh, Dennis van den Broek
Topic: Liver transplantation in patients with primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD)
Chairs: Luca Belli, Silvio Nadalin
Steering committee: Annika Bergquist, Marco Carbone, Eleonora De Martin, Andrea Della Penna, Pal Dag Line, Chiara Mazzarelli, James Neuberger, Palak Trivedi
Topic: Clinical endpoints in liver transplantation according to value based care
Chairs: Umberto Cillo, Mario Strazzabosco
Steering committee: Marco Carbone, Agostino Colli, Costantino Fondevila, Anna Forsberg, Lorenzo Mantovani, Sandor Mihaly, Alessandra Nardi, James Neuberger, Wojtek Polak, Karen Rockell, Ian Rowe, Liz Schick
Topic: Downstaging, bridging and immunotherapy in liver transplantation for HCC
Chair: Christian Toso
Steering committee: René Adam, Sherrie Bhoori, Umberto Cillo, Marco Claasen, Constantino Fondevilla, Bastiaan Rakke, Maria Reig, Gonzalo Sapisochin, Dimitri Sneiders, Parissa Tabrizian
Topic: Role of pancreas machine perfusion to increase the donor pool for beta cell replacement
Chair: Joana Ferrer
Steering committee: Julien Branchereau, Jason Doppenberg, Cinthia Drachenberg, Marten A Engelse, Paul Johnson, Henri G. D. Leuvenink, Benoît Mesnard, Franka Messner, Ann Etohan Ogbemudia, Vassilios Papalois, Trevor Reichman, Fabio Vistoli, Steve White
Topic: Prehabilitation for solid organ transplant candidates
Chairs: Diethard Monbaliu, Sharlene Greenwood
Steering committee: Coby Annema, Ellen Castle, Stefan De Smet, Pisana Ferrari, Tania Januadis- Ferreira, Joost Klaasen, Evangelia Kouidi, Sunita Mathur, Yasna Overloop, Maria José Perez Saez
Topic: Molecular biology testing for non-invasive diagnosis of allograft rejection
Group: heart, Chair: Luciano Potena
Steering committee: Ingvild Birschmann, Maria Crespo Leiro, Kiran Khush, Annamaria Minervini, Andrianna Nikolova, Javier Segovia
Group: kidney, Chair: John Friedewald
Steering committee: Dany Anglicheau, Oriol Bestard, Sook Park, Joana Sellares, Claire Tinel
Group: liver, Chair: Marina Berenguer
Steering committee: Eleonora de Martin, Amelia Heissheimer, Josh Levitsky, Alina Lutu, Valeria Mas, Nabeel Wahid, Haseeb Zubair

SC members, consideration was given to: 1) representation of different disciplines and expertise; 2) gender balance; 3) broad geographic representation; 4) involvement of all health care professionals, if indicated and possible; 5) involvement of patient and public representatives if indicated; 6) involvement of members of ESOT YPT (young professionals in transplantation); 7) involvement of methodologists when indicated.

Some of the consensus topics are developed jointly with other international organizations. In those cases, representatives suggested by the partner organization were included as members of the SC and involved throughout the entire process.

The composition of the nine SC, including roles, is illustrated in **Table 1**.

Steering committee members participate on a voluntary basis and are not paid for their contribution. Travel and

accommodation costs for meetings are reimbursed according to the relevant ESOT travel and meetings policy.

Consensus Questions, Evidence Review and Formulation of Recommendations

A number of virtual meetings were held by the SC to define the scope and aims of their topics and to work on their particular consensus process. Further meetings are scheduled in the upcoming months. Key issues were identified and implemented in the process to be worked on. The agreed clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome) (13). All PICO questions are listed in **Supplementary Appendix S1**. In some cases (i.e., VBHC endpoints in liver transplantation), the strict PICO format was methodologically not applicable (see below). PICO eliminations will be decided upon full agreement during the open discussion that will precede the conference or in the context of the meeting itself. All these changes will be accurately recorded and reported to assure full transparency of the process.

Following the definition of the PICOs, for each topic, literature searches were developed by expert staff from the CET who have expertise in conducting systematic reviews. The searches were conducted in the Transplant Library, Medline, and Embase with or without a date limit (dates differed for each of the groups) and the exact search date of each search was recorded (and will be reported in each consensus-dedicated publication). Bibliographic searches consisted of a combination of Medical Subject Headings and keywords. Search terms and strategies will be provided in the specific topic related publications. Searches, excluding grey literature (some SC included congress abstracts upon request) and following removal of duplicate references, resulted in unique references which were selected for title/abstract screening. If titles/abstracts appeared relevant to the PICO question, corresponding full texts were acquired and reviewed for possible inclusion and interactive reading, and to support the development of consensus statements. Due to the breadth of topics included, a full systematic review process for article review was not performed at this time. Rather, titles and abstracts were reviewed by CET members.

PRISMA flowcharts describing the number of studies identified by the literature search and number of studies selected for inclusion in the consensus statement will appear in the following topic-specific publications.

A short summary of the evidence addressing each key question by the included studies was prepared in an evidence table. The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. In particular, in the evaluation of the quality of evidence according to GRADE the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance (14). Strength of recommendation was rated as 1 (strong) or 2 (weak).

Jury Selection

The ESOT GT decided to maximize community involvement and inclusion of different perspectives while maintaining a high level of quality by assigning a panel to assess the documents prior to finalization. To establish these panels, an open call to attract jury members was launched in July 2022 *via* the ESOT webpage (15). Jury applicants register for the conference and specify their wish to be part of the recommendation voting process and the specific topic of interest. Jury member applicants' CVs are subsequently evaluated by the GT before acceptance to ensure they have the necessary experience allowing them to fairly assess the recommendations. Furthermore, due to the focus on patient-centered medicine, patients and patients' representatives are eligible to apply as jury members. Trainees will have the opportunity to follow the work of all included TLJ 3.0 panels as observers according to their particular interests (15). When jury members are appointed by the GT, conflicts of interests must be disclosed.

Jury members will receive the selected evidence as well as a preliminary version of the recommendations before the conference. They will be asked to provide the SC with comments and suggestions for potential changes and refinements before the start of the in-person meeting in Prague. In this way, a constructive discussion can take place during the face-to-face meeting.

Consensus Format

Working groups will include SC members and jury members. Working group processes will consist of the following: 1) SC leaders will introduce and present their topic to an extended panel composed of all working group members in addition to conference participants registered to participate in the in-person consensus discussion; 2) a single SC member, will provide an overview of the evidence for each key question and present the proposed recommendations; 3) feedback will be provided by working group members and conference participants with particular attention to the generation of clear and concise consensus statements taking into account the suggestions emerged by the discussion 4) the following day the consensus recommendations will undergo the jury vote. Consensus will be considered achieved will be considered as reached if an agreement rate of >80% is achieved; topic lectures and proposed consensus statements will be presented to the entire TLJ 3.0 audience in a dedicated session on the last day of the in-person meeting in Prague.

Consensus conference participants are selected and distributed amongst the working groups by the GT members. Complete information including the list of consensus conference working group domains, processes regarding consensus conference participant selection, development and refinement of consensus statements, and modified Delphi methodology including consensus polling will be also reported in Transplant International after the face-to-face meeting in Prague.

Validation Committee and AGREE

A validation committee, including experts in validation procedures, will be formed after the jury members have been

finalized. Consensus and recommendations will be reviewed by experts in validation according to the AGREE II guidelines: Appraisal of guidelines for research and evaluation II (16, 17). The complete validation and appraisal process will be published in due course after the in-person meeting in Prague.

SUMMARY AND NEXT STEPS

The 2022 ESOT Consensus Conference, as part of TLJ 3.0, will be the first consensus and guideline conference initiated by ESOT covering the entire field of organ transplantation including organ-specific as well as cross-cutting, inter- and multidisciplinary topics. This in-person event represents the impetus for the foundation of an ongoing consensus, recommendation, and guideline production process which launches also a permanent area, like a standing committee, within ESOT. All guidelines and recommendations produced and published by ESOT and its involved representatives will undergo a continuous review process to stay up to date. Pre-meeting responsibilities and activities included constitution of a taskforce, steering committees and their working group members, opening of the jury applications and their selection process. The guideline development process started with the identification of the topics of interest, formulation of PICO questions and the identification of the relevant evidence.

The consensus conference during the TLJ 3.0 consists out of discussion session on statements and generating recommendations including Delphi rounds in some cases, as well as a voting and a discussion session, on the last day during the in-person meeting (10). The TLJ 3.0 program, however, also includes educational sessions training on guideline and consensus statement production.

All recommendations and consensus statements produced for the nine selected topics will be published including the entire evidence-based consensus-finding process.

AUTHOR CONTRIBUTIONS

Involved in the conception or design of the work: all contributing authors. Literature screen and review: LP and CET (Centre for Evidence in Transplantation). Drafted the article: UC and AW. Critically revised the article: all contributing authors. Finally approved the version to be published: all contributing authors.

FUNDING

All costs related to taskforce and workgroup meetings were covered by ESOT, without external funding.

CONFLICT OF INTEREST

IJ received speaker fees from XVIVO perfusion paid to her institution. IJ is an ESOT Councilor for which she receives no reimbursement. JFe is recipient of a grant supported by Instituto de Salud Carlos III (ISCIII) through the project "PI18/00161 (Optimization of pancreas transplant graft: A multicentric study of histo-morphological and functional characteristics of unaccepted organs.)" and co-funded by the European Union. AdV received in the past speaker and consultation fees from Astellas, Chiesi, Hansa, Novartis, Sandoz, CSL Behring all of which paid to his institution. AdV is chair of the Dutch Kidney Advisory Committee (Landelijk Overleg NierTransplantatie LONT) for which he receives no reimbursement.

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10915/full#supplementary-material

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European Society of Organ Transplantation (ESOT) Consensus Statement on Machine Perfusion in Cardiothoracic Transplant

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The machine perfusion (MP) of transplantable grafts has emerged as an upcoming field in Cardiothoracic (CT) transplantation during the last decade. This technology carries the potential to assess, preserve, and even recondition thoracic grafts before transplantation, so it is a possible game-changer in the field. This technology field has reached a critical turning point, with a growing number of publications coming predominantly from a few leading institutions, but still need solid scientific evidence. Due to the increasing need to expand the donor pool, especially in Europe, where the donor age is steeply increased, a consensus has been established to address the growing need and knowledge of machine perfusion in cardiothoracic transplantation, targeting the unmet scientific need in this growing field but also, priorities for development, and regional differences in utilization rates and organizational issues. To address MP in CT, the European Society of Organ Transplantation (ESOT) convened a dedicated Working group comprised of experts in CT to review literature about MP to develop guidelines that were subsequently discussed

Abbreviations: CET, Center of Evidence in Transplantation; CHD, Congenital heart defects; CT, Cardiothoracic; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; ECMO, Extra-Corporeal Membrane Oxygenation; ESOT, European Society of Organ Transplantation; EVLP, *Ex-vivo* Lung Perfusion; ICU, Intensive Care Unit; LVAD, Left Ventricular Assist Device; HT, Heart transplantation; MP, Machine perfusion; OCS, Organ Care System; PGD, Primary Graft Dysfunction; PICO, Population, Intervention, Comparator, and Outcome; RCTs, Randomized Controlled Trials; SCS, Static cold storage; uDCD, Uncontrolled Donation after Circulatory Death.

and voted on during the Consensus Conference that took place in person in Prague during the TLJ 3.0 in November 2022. The findings and recommendations of the Cardiothoracic Working Group on MP are presented in this article.

Keywords: machine perfusion, ex-situ heart perfusion, ex-situ lung perfusion, graft preservation, cardio-thoracic transplantation

INTRODUCTION

Heart and lung transplantation are the most commonly used therapies for patients with end-stage lung and heart failures.

In 2019, a record number of more than 4,500 lung transplant procedures were performed at over 260 lung transplant centers worldwide, thanks to clinical and scientific advancements, new types of donations like donation after cardiac deceased controlled and uncontrolled or *Ex-vivo* Lung Perfusion (EVLP) technique [1].

EVLP allows the assessment, reconditioning before transplantation and the use of grafts that would have discharged.

Heart transplantation (HT) is the most commonly used therapy for patients with end-stage heart failure. Despite over 20,000 patients in the United States being eligible for HT each year, only a small percentage of them actually undergo transplantation. Additionally, donor heart non-utilization rates in the United States are high, with an estimated 60%–65% of viable hearts being discarded, further limiting the impact of HT [2]. The low donor heart acceptance rate may be due to the expectation that using marginal donors will result in poor outcomes.

Preservation of thoracic grafts is crucial to maintain their function during storage. The mainstream method of organ preservation during the last 40 years has been hypothermic preservation by static cold storage (SCS). However, the extension of donor ages has led to the use of grafts that are more vulnerable to ischemic damage. This epidemiologic change has prompted the need for new technologies to recondition the organs and expand the acceptability criteria for heart donation [3].

Ex-situ machine perfusion (MP), or *ex-vivo*, is an emerging technique to preserve solid organs explanted for allogeneic organ transplantation. MP provides a more "physiologic" alternative to the standard of care static-cold preservation, allowing for prolonged preservation and real-time monitoring of organ quality. It can also reduce or prevent ischemia-reperfusion injury and potentially convert the time of transport into a potential benefit for the organ, during which the organ can be reconditioned or even healed. Moreover, it has enabled the expansion of donor criteria, including after circulatory death, thereby increasing the organ pool. The MP platform has the potential to be a game-changer by providing reconditioning, modification of diseased organs, and regenerative approaches [4].

In recent years, due to changes in allocation policies and the complicated clinical and surgical profile of cardiac and lung recipients, graft preservation in organ transplantation has once again become a research priority. Improvements in the medical management of outpatients suffering from chronic heart failure and the availability of left ventricular assist devices (LVADs) and ECMO have shifted the allocation of organs to urgent candidates. However, this has led to an increase in ischemic times and an increased chance of primary graft dysfunction (PGD) due to the rise of surgical complexity and the addition of donor and recipient risk factors [5, 6].

The issue of organ preservation in heart transplantation has been flawed by assessing donor quality and possible modifications due to brain death and its management. The graft function after 24–48 h from reperfusion is quite worse than that seen during the evaluation of the graft during retrieval [7]. Within these changes, there are several factors to consider, such as the intrinsic quality and function of the graft during retrieval, the amount of ischemic damage, the amount of damage due to freezing, rewarming, and reoxygenating injury, and the amount of reperfusion injury, which could be related to ischemia and immunologic reasons.

PGD has a dreadful course, affects postoperative ICU stays, and may require expensive treatments like ECMO and temporary circulatory support, affecting ICU stay, costs, morbidity, and mortality. Therefore, alternative sustainable paradigms to improve CT organ preservation are being researched.

Despite initial encouraging data, preservation technologies still await a breakthrough. Optimal assessment parameters are required to evaluate organ quality and viability and must be agreed on.

There is a solid unmet scientific need for well-designed trials or granular data to ascertain the real benefit of MP in each specific subset of donors and recipients. This consensus report was considered timely to define the role of Cardiothoracic machine perfusion and the level of evidence supporting their use in everyday clinical practice. Furthermore, these data are required to support decision-making, pharmacoeconomic evaluations, and logistical and organizational models that may be sustainable in different social and healthcare systems. Moreover, MP could provide:

- An organizational paradigm shift to increase the number of transplants.
- Providing opportunities for assessment.
- Drug therapies.
- Cellular therapies.
- Facilitating further research and innovation.

Aim of the guidelines: To address Machine perfusion in cardiothoracic transplant, ESOT convened a consensus conference comprised of a global panel of experts involving six transplant experts for the heart and six for the lung to develop expert opinion on key aspects of MP in CT transplant and to help define future needs for research. Summaries of the evidence were presented to the entire group of panelists and jury (MB). The consensus findings and recommendations of the ESOT

TABLE 1 | Heart and Lung Pico's proposed to CET.

Heart	
PICO 1: Heart	In heart transplantation, for which heart should machine perfusion be performed?
PICO 2: Heart	Heart In heart transplantation, which protocol/perfusate/perfusion strategy for ex-vivo/ex-situ heart perfusion leads to the best clinical outcomes post-transplant?
PICO 3: Heart	In heart transplantation, which biomarker/parameter is capable to predict the graft survival, graft function, primary non-function during ex vivo heart perfusion?
PICO 4: Heart	In heart transplantation, which recipients will benefit from a heart assessed by machine perfusion?
Lung	
PICO 1: Lung	In lung transplantation, for which type of lung should ex vivo lung perfusion be performed?
PICO 2: Lung	In lung transplantation, which protocol/perfusate/ventilation strategy for ex-vivo/ex-situ lung perfusion leads to optimal outcomes?
PICO3: Lung	In lung transplantation, which parameters (physiological, biomarkers) should be used to determine graft quality during ex vivo lung perfusion?
PICO4: Lung	In lung transplantation, which recipients should benefit from a lung assessed by ex vivo lung perfusion?

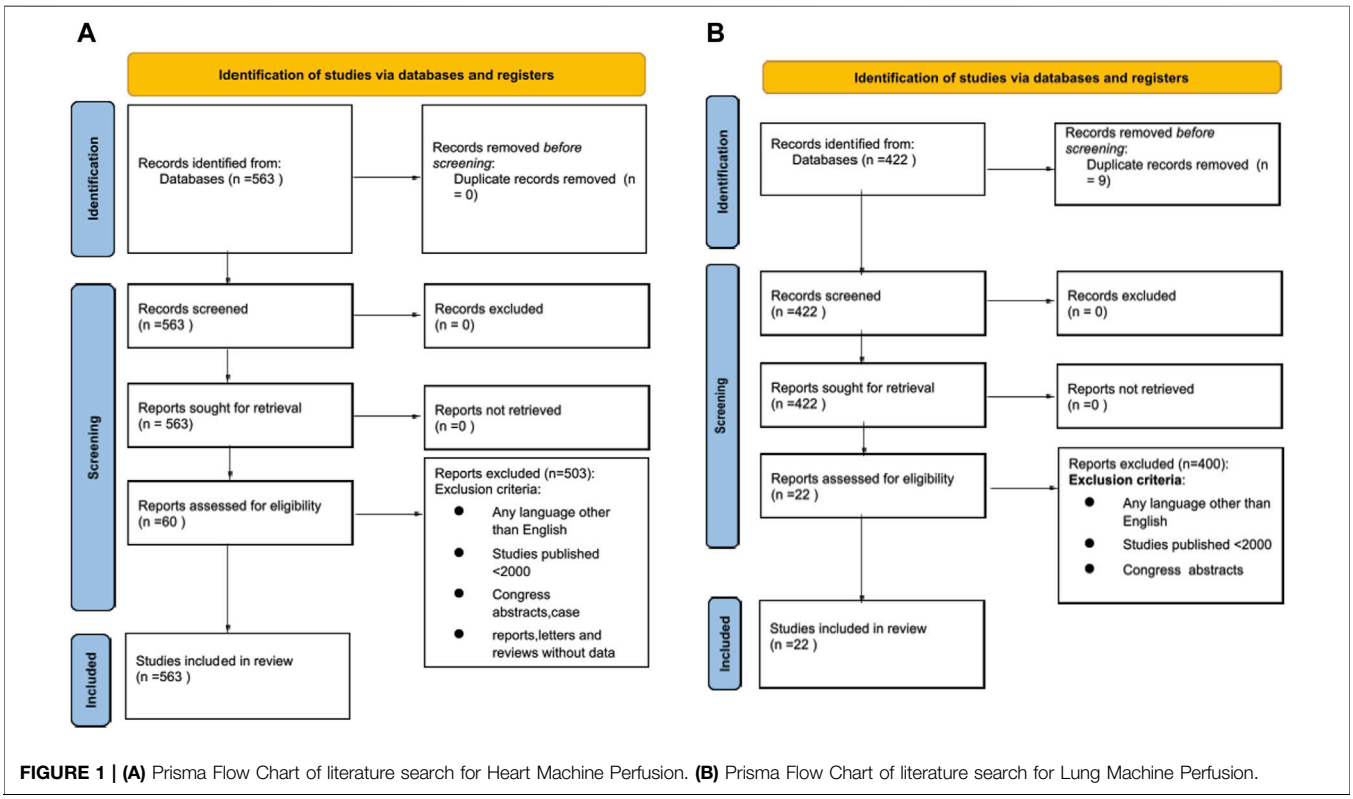

FIGURE 1 | (A) Prisma Flow Chart of literature search for Heart Machine Perfusion. **(B)** Prisma Flow Chart of literature search for Lung Machine Perfusion.

Consensus guidelines on MP are presented in this document. This document, which will be updated to reflect new evidence as it becomes available, is intended for healthcare providers.

METHODS

A dedicated Guidelines Taskforce within ESOT organized the consensus development process and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members, and patient representatives. A detailed description of the methodology used has been reported previously [8].

Briefly, key issues related to MP in CT transplant topics were identified by each working group, and specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator, and Outcome) [9]. All PICO questions are listed in **Table 1**. Following the definition of the PICOs, literature searches were developed by expert staff from the CET (Center of Evidence in Transplantation) who have expertise in conducting systematic reviews and subsequently integrated, when needed, by the steering committee experts.

A PRISMA flowchart describing the number of studies identified by the literature search and the number of studies selected for inclusion in the consensus statement appears in **Figures 1A, B**.

A

Statement	Quality of evidence	Recommendation strength	Consensus
PICO 1: In lung transplantation, for which type of lung should machine ex vivo lung perfusion be performed?			
1.1: Compared with cold storage preservation, ex vivo lung perfusion is technically safe for standard donor lungs.	Moderate	Strong for	100% agree CONSENSUS REACHED
1.2: Compared with cold storage preservation, ex vivo lung perfusion is technically safe and might lead to increased donor utilisation in non- standard donor lungs.	Moderate	Strong for	100% agree CONSENSUS REACHED
2.1: Ex vivo lung perfusion is safe for re-evaluation in situations with impaired/questionable graft function in DCD/DBD grafts.	Low	Weak for	100% agree CONSENSUS REACHED
2.2: Ex vivo lung perfusion is safe for logistical reasons.	Low	Weak for	100% agree CONSENSUS REACHED
2.3: Ex vivo lung perfusion is safe for standard preservation.	Low	Weak for	70% agree CONSENSUS NOT REACHED
2.4: Ex vivo lung perfusion is safe for long expected ischaemic times.	Low	Weak for	100% agree CONSENSUS REACHED
PICO 2: In lung transplantation, which protocol/perfusate/ventilation strategy for ex vivo/ ex situ lung perfusion leads to optimal outcomes?			
3: The current three major protocols (LUND/TORONTO/OCS) have been validated for clinical use.	Moderate	Strong for	100% agree CONSENSUS REACHED
4: Further individualisation of the ex vivo lung perfusion protocols is required.	Low	Strong for	100% agree CONSENSUS REACHED
5: The physiological parameters (perfusion/ventilation/gas exchange) have been sufficiently validated to accept/decline a donor lung after ex vivo lung perfusion in clinical practice.	Low	Weak for	100% agree CONSENSUS REACHED
PICO 3: In lung transplantation, which parameters (physiological, biomarkers) should be used to determine graft quality during ex vivo lung perfusion?			
6: The assessment of the graft quality to accept/decline the donor lung using physiological parameter cannot be done using one single parameter.	Moderate	Strong for	100% agree CONSENSUS REACHED
7: The use of parameters other than the standard physiological parameters should be further developed into clinical practice to define the acceptance/ decline of a pulmonary graft.	Moderate	Strong for	100% agree CONSENSUS REACHED
PICO 4: In lung transplantation, which recipients should benefit from a lung assessed by ex vivo lung perfusion?			
8: Currently, there is consensus on recipient criteria that might indicate the need to perform machine perfusion.	Very low	Strong for	70% agree CONSENSUS NOT REACHED
9: The risk/benefit ratio to transplant the recipient can justify the acceptance of questionable lungs after ex vivo lung perfusion assessment.	Low	Weak for	100% agree CONSENSUS REACHED

B

Statement	Quality of evidence	Recommendation strength	Consensus
PICO 1: In heart transplantation, for which type of heart should machine perfusion be performed?			
1: The technique of machine perfusion is safe (non-inferior) for heart preservation in transplantation.	Moderate	Strong for	100% agree CONSENSUS REACHED
2: The use of machine perfusion reduced the cold ischaemic time and, therefore, offers the possibility to prolong preservation time.	Moderate	Strong for	100% agree CONSENSUS REACHED
3.1: Machine perfusion is a valuable tool in DBD to re-evaluate organ viability before implantation.	Moderate	Strong for	83% agree CONSENSUS REACHED
3.2: Machine perfusion is a valuable tool in DCD to assess and re-evaluate organ viability before implantation.	Moderate	Strong for	100% agree CONSENSUS REACHED
4: Other devices for advanced graft preservation are under clinical investigation to extend the safe ischaemic time.	Low	Strong for	100% agree CONSENSUS REACHED
PICO 2: In heart transplantation, which protocol/perfusate/perfusion strategy for ex vivo/ ex situ heart perfusion leads to the best clinical outcomes post-transplant?			
5.1: The current machine perfusion protocol(s) have been validated for clinical use in adult recipients.	Moderate	Strong for	100% agree CONSENSUS REACHED
5.2: The current machine perfusion protocols are feasible for clinical use in paediatric recipients.	Moderate	Strong for	100% agree CONSENSUS REACHED
PICO 3: In heart transplantation, which biomarker/parameter is capable of predicting graft survival, graft function and primary non-function during ex vivo heart perfusion?			
6: Angiography is a possible tool to assess coronary arteries of the heart during machine perfusion.	Low	Strong for	100% agree CONSENSUS REACHED
7: Lactate is the most commonly used parameter to assess heart preservation during machine perfusion.	Low	Strong for	100% agree CONSENSUS REACHED
8: Other biological/functional tools have to be developed to assess heart quality during machine perfusion.	Low	Strong for	100% agree CONSENSUS REACHED
PICO 4: In heart transplantation, which recipients will benefit from a heart assessed by machine perfusion?			
9: The use of machine perfusion is non-inferior to perform heart transplantation in VAD patients.	Moderate	Weak for	100% agree CONSENSUS REACHED
10: Currently, there is consensus on recipient criteria that might indicate the need to perform machine perfusion.	Very Low	Strong for	100% agree CONSENSUS REACHED

FIGURE 2 | (A) Statements with quality of evidence, strength, and level of agreement during the Votation (heart) **(B)**. Statements with quality of evidence, strength, and level of agreement during the Votation (lung).

A summary of the evidence addressing each key question by the included studies was prepared in evidence (**Supplementary Tables S2, S3**). The workgroup proposed a recommendation for each key question based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. For evaluation of the quality of evidence according to GRADE [10], the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance, and publication bias. The strength of recommendation was rated as 1 (strong) or 2 (weak).

The Delphi method was applied to arrive at a group opinion during the consensus conference.

Complete information, including the list of consensus conference workgroup domains (and topics noted below), and process regarding consensus conference participant selection, development and refinement of consensus statements, and modified Delphi methodology, including consensus polling, are previously reported in beforehand the in-person conference held in Prague, Czech Republic, Nov 13–15, 2022 [8].

RESULTS

Heart Results

PICO 1: Heart (4 Statements)

In heart transplantation, for which heart should machine perfusion be performed?

- 1. The machine perfusion technique is safe (non-inferior) for heart preservation in transplantation.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

The original statement proposed was: The machine perfusion technique is safe and effective for heart preservation in transplantation." but reached a low quality of evidence and recommendation strength. The statement was

rewritten based on the fact that even if same retrospective data show optimal organ preservation and clinical results [11, 12], randomized trials obtained non-inferior results [13, 14] and metanalysis were too heterogeneous (DBD and DCD together) to add meaningful data [15]. So, the new statement was changed highlighting non-inferiority, and the recommendation strength was increased from moderate to strong.

- 2. The use of machine perfusion reduces the cold ischemic time and, therefore, offers the possibility to prolong preservation time.

Quality of Evidence: [moderate] Recommendation strength [strong for].

The employment of MP limits the ischemic time to the time necessary for graft procurement, device instrumentation and heart transplantation independently by the transportation time that in this way can safely exceed the 4 h. Some reports describe very long support >16-17 h [16]. Recent data in DCD organ donation suggests further safe extension of the ischemic time in a wide variety of clinical settings [17].

- 3.1. Machine perfusion is a valuable tool in DBD to re-evaluate organ viability before implantation.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

Lactates analysis permits to access organs during transportation, coronary angiography is possible when the heart is placed in the MP.

- 3.2. Machine perfusion is a valuable tool in DCD to assess and re-evaluate organ viability before implantation.

Whether normothermic regional perfusion is not feasible or available due to ethical and legal constraints, MP is the only possibility to assess DCD organs. DCD programs when a MP is employed permitted to obtain non inferior results compared to DBD programs [18].

Quality of Evidence: [moderate] Recommendation strength: [strong for].

- 4. Other devices for advanced graft preservation are under clinical investigation to extend the safe ischemic time.

Quality of Evidence: [low] Recommendation strength: [strong for].

The Guardian Registry showed valuable data about PGD reduction when controlled hypothermia is used for graft transportation compared with standard icebox [19, 20] also in extended donors [21].

PICO 2: Heart (1 Statement)

Heart In heart transplantation, which protocol/perfusate/perfusion strategy for *ex-vivo*/*ex-situ* heart perfusion leads to the best clinical outcomes post-transplant?

- 5.1. The current machine perfusion protocol(s) have been validated for clinical use in adult recipients.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

In heart transplantation the availability of different protocols and perfusion strategies has been reduced by the presence of a single device for warm ESHP commercially available. The need of a standardization of the protocols of this commercially available MP has limited the possibility to have multiple protocols so there is a strong recommendation strength to strictly adhere to the unique methods utilized for all the trials on OCS.

- 5.2. The current machine perfusion protocols are feasible for clinical use in pediatric recipients.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

No sufficient data regarding the use in pediatric recipients, however, the actual devices are recommended for donors >15 kg. In adult recipients suffering from end stage biventricular and univentricular congenital heart defects (CHD) machine perfusion is non-inferior compared to adult non-CHD patients [22].

PICO 3: Heart (3 Statements)

In heart transplantation, which biomarker/parameter is capable to predict the graft survival, graft function, primary non-function during *ex vivo* heart perfusion?

- 6. Angiography is a possible tool to assess coronary arteries of the heart during machine perfusion.

Quality of Evidence: [low] Recommendation strength: [strong for].

Angiography during MP is anecdotal and may be useful to evaluate anatomy more than quality. When concerns emerge during perfusion may be considered to rule-out organs with hidden coronary damages [12].

- 7. Lactate is the most commonly used parameter to assess the heart preservation during machine perfusion.

Although data from leading institutions [23] show that lactate levels doesn't correlate with outcome the use is suggested by the consolidate use of the only warm ESHP commercially available. Data on DCD [24] seem to lower the importance of lactate in DCD donors.

Quality of Evidence: [low] Recommendation strength: [strong for].

- 8. Other biological/functional tools have to be developed to assess heart quality during machine perfusion

Quality of Evidence: [low] Recommendation strength: [strong for].

Although based on a single paper [25, 26] on current and future biomarkers the availability of new biomarkers to better

evaluate the organ quality appears a possible gamechanger of the future of the technology thus improving the quality of the prediction of organ function and reducing the risk for PGD.

PICO 4: Heart (2 Statements)

In heart transplantation, which recipients will benefit from a heart assessed by machine perfusion?

- 9. The use of Machine perfusion is non-inferior to perform heart transplantation in VAD patients.

LVAD patients may be a surgical challenge and appear patients in which the MP technology may warrant superior outcomes permitting the surgeon to work without the hurry [26] in an elective setting. Many small retrospective reports support the safety of MP in this setting [27–29] but there is still a lack for well-designed trials in this setting,

Quality of Evidence: [moderate] Recommendation strength: [weak for].

- 10. Currently, there is consensus on recipient criteria that might indicate the need to perform machine perfusion

Quality of Evidence: [very low] Recommendation strength: [strong for].

The weight of the recipient's features in Heart transplant appears a crucial factor for choosing the right way to preserve the donor graft. However, few small retrospective studies supported the use of MP in selected high-risk recipients as LVAD and CHD [30]. These patients however carry a high risk of mortality and ECMO support. Pediatric recipients might receive adult donor heart organs evaluated for transplantation in pediatric recipients. DCD donors over 15 kg are often preserved with ESHP [31]. The utilization of scores for selecting the right graft preservation strategy could represent a valuable attempt to justify the additional costs of MP in some healthcare systems with economic constraints.

Lung Results

PICO 1: Lung (2 Statements)

In lung transplantation, for which type of lung should *ex vivo* lung perfusion be performed? (**Figure 2B**)

- 1.1. Compared to cold storage preservation, *ex vivo* lung perfusion is technically safe for standard donor lungs.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

Different clinical studies have investigated the use of EVLP for standard donor lungs [32–37]. The definition of standard vs nonstandard lung donors was strongly discussed since it appears a crucial limitation of the current literature since different manuscript tend to adopt different definitions [38–41]. The group agrees on the lack of robust data until now on the definition of the marginal or extended donors [42]. This definition should keep in consideration the differences between DBD, cDCD and uDCD. Also, based on local practices, not every DCD donor lung should be considered marginal or extended.

- 1.2. Compared to cold storage preservation, *ex vivo* lung perfusion is technically safe and might lead to increased donor utilization in non-standard donor lungs

Quality of Evidence: [moderate] Recommendation strength: [strong for].

Reported donor utilization rate after *ex vivo* lung perfusion from non-standard donor lungs ranges from 60%–90% based on case series and reported trials [34, 43–46]. The dynamic process of the quality of the organ during *ex vivo* lung perfusion may further complicate the definition of the advantage of MP to increase the donor utilization in non-standard donors. Recently, new evidence indicate also a paradigm shift in cold static storage preservation, where higher temperatures (avoiding freezing of the graft) are being investigated with promising results. The role of this new strategies for standard and non-standard donor lungs and the interaction with *ex vivo* lung perfusion should be investigated [47–50].

- 2.1. *Ex vivo* lung perfusion is safe for re-evaluation in situations with impaired/questionable graft function in DCD/DBD grafts.

Quality of Evidence: [low] Recommendation strength: [weak for].

Looking at the literature the heterogeneity of the reasons behind the use of MP [33, 34] in lung transplantation was debated and there was an agreement on analyzing separately the different indications for its usage. The recommendation strength behind the usage for reassessing the quality of the organ based on the current literature was considered low despite the clinical rationale that appears solid.

- 2.2. *Ex vivo* lung perfusion is safe for logistical reasons.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

Currently standard use of *ex vivo* lung perfusion for logistical reasons is driven by local practices and clinical protocols and is based on the principle to prolong preservation times. The evidence for systematic use of *ex vivo* lung perfusion for extending preservation times is limited and needs further investigation. Some systems are portable and can be transported to the donor hospital. We have observed a tendency towards centralization of *ex vivo* lung perfusion which may impact the logistical use based on higher efficiency, reduced costs and centralization of expertise [51].

Also, new innovations in static cold preservation might need to redefine the role of *ex vivo* lung perfusion for logistical reasons alone.

- 2.3. *Ex vivo* lung perfusion is safe for standard preservation.

Quality of Evidence: [low] Recommendation strength: [weak for].

This statement didn't reach the sufficient consensus (70%), further supporting the need for well-designed data in support of the use of MP in standard donors.

- 2.4. *Ex vivo* lung perfusion is safe for long expected ischemic times.

Quality of Evidence: [low] Recommendation strength: [weak for].

Based on the same discussion regarding logistical reasons for *ex vivo* lung perfusion, the clinical evidence to prolong ischemic times based on *ex vivo* lung perfusion is limited [52]. Further investigation to prolong the homeostasis of the graft is needed and experimental evidence is increasing to adjust the systems and protocols towards longer perfusion times [53]. Also, the combination of different intervals using *ex vivo* lung perfusion and static preservation strategies should be further investigated [50].

PICO 2: Lung (2 Statements)

In lung transplantation, which protocol/perfusate/ventilation strategy for *ex-vivo*/*ex-situ* lung perfusion leads to optimal outcomes?

- 3. The current 3 major protocols (LUND/TORONTO/OCS) have been validated for clinical use.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

During the Consensus the 3 major protocols were described [33, 34, 54, 55], and the group agreed on the effectiveness of all of them to warrant optimal outcomes although no data could support the choice between each of them and direct comparisons are not possible.

- 4. Further individualization of the EVLP protocols is required.

Quality of Evidence: [low] Recommendation strength: [strong for].

The importance of cost-effectiveness studies to select the right preservation strategy based on clinical profile of donor and recipients was debated. The group agreed on the need of cost-effectiveness analysis to avoid the wasting of resources.

- 5: The physiological parameters (perfusion/ventilation/gas exchange) have been sufficiently validated to accept/decline a donor lung after *ex vivo* lung perfusion in clinical practice.

Quality of Evidence: [low] Recommendation strength: [weak for].

Although there is enough clinical data about the commonly accepted values of perfusion, ventilation and gas exchange parameters to decide whether an organ is usable or not after EVLP, the reality is that each group applies their own criteria, based on clinical practice, without robust evidence-based data to define the threshold to accept or reject a perfused graft [56].

PICO 3: Lung (2 Statements)

In lung transplantation, which parameters (physiological, biomarkers) should be used to determine graft quality during *ex vivo* lung perfusion?

- 6: The assessment of the graft quality to accept/decline the donor lung using physiological parameter cannot be done using one single parameter.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

When evaluating the quality of a donor lung during *ex situ* lung perfusion, relying on a single physiological parameter is insufficient [57, 58]. Many different parameters and scores were presented during the session showing the potential room for moving from single parameters to multiparametric evaluations to discriminate the quality of the organ. Instead, a comprehensive assessment that considers multiple parameters (flow rate, compliance, gas exchange, airway pressures, lung weight) is essential to make informed decisions regarding the suitability of the lung for transplantation [59].

- 7: The use of parameters other than the standard physiological parameters should be further developed into clinical practice to define the acceptance/decline of a pulmonary graft.

Quality of Evidence: [moderate] Recommendation strength: [strong for].

There is a need for expanding beyond standard physiological parameters when assessing pulmonary grafts during *ex situ* lung perfusion. While traditional parameters like compliance, pulmonary vascular resistance (PVR), and oxygenation remain crucial, there's a call to develop and incorporate additional parameters like biomarkers for inflammation or cellular damage [57, 58]. These novel indicators could enhance the accuracy of decisions regarding acceptance or rejection of donor lungs for transplantation [60]. The possibility to implement Machine-learning and AI technology was also highlighted as a future perspective.

PICO 4: Lung (2 Statements)

In lung transplantation, which recipients should benefit from a lung assessed by *ex vivo* lung perfusion?

- 8: Currently, there is consensus on recipient criteria that might indicate the need to perform machine perfusion.

It appears that the statement in question did not receive the required consensus of 70%. This reinforces the need for well-designed evidence to support the selection of recipients candidates for donors preserved with MP. The weight of the recipient's features in Lung transplant still seems to be a challenging factor to consider.

- 9: The risk/benefit ratio to transplant of the recipient can justify the acceptance of questionable lungs after *ex vivo* lung perfusion assessment.

The discussion focused on the need to gather data to facilitate informed shared decision-making with patients to improve their experience and move towards person-centered care planning.

DISCUSSION

MP has been advocated as a tool to revolutionize the field of transplantation by:

- Increasing the number of organs,
- Improving the safety of the procedure,
- Reducing the burden of PGD,
- And converting an emergent procedure in a safe and calm elective procedure [55].

The technology has been separately developed for the heart and lung, with the lung as a trailblazer and a few groups in the world (Lund, Papworth, Toronto) as an upfront participant in clinical development. Given the possibility of assessing organ quality and widening the donor pool, the DCD has immediately become the natural clinical arena for growing the experience in the field until the possibility of reperfusing the organs in the donors through Normothermic Regional Perfusion has been envisioned [61].

CT MP has been developed as an alternative to the standard static-cold preservation method. The longer preservation of organs and real-time monitoring of organ quality may allow to redesign the allocation while also reducing or preventing ischemia-reperfusion injury. Ongoing improvements in MP protocols, particularly in extending the preservation duration, have opened up new possibilities for reconditioning and modifying diseased organs, as well as for tumor and infection therapies and regenerative approaches [62]. Lastly, the implementation of MP for in vivo-like preclinical studies that improve disease modeling has generated significant interest, creating an ideal interface for bioengineering and genetic manipulation [63]. In this perspective, large part of the innovation in the field of CT transplantation depends on how rapidly the research in this technology will evolve. Despite all these promises it is necessary to establish a methodological environment to warrant the use of this technology based on the unmet clinical needs of the patients and aimed at making the system economically sustainable in different healthcare systems.

HEART

The change in the donor profile with the impressive increase of DCD [64] in many healthcare systems and the increase of mean donor age in Europe represents the first call for action to identify in which donors and in which recipients MP is necessary and when it may be helpful to warrant an improvement of patient's outcomes. The PICOs of this consensus conference were designed to assess the heart and the lungs using the same methodology. Until the consensus, the only licensed system of MP for the heart was the OCS, with some upcoming data of the XVIVO coming from the first clinical application of this new technology [65]. PICO 2 and 3 for the heart were, therefore, mainly related to the protocol standardization coming from the OCS system.

The difference between DBD and DCD donors in terms of need of assessment and preservation was intensely discussed, and without envisioning the role of NRP [61] as an alternative for perfusing and evaluating organs, the MP was considered a valuable means to preserve and assess the donor hearts coming from DCD donors. The experience from all around the world with the NRP leave now opens the possibility of evaluating the heart with NRP and preserving the donors with SCS [66, 67]. During the discussion on the controversies around the utility of MP in the extended donors, one of the more controversial points was the demonstration of marginality for the extended donors and the demonstration of the reduction of the intrinsic risk (of PGD) carried from the donor. Scores like the Eurotransplant donor score [68] or the adapted Donor Risk score [69] have been advocated to demonstrate the complexity of the donor pool. Recently, the Donor Utilization Score [70] has shown differences between the European and US donor pools. Using a similar score to identify donors benefiting from preservation with MP could be a way to justify the additional costs carried by this technology. On the other hand, the authors shared the need to have well-designed RCTs or registries for LVAD recipients and CHD recipients to support the benefit of MP in this setting. After the impressive data coming from first XVIVO animal, experimental and clinical experiences [71–73], the next horizon will be to clarify the organs in which extending donor preservation (by Sherpapak or by XVIVO) may be sufficient to provide an improved outcome to the recipient and to which extent of extension the clinician may push the preservation time with each technology. Until now, the OCS has been the only technology that permits the assessment of the quality of the preservation and the intrinsic quality of the organ, and this retails a unique place to expand the donor pool.

The role of visual assessment is strongly dampened by the unloading of the heart, even if recently has been postulated a computerized system to assess the kinematics of *ex vivo* beating hearts undergoing normothermic perfusion on the TransMedics OCS [74]. This and similar tools may further fortify the possibility of the OCS to certify the quality of the graft.

The possible role of biomarkers [75] in this setting is another target for research to innovate the field of MP. The availability of a biomarker capable of appropriately predicting the hazard of PGD and delayed graft function may render the visual assessment unrelevant but also strengthen the advantage of dynamic strategies of perfusion over the impressive amount of data coming from the more reliable comparator that appears today, the Sherpapak.

One of the weaknesses of all the consensus was, in fact, the absence of a clear, unique comparator since icebox preservation has been poorly standardized and based on different cardioplegic solutions and delivery modalities (single shot, repeated before declamping, etc.).

The anecdotal demonstration that the *ex-vivo* preservation could mitigate the tissue damage that is expected after long ischemic times thus reverting the myocardial disarray is one of the most appealing issues supporting the possibility to expand donor pool through the implementation of MP [76].

In conclusion, MP appears the most attracting Innovation in a field that until now has been constraint by the lack of donors. MP has

the possibility to exploit the number of CT transplants and redesign the field. Obviously, one of the variables in the pot is if the system will result sustainable and able to improve the outcomes of CT transplantation not only in terms of immediate outcomes but also during the mid and long-term thank to the possibility of modifying the immunogenicity of the grafts [77]. Having a certification of quality of the organ, the evolution from a center-based organization toward a national (or supranational in Europe) organization will be probably the natural evolution of the logistical and organizational pathways of CT transplantation permitting a broader allocation accounting also for HLA. The NOP in US and the Bridge in Sweden open the clear road from the center providing its own preservation strategy toward and Amazon-like organization where the organ may be evaluated at the arrival in the hospital before deciding to carry-on or not the operation for the single recipient identified with designed algorithms.

The recent perspective to prolong perfusion over 24 h [78] will further modify the pathways for organ allocation from the current standard toward a new model in which organ repair centers could also play a significant role.

LUNG

Ex vivo lung perfusion (EVLP) is a promising technology [56, 79] that allows donor lungs to be evaluated in a closed circuit outside of the body and extends lung donor assessment prior to final acceptance for transplantation. Compared to cold storage preservation, EVLP is technically safe for standard donor lungs and might lead to increased donor utilization in non-standard donor lungs. EVLP is also safe for re-evaluation in situations with impaired/questionable graft function in DCD/DBD grafts, logistical reasons, standard preservation, and long expected ischemic times. However, the evidence for the safety of EVLP for these situations is weak. The current three major protocols (LUND/TORONTO/OCS) have been sufficiently validated and have shown to be safe to accept/decline a donor lung after *ex vivo* lung perfusion in clinical practice. However, the assessment of the graft quality to accept or reject an organ should be performed in a holistic manner, taking into consideration different objective physiologic parameters (perfusion rate, vascular resistance, airway pressure, compliance, gas exchange, compliance, weight gain). Moreover, the use of parameters other than the standard physiological parameters (biomarkers) should be further developed into clinical practice to define the acceptance/decline of a pulmonary graft. Recent studies have shown that EVLP has diagnostic capabilities as an organ monitoring device and therapeutic potential to improve lung allograft quality when specific issues are encountered. An important aspect is the future development of EVLP as a reconditioning platform to translate and personalize different treatment strategies prior to transplantation.

The safety of EVLP for standard preservation statement did not reach a consensus. Despite clinical trials and retrospective studies have shown that recipients of EVLP-treated lungs have similar post-transplant survival rates compared to those who received conventionally preserved lungs, indicating that EVLP is non-detrimental in terms of mortality and retransplantation rates [80], they did not demonstrate superiority in standard donors, but it increases significant the costs and the optimal perfusion protocol and perfusate composition remain subjects of ongoing research. EVLP appears to be a safe and effective method for lung preservation, offering several advantages over traditional methods in selected cases, although further optimization and cost management are needed to fully realize its potential.

There is no absolute consensus on specific recipient criteria that indicate the need to perform EVLP. It is primarily employed to address the shortage of viable donor lungs by allowing the assessment, preservation, and reconditioning of marginal or high-risk donor lungs, which would otherwise be deemed unsuitable for transplantation.

It is important to note that EVLP is a relatively new technology, and its long-term effects are still being studied. Furthermore, the cost-effectiveness of EVLP compared to other methods of lung preservation is still being evaluated. Despite these limitations, due to ongoing improvements, EVLP has the potential to improve the quality and number of donor lungs available for transplantation, particularly through possible regenerative approaches to reprocessing and modifying originally marginal donor organs and in the use of DCD donors, but also in the future following the cardiac approach in the context of xenogeneic transplantation.

SUMMARY AND NEXT STEPS

The current evidence on MP is still weak, as stated in this document; however, there is a large consensus regarding the tremendous challenge that this technology offers to the expansion of the donor pool and to the reshaping of the logistics of CT transplantation. Facing the weaknesses of the current data, the group of experts agreed on the necessity of work in the direction of a European Registry for machine perfusion and DCD donation and on the need of cost-effectiveness studies to support the use of MP in CT transplantation.

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

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.

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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.2024.13112/full#supplementary-material

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European Society for Organ Transplantation (ESOT) Consensus Statement on the Use of Non-invasive Biomarkers for Cardiothoracic Transplant Rejection Surveillance

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While allograft rejection (AR) continues to threaten the success of cardiothoracic transplantation, lack of accurate and repeatable surveillance tools to diagnose AR is a major unmet need in the clinical management of cardiothoracic transplant recipients. Endomyocardial biopsy (EMB) and transbronchial biopsy (TBBx) have been the cornerstone of rejection monitoring since the field's incipience, but both suffer from significant limitations, including poor concordance of biopsy interpretation among pathologists. In recent years, novel molecular tools for AR monitoring have emerged and their performance characteristics have been evaluated in multiple studies. An international working group convened by ESOT has reviewed the existing literature and provides a series of recommendations to guide the use of these biomarkers in clinical practice. While acknowledging some caveats, the group recognized that Gene-expression profiling and donor-derived cell-free DNA (dd-cfDNA) may be used to rule out rejection in heart transplant recipients, but they are not recommended for cardiac allograft vasculopathy screening. Other traditional biomarkers (NT-proBNP, BNP or troponin)

do not have sufficient evidence to support their use to diagnose AR. Regarding lung transplant, dd-cfDNA could be used to rule out clinical rejection and infection, but its use to monitor treatment response is not recommended.

Keywords: heart transplantation, lung transplant, biomarker, rejection, guidelines

INTRODUCTION

Despite major advances in the management of immunosuppression, allograft rejection (AR) continues to threaten the success of cardiothoracic transplantation. AR can lead to acute immune-mediated graft dysfunction, as well as chronic multifactorial graft-specific diseases, such as cardiac allograft vasculopathy (CAV) and chronic lung allograft dysfunction (CLAD), both ultimately leading to graft failure and death.

Lack of accurate and repeatable surveillance tools to diagnose AR is a major unmet need in the clinical management of cardiothoracic transplant recipients. Endomyocardial biopsy (EMB) and transbronchial biopsy (TBBx) have been the cornerstone of rejection monitoring since the field's incipience. Long considered the "gold standard," both suffer from significant limitations, including sampling error, high cost, potential complications, and patient discomfort. Moreover, prior studies have shown poor overall concordance of biopsy interpretation among pathologists [1].

The vast majority of TBBx and EMB performed during surveillance do not show signs of clinically meaningful AR, hence highlighting the need for reliable non-invasive biomarkers to screen for AR and to reduce the frequency of invasive procedures [2, 3].

A multitude of biomarkers for rejection diagnosis have been developed over the past few decades and are at different stages of commercial development and clinical validation. Given these advances in the field, a working group was convened by the European Society of Organ Transplantation (ESOT) that included healthcare professionals from across Europe and North America with expertise in the field. The panel has reviewed the existing literature for the degree of evidence supporting the use of these assays in clinical practice in order to provide clinical practice recommendations for the clinical use of biomarkers in cardiothoracic transplant rejection surveillance, and to highlight knowledge gaps that need to be fulfilled by future research.

In this context, the working group has chosen to focus the discussion and recommendations mainly on emerging biomarkers assayed by molecular biology techniques (i.e., the gene expression profiling (GEP) test AlloMap and donor-derived cell-free DNA [dd-cfDNA] assays), given their commercial availability as diagnostic tests and the initial use in clinical practice (**Table1**). In addition, two cardiac biomarkers [troponin and B-type natriuretic peptides (BNP)] which have been re-examined in recent studies as to their utility for rejection surveillance in heart transplantation, are discussed.

The hallmark of allograft rejection is immune-mediated cell necrosis. Transplantation introduces genomic admixture with donor and recipient genomes. During allograft rejection, cell-free DNA fragments are released into the recipient's bloodstream from the donor allograft. Leveraging transplant genomic admixture, the dd-cfDNA fraction can be identified with modern genomic techniques, which may serve as a biomarker of allograft injury and rejection. Similarly, in heart transplantation, troponin as marker of injury, and BNP as marker of graft dysfunction, may be detected in the recipient's circulation.

On the other hand, the GEP of circulating peripheral blood mononuclear cells (PBMCs) is thought to reflect host immune responses towards the allograft, which could thus also serve as a biomarker for rejection surveillance.

In order to be useful for accurate rejection surveillance in clinical practice, a biomarker should ideally have the following characteristics: minimally invasive (blood based), quick turn-around time, good inter-sample and inter-laboratory reproducibility, affordable, accessible (in terms of technology and staff requirements), high negative predictive value (NPV) for rejection monitoring, able to categorize common transplant complications, such as acute cellular rejection (ACR), antibody-mediated rejection (AMR) and infection, and not influenced by patient or treatment factors.

In the consensus statements, available evidence on these potential biomarkers is summarized and recommendations are made on the use of non-invasive biomarkers for cardiothoracic transplant rejection surveillance.

METHODS

This consensus document follows a process that has been organized and supervised by a dedicated ESOT guidelines taskforce as outlined in a dedicated guideline [4].

Using the PICO (Population, Intervention, Comparison, Outcome) model, clinical questions were formulated, around which the expert panel's recommendations are focused (**Table 2**). The rationale for the PICO questions is based on the need to provide guidance on three general domains: 1. Diagnosis/surveillance of acute rejection 2. Diagnosis/surveillance of chronic rejection 3. Prognostic stratification.

For each question, bibliographic searches were developed by experienced staff from the working group. Different members of the group drafted each chapter, which was then reviewed by the whole working group. The panel convened on 13–15th November 2022 (in conjunction with

TABLE 1 | Practical considerations in the use of the currently commercially available GEP and dd-cfDNA assays.

Assay	Type of rejection monitoring and diagnostic thresholds	Validation studies	Timing of initiation	Suitable patient populations to be applied to	Caveats
Allomap (CareDx)—peripheral blood mononuclear cells–based 11-gene expression panel (ITGAM, FLT3 and IL1R2 are steroid responsive)	Validated only for ACR monitoring (not for AMR) with score range 0–40 >99% NPV for ACR with the following diagnostic thresholds ≥30 for patients 2–6 months post HT and ≥34 after 6 months post HT	Validated in 2 randomized (IMAGE and eIMAGE) clinical trials and large prospective observational cohort studies (both US and European based- OAR and CARGO II) as non-inferior to EMB for rejection surveillance	Per the IMAGE and eIMAGE studies, eligible patients include those ≥55 days post HT and on <20 mg of daily prednisone dose, and up to 5 years post HT.	-HT recipients >15 years of age with normal graft function (LVEF≥50%) and asymptomatic -No history of AMR≥1 or treated ACR Grade ≥2R -Absence of DSAs -On corticosteroid dose <20 mg/day -Have not received hematopoietic growth factors or blood transfusions during the previous 30 days -Are not pregnant -No history of severe CAV -Absence of CMV infection (both asymptomatic viremia or CMV disease)	-Different test thresholds can be chosen to maximize either sensitivity or specificity per the clinicians' needs -The GEP test has not been validated against intragraft gene expression -Affected by other factors leading to immune activation (steroid dose, infections, leukopenias, etc.) -In the USA, it is processed in centralized laboratories -Adoption of the test in Europe is limited by cost considerations and establishing laboratory infrastructure for testing
Allosure (CareDx), Prospera (Natera) Allonext (Eurofins)—dd-cfDNA assays measuring the fraction of donor derived cfDNA compared to the recipient's cfDNA	Can be applied to both AMR and ACR monitoring >97% NPV for AR with the following diagnostic thresholds Allosure ≥0.20% and Prospera ≥0.15% Allonext >0.15	No randomized controlled trials have tested the non-inferiority of dd-cfDNA-based vs. EMB-based monitoring for AR (upcoming DETECT trial (NCT05081739 will address that – uses Prospera) Validated in 3 large prospective cohort studies conducted in North America (upcoming FreeDNA-CAR (NCT04973943) will compare cdDNA vs. EMB based surveillance in centers in Spain)	In the GRAfT study (uses research-grade assay), patients were enrolled at ≥28 days post HT, in D-OAR (uses Allosure)—>55 days post HT and in DEDUCE (uses Prospera)—≥28 days post HT. Most centers implement dd-cfDNA testing starting 1–3 months post HT Threshold values for HT recipients monitoring >2 years post HT are undefined	-Single HT organ recipients only (not tested in multi-organ transplants) -Exclude pregnant patients -Exclude patients with known malignancy -Dd-cfDNA testing should not be performed within 24 h of EMB -Most cohort studies included subjects at low rejection risk (only a single center substudy of D-OAR included patients at elevated AMR risk)	-Different test thresholds can be chosen to maximize either sensitivity or specificity per the clinicians' needs -Dd-cfDNA elevation is not specific to rejection and the assays cannot discriminate AMR from ACR, hence EMB is needed for diagnosis and to guide therapy -Assays have not been validated in European cohorts of HT recipients -Adoption of this assay in Europe faces many challenges, including cost, creation of local laboratory infrastructure with good inter-laboratory reproducibility, and obtaining approval by local regulatory agencies

the ESOT TLJ 3.0 meeting in Prague and virtually), when a draft of the final recommendations with supporting evidence was presented and discussed, with further subsequent refinements.

Recommendations were graded according to the strength of the recommendation [strong (1) or weak (2)] and the quality of the evidence [high (A), moderate (B), low (C) or very low (D) (2)].

TABLE 2 | Summary of the PICO questions and recommendations.

	PICO question		Recommendation	Level of evidence
1	*In heart transplant patients with stable graft function, are GEP and dd-cfDNA reliable surveillance tools for subclinical acute rejection monitoring, compared to endomyocardial biopsy?*	GEP	Strong for	Moderate
		Cf-ddDNA	Weak for	Low
2	*In heart transplant patients, are dd-cfDNA and GEP reliable methods to monitor for cardiac allograft vasculopathy as compared with standard diagnostic methods?*		Weak against	Very Low
3	*In heart transplant patients with stable graft function, is dd-cfDNA or GEP a reliable marker to stratify prognosis as compared to standard clinical classifiers?*		Weak against	Very Low
4	*In heart transplant patients with stable graft function, are cardiac biomarkers (NT-pro BNP, BNP, troponin) reliable surveillance tools for subclinical acute rejection monitoring, compared to EMB?*	Troponin	Weak neutral	Very Low
		BNP	Weak against	Very Low
5	*Is dd-cfDNA a reliable marker to diagnose/monitor a) clinical and subclinical acute rejection or b) infection of the graft in lung transplant patients, compared with standard diagnostic methods (surveillance bronchoscopy with TBB for histopathology and bronchoalveolar lavage for microbiology testing)?*	5A	Weak for	Low
		5B	Weak for	Very Low
6	*Is dd-cfDNA a reliable therapeutic marker to monitor treatment response for acute rejection or infection of the graft in lung transplant patients, compared with standard diagnostic methods (i.e., follow-up surveillance TBBx)?*		Weak against	Very Low
7	*Is dd-cfDNA a reliable marker to stratify prognosis of lung transplant recipients for chronic lung allograft dysfunction (CLAD), as compared to standard clinical classifiers?*		Weak for	Very Low

Other emerging biomarkers are briefly described below as an overview of the scientific landscape and the pipeline of discovery.

PICO QUESTIONS AND RECOMMENDATIONS

Heart Transplantation

Question 1A. In heart transplant patients with stable graft function, is GEP a reliable surveillance tool for subclinical acute rejection monitoring, compared to endomyocardial biopsy?

Recommendation: Peripheral blood GEP assay (marketed in United States as Allomap®) is a reliable non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk heart transplant recipients >15 years of age who are >55 days post HT.

- Level of evidence—moderate
- Strength of recommendation—Strong for

Warnings: This test is currently unavailable for clinical use in Europe.

Supporting Evidence

The Allomap® test by CareDx Inc., United States, utilizes GEP of PBMCs to reflect host responses towards the target organ (**Table 1**).

Randomized studies have shown non-inferiority of Allomap-based surveillance compared to traditional biopsy-based approaches. The IMAGE study defined an abnormal score as ≥34 for adult heart transplant recipients 6 months to 5 years post-transplant, allowing to substantially reduce the number of surveillance EMBs performed, and the eIMAGE study confirmed Allomap non-inferiority in the earlier post-transplant period (55–185 days) [5, 6]. The main limitation of these studies, however, was the very low number of biopsy-proven AR.

Previously, Allomap had been rigorously validated in large observational studies, including Cardiac Allograft Rejection Gene Expression Observational (CARGO II), which included 499 heart transplant recipients from 17 predominantly European centers, and the Outcomes AlloMap Registry (OAR), which included 1,504 subjects from 35 US centers [7, 8]. Both studies showed non-inferiority of Allomap as compared to EMBs for ACR monitoring up to 5 years post-transplant, with respect to the composite outcome of rejection, graft dysfunction, death or re-transplantation, with robust NPV (>98%) and modest PPV (4%–7% among studies) [7, 8]. The OAR study additionally showed no association between higher Allomap scores and CAV, cancer or non-cytomegalovirus infection [8]. Furthermore, GEP scores did not differ between dual organ and heart alone recipients, but there are no randomized trials testing Allomap's performance in the setting of multi-organ transplantation [8].

Allomap has received endorsement in the 2023 International Society of Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients, where it was given a class IIa, level B recommendation for ACR surveillance [9].

Caveats

The Allomap algorithm was developed about 20 years ago, when the diagnosis and surveillance of AMR was not standard clinical practice. Implementation in Europe has been limited by cost considerations and the need to establish laboratory infrastructure for testing; as such, this test is currently unavailable for clinical use. The strength of recommendation is based on the robustness of evidence of consistent high NPV in two randomized clinical trials, in which, however, ACR was detected in less than 4% of all EMBs.

Question 1B. In heart transplant patients with stable graft function, is dd-cfDNA a reliable surveillance tool for subclinical acute rejection monitoring, compared to endomyocardial biopsy in stable recipients?

Recommendation: Beyond 4 weeks after transplantation, in addition to routine clinical care, dd-cfDNA measurements could be used to rule out clinical and subclinical rejection, given its high NPV.

- Level of evidence: low
- Strength of recommendation: weak for

Warning: Current data are based on centralized laboratory analyses; therefore, caution should be used when using assays performed in local laboratories.

Supporting Evidence

DD-cfDNA assays for rejection surveillance have never been compared head-to-head with EMBs in a randomized clinical trial. The current evidence for their utility in AR monitoring comes primarily from three large cohort studies that were conducted mainly in the United States—the GRAfT study used a research-grade assay, D-OAR used Allosure (CareDx) and the DEDUCE study employed Prospera Heart (Natera). Several dd-cfDNA assays by different vendors have been developed (**Table 1**).

The GRAfT study used a threshold of ≥0.25% and showed that the test had sensitivity 81%, specificity 85%, PPV 19.6%, NPV 99.2% for rejection detection, defined as ACR≥2R and pAMR ≥1 [3]. The study demonstrated that using a dd-cfDNA-based monitoring strategy could have safely avoided 81% of all routine EMBs [3]. Allosure has been validated in a US-based prospective observational cohort study (D-OAR) of 740 heart transplant recipients in the first 2 years post HT(10). At a 0.2% threshold, the test had sensitivity 44%, specificity 80%, PPV 8.9% and NPV 97.1% to differentiate AR from no rejection [10]. The D-OAR study also included a parallel arm single-center cohort of 33 heart transplant recipients at high risk for AMR and showed that the test had similar performance in this group [10].

The DEDUCE study was an observational 2-center study with retrospective and prospective components testing the performance of the Prospera dd-cfDNA assay for AR surveillance [11]. It included 811 samples from 223 heart transplant recipients [11]. Using a proposed threshold of ≥0.15% for the assay, it had sensitivity 79%, specificity 77%, PPV 25% and NPV 97% for AR. In biopsy-matched non-rejection samples the dd-cfDNA fraction was stable up to 24 months post-transplant, and increased after 24 months [11].

The FreeDNA-CAR was an observational study including 206 patients from 12 Spanish transplant centers. By using the Allonext® assay (Eurofins Genome) a threshold of ≥0.15% had a 97% NPV for AR. This study was presented at the ESOT 2023 congress and is not yet available as peer reviewed publication.

Caveats and Unmet Needs

Different dd-cfDNA thresholds have been tested in available studies, ranging from 0.15% to 0.25%. Assay variability, limit of detection, and other characteristics vary between commercially available tests, and the rate of rejection in the study populations may affect the resulting test performance. These test characteristics, in addition, have been determined in studies with centralized laboratory measurements. It is unknown how they might be applicable in clinical practice when the assay is performed in local laboratories.

It must be noted that, as compared to kidney and lung transplantation studies, the dd-cfDNA threshold value is much lower in heart transplantation. An important question which remains unaddressed is whether the accuracy of the dd-cfDNA test, often reported as coefficient of variability, is applicable at different dd-cfDNA thresholds. However, even with constant standard variation, the coefficient of variability of an assay is inversely related to the mean value at different ranges of the data. In addition, variability of assay measures generally increases with lower concentration of the analyte. Thus, a coefficient of variability measured at high dd-cfDNA thresholds used for lung and kidney transplantation may not be applicable in heart transplantation with lower dd-cfDNA thresholds. The coefficient of variability should be computed around all desired thresholds.

Some data suggest that absolute dd-cfDNA quantity may be a better marker than dd-cfDNA fraction, as it is independent of changes in background (recipient) cfDNA levels. In the DEDUCE study, a *post-hoc* analysis using dd-cfDNA quantity indicated that incorporation of this measure could increase the sensitivity of the assay [11].

There is a paucity of data on whether dd-cfDNA assays can be employed for monitoring treatment response during and after AR. Small studies have shown a reduction in cfDNA levels after rejection treatment; however, the assays have not been validated for therapeutic guidance. Additionally, dd-cfDNA levels have been shown to be elevated in patients with *de novo* donor-specific antibodies (DSAs), raising the possibility of identifying pathological DSAs using these assays. However, these preliminary findings are hypothesis-generating and must be verified in large studies.

Question 2. In heart transplant patients, are dd-cfDNA and GEP reliable methods to monitor for cardiac allograft vasculopathy as compared with standard diagnostic methods?

Recommendation: We do not recommend the use of either dd-cfDNA or GEP (Allomap) as surveillance strategies for cardiac allograft vasculopathy post-heart transplantation.

- Level of evidence: very low
- Strength of recommendation: weak against

Supporting Evidence

A small single-center pilot study performed in the US showed that dd-cfDNA is elevated in cardiac allograft vasculopathy (CAV) and suggested endothelial injury and ischemia as possible mechanisms [12]. However, another study from Spain using different dd-cfDNA detection methods and thresholds did not confirm the association between dd-cfDNA and CAV [13].

These divergent findings underscore the need for larger prospective studies to define the role of dd-cfDNA in screening for CAV (the SHORE registry is exploring this question). Similarly, a retrospective study did not support the use of GEP for CAV surveillance [8].

Question 3. In heart transplant patients with stable graft function, is dd-cfDNA or GEP a reliable marker to stratify prognosis as compared to standard clinical classifiers?

Recommendation: We do not suggest the use of dd-cfDNA or GEP to stratify prognosis after heart transplantation, despite several studies showing associations of these biomarkers with long-term clinical events

- Level of evidence: Very low
- Strength of recommendation: Weak against

Supporting Evidence

There are no studies specifically powered and designed for exploring the prognostic role of either GEP or dd-cfDNA in heart transplantation. Major studies on these biomarkers were performed in stable low-risk patients, with very low mortality rates during their limited follow-up (up to 3-year) [7, 14]. Moreover, the few available post-hoc analyses reporting combined clinical outcomes provide contradictory results.

No association has been found between GEP scores and mortality during follow-up in different studies. Two sub-studies of major trials (IMAGE [15] and CARGO II [7]) published by Deng et al in 2014 [16] and Crespo-Leiro et al in 2015 [17], tested the performance of AlloMap™ as a predictor of major adverse cardiac transplant events (MACTE, a composite of acute rejection with hemodynamic compromise, graft dysfunction, death or retransplantation). In both cases, intraindividual variability (standard deviation of ≥4 GEP scores) predicted a higher incidence of MACTE in the next 2-3 years, with a hazard ratio of 1.76 per unit increase in variability in one of the studies [16]. Other ways of measuring repeated individual GEP scores (ordinal score, scores above a given threshold) did not show a similar predictive ability. Moreover, in the OAR study (Moayedi 2019) [8], no meaningful changes in GEP were seen in relation to specific heart transplant complications such as CAV, cancer or non-cytomegalovirus infections.

However, the existence of 2 sub-studies [16, 17] of major GEP trials with reasonably sized populations (369 and 91 patients, respectively) and differing characteristics (one USA-based, the second mainly European) with coincidental findings should not be dismissed to potentially identify GEP score change as a predictor of adverse outcomes. The main limitation of these studies is the need for ≥4 consecutive GEP scores to evaluate variability (standard deviation of all scores).

As for dd-cfDNA, a preliminary study (Zangwill, 2020) [18] focused on the first 10 days after heart transplantation in a small pediatric population showed that a blunted decline of initially elevated dd-cfDNA may be associated with early death. Two other studies found that total cfDNA levels greater than 50 ng/mL were associated with increased risk of major events (composite outcome of cardiac arrest, mechanical circulatory support, death) (Zangwill 2022 [19]), death (Scott, Zangwill 2022 [19, 20]) and treatment for infection [19, 20].

Only one exploratory abstract (Crespo-Leiro, 2017) [21] has been directed to evaluate the prognostic value of dd-cfDNA in stable HT recipients. It included 48 patients and 166 samples from the CARGO-II trial, and showed an association between the median of several individual dd-cfDNA values and subsequent incidence of MACTE (as defined above), $p = 0.02$, AUCOR = 0.77. Other dd-cfDNA measures, such as maximum value, individual measures, or variability of intraindividual measures did not predict MACTE.

Of note, several groups have found clear relationships between "total or nuclear cfDNA" (derived both from recipient and donor tissues) and several near-term events, such as death, cardiac arrest, and need for mechanical circulatory support [19]. Total cfDNA seems to be a marker of more extensive tissue damage, and has demonstrated prognostic value in different ICU patient populations. Total cfDNA elevations have also been seen in patients with infections after heart transplantation [20]. The same is true for sepsis, inflammatory diseases and cancer in non-transplant populations.

Caveats and Unmet Needs

Despite current available data do not support the use of dd-cfDNA as a biomarker predictive for subsequent clinical events, in the GRAfT study [3] dd-cfDNA elevations associated with negative EMB were predictive of subsequent biopsy-proven AR or allograft dysfunction. These findings suggest that asymptomatic dd-cfDNA elevation represents an opportunity for additional testing (e.g., donor-specific antibodies) and early intervention prior to detection of histopathological rejection. Current data do not support use of dd-cfDNA to titrate immunosuppressive medications, but the above preliminary findings suggest that patients with elevated dd-cfDNA in the absence of biopsy-proven rejection may benefit from closer monitoring. It remains to be investigated if intensification of immunosuppression in the setting of elevated dd-cfDNA and absence of histologic rejection could mitigate future episodes of biopsy-proven rejection, graft injury and/or graft dysfunction. On the other hand, we may hypothesize that low dd-cfDNA levels can be used to guide safe weaning of immunosuppression, thus decreasing lifelong risks of infections, malignancies and renal dysfunction, among complications. HeartCare Immuno-optimization in Cardiac Allografts (MOSAIC) (NCT05459181) is one such study aimed to determine whether patients at low risk of acute rejection can safely wean their post-transplant immunosuppressive medications using a combination of tests

that include DSA, histology, donor-derived cell-free DNA (AlloSure), and gene expression profiling (AlloMap). The study is in the planning stages and is designed as an unblinded randomized controlled study of 930 HT recipients enrolled within 2 weeks of HT.

Question 4. In heart transplant patients with stable graft function, are cardiac biomarkers (NT-pro BNP, BNP, troponin) reliable surveillance tools for subclinical acute rejection monitoring, compared to EMB?

Recommendation 4.A: There is inadequate evidence to support the routine use of cardiac troponin (or high-sensitivity troponin) for the diagnosis of AR after heart transplantation, due to conflicting data.

- Level of evidence: very low
- Strength of recommendation: weak neutral

Supporting Evidence

Cardiac troponin (cTn) is the hallmark biomerker of cardiac damage and bears a central role in general cardiology for the diagnosis of acute coronary syndromes and to stratify cardiovascular prognosis. However its role in the setting of heart transplantation is controversial. Myocyte damage is the pathologic hallmark of moderate to severe ACR, so an elevated cTn level would be expected during an episode of ACR, in particular for high-sensitivity assays (hs-cTn) [22–24]. However, the results of different studies are conflicting, with some reporting no association between cTn and EMB-proven ACR [24–26] and others finding that cTn levels [27] were significantly higher in patients with ACR [28–32].

A systematic review with meta-analysis of 27 studies with 1,684 patients confirmed a poor diagnostic accuracy [33].

A systematic review by Fitzsimons et al [34] *showed that cTn assays did not have sufficient specificity to diagnose ACR in place of EMB, but hs-cTn assays may have sufficient sensitivity and negative predictive value to exclude ACR and limit the need for surveillance EMB.*

Caveats and Unmet Needs

Studies about cTn in the diagnosis or surveillance of rejection are mostly small-sized, retrospective and single center, leading to conflicting results. No randomized or prospective observational multicenter studies are available. Nevertheless, given the universal availability and low cost of the assay, and the proven reliability of this biomarker for cardiac injury, it may provide support to the complete clinical evaluation in ruling out acute cardiac injury in stable patients.

Recommendation 4.B: We do not suggest the routine use of natriuretic peptides (BNP, NT-pro BNP) to monitor for subclinical AR in stable heart transplant patients, due to the many clinical factors that can affect BNPs levels.

- Level of evidence: very low
- Strength of recommendation: weak against

Supporting Evidence

Natriuretic peptides (NPs) are hormones produced by the myocardium in response to atrial and ventricular wall stress. BNP and its pro-hormone NT-proBNP are widely used in the diagnosis and prognostic stratification of heart failure patients. These biomarkers are sensitive to treatment and have also been used as surrogate endpoints for drug efficacy. Despite the fact that they are widely studied in the context of heart failure, evidence in the setting of heart transplantation is sparse and of poor quality.

Most observational studies showed that BNP/NT-proBNP levels were significantly higher in patients with graft rejection [32, 35–39]; however, they had low discriminating power to detect clinically significant episodes of rejection. There was a considerable overlap in BNP/NT-proBNP levels in patients with and without significant ACR.

BNP levels are reported to be higher in heart transplant recipients than in the general population, and are sensitive to higher grades of rejection and left ventricular dysfunction [35]. Klingenberg et al observed that changes in BNP levels compared to baseline were more useful, as BNP values could be influenced by patient variables such as sex or renal function, or transplant variables such as post-transplant time [40]. The association of BNP with AR and the usefulness of serial measurements were corroborated by other studies [41–43]. Prior studies have also demonstrated a decrease in NP levels in the first 6 months after transplant, which then reach a plateau [44, 45]. NP levels have further been shown to correlate with allograft dysfunction, cardiac allograft vasculopathy and cardiovascular death [46, 47].

However, other studies have found that BNP levels lack sufficient discriminatory ability to guide the performance of EMBs [48, 49]. In summary, despite initial promising studies, later studies did not find any association between AR episodes and BNP [50] or NT-proBNP [25, 51].

Caveats and Unmet Needs

The low quality of available evidence, the heterogeneity of factors affecting NP levels, and the conflicting results of published studies do not support the use of NPs for non-invasive surveillance of acute rejection. However, high levels of NPs are associated with poor long-term post-transplant prognosis, and in the context of multiparametric clinical evaluation, NP levels may help guide the assessment of graft function in asymptomatic patients.

Lung Transplantation

Question 5. Is dd-cfDNA a reliable marker to diagnose/monitor a) clinical and subclinical acute rejection or b) infection of the graft in lung transplant patients, compared with standard diagnostic methods (surveillance bronchoscopy with TBB for histopathology and bronchoalveolar lavage for microbiology testing)?

Recommendations:

A) Beyond 6 weeks of transplantation, in addition to routine clinical care, dd-cfDNA measurements could be used to rule

TABLE 3 | Caveats regarding interpretation of dd-cfDNA in lung transplant recipients.

- Donor fraction vs. absolute dd-cfDNA levels: no available data in lung transplantation. There is a need for studies to elucidate this point
- Prognostic role of asymptomatic dd-cfDNA elevation: in the GTD, ALARM and GRAfT Studies [11, 15, 16], high dd-cfDNA levels were observed up to 6 months prior to clinically significant events (graft dysfunction, pathological rejection diagnosis, etc.). This represents an opportunity for early diagnosis and treatment. However, no studies have been performed to date to determine the prognostic value of asymptomatic dd-cfDNA elevations
- dd-cfDNA for surveillance of acute rejection treatment response: studies have shown reduction in cfDNA levels with initiation of acute rejection treatment [11, 12, 15, 16]; however, the correlation of the dd-cfDNA trends and treatment response remain undefined
- dd-cfDNA assays are unable to differentiate AMR from ACR and hence, the need for TBBx (+/– more advanced gene expression testing) to determine rejection type, as this guides treatment approach. Fortunately, novel cfDNA approaches show promise, being able to target tissue-specific cfDNA to identify the cells and tissue involvement and/or identify disease molecular pathways. Perhaps these novel cfDNA approaches show improved specificity to differentiate AMR from ACR.
- dd-cfDNA assays are currently processed in central laboratories in the USA with relatively slow turn-around time of up to 72 h (Allosure and Prospera); the adoption of this technology in Europe is limited by cost considerations, regulatory approval by local agencies and the availability of the appropriate equipment and technology at local centers
- dd-cfDNA levels are affected by multi-organ transplants, active malignancy, prior bone marrow transplant, pregnancy, <24 h following an TBBx, and sepsis, for example,

out clinical and subclinical rejection, given its high NPV for rejection diagnosis.

- Level of Evidence = low
- Strength of recommendation = weak for

B) Beyond 6 weeks of transplantation, in addition to routine clinical care, dd-cfDNA measurements could be used to rule out infection.

- Level of Evidence = very low
- Strength of recommendation = weak for

Supporting Evidence

In cohort studies, dd-cfDNA increased with histologically documented ACR and clinical AMR [14, 52–59] The cohort studies reported good test performance of dd-cfDNA with a high NPV to detect rejection. Indeed, levels of dd-cfDNA increased up to 2–4 months prior to the diagnosis of AMR [52, 57].

Some studies reported [14, 54] that dd-cfDNA also increased in patients with infections, while other studies found no correlation [55, 58, 59]. While dd-cfDNA levels were often similar between pathogen positive and pathogen negative timepoints across lung transplant studies, two studies examined the association between the presence of pathogens with or without concomitant infectious symptoms and dd-cfDNA levels (infection was defined as detection of pathogens plus a reduction in pulmonary function test or presence of pulmonary symptoms. The studies showed higher dd-cfDNA levels at infection compared to stable controls or pathogens without signs or symptoms of infection; levels were similar for infection and acute rejection [52, 58].

For patients with serial dd-cfDNA levels and with dd-cfDNA levels <1%, fluctuations, increases in dd-cfDNA from baseline or less are normal [60]. From one multicenter study, monthly dd-cfDNA was used in routine care for surveillance for acute lung allograft dysfunction (ALAD). In total 175 patients were enrolled and followed over 6 months. A 1% dd-cfDNA level was used as a rule out threshold with a sensitivity of 74%, specificity of 88%, a PPV of 43% and NPV of 97% to detect ALAD, a composite endpoint of infection and acute rejection [58].

Optimal dd-cfDNA Thresholds and Relevance

Considerations in selecting a dd-cfDNA threshold as a rule out test include the test characteristic being prioritized (sensitivity, specificity, and PPV and NPV) or whether the patient has a single versus double lung transplant.

In the GRAfT and ALARM Studies, defining acute rejection as histopathology ACR grade ≥2 or histopathology grade 1 plus a reduction in forced expiratory volume in one second (FEV1) by at least 10% or presence of pulmonary symptoms and/or clinical AMR defined by the 2016 International Society for Heart and Lung Transplantation Consensus criteria, a 1% dd-cfDNA threshold showed sensitivity of 74%–77%, specificity of 84%–88%, NPV of 90%–97% and PPV of 43%–64% [52, 58]. In one study, defining acute rejection as only grade 3 and 4 ACR, a 1% dd-cfDNA showed a sensitivity of 100% [53]. These studies did not differentiate clinical from subclinical acute rejection.

In the GRAfT Study, a lower threshold of dd-cfDNA, 0.5%, showed sensitivity of 95%, specificity of 65% and NPV of 96% and a PPV of 64% [3]. In two other studies, a 0.85% or 0.87% threshold showed sensitivity of 76% and 73%, specificity of 53% and 56%, PPV of 34% and 43% and NPV of 84% and 86%, respectively [55, 59].

The optimal dd-cfDNA threshold for detection of acute rejection is lower in single lung (0.54%) vs. double lung transplant (1.1%): differences in dd-cfDNA in single versus double lung transplant is key for the interpretation of dd-cfDNA testing in research and clinical settings [56].

Timing of Initiation of Surveillance With dd-cfDNA Monitoring

We suggest use of dd-cfDNA starting from week 6 of transplantation and until 18–24 months after transplant. In the GRAfT Study, dd-cfDNA levels are high after transplant surgery, followed by a decay to reach low stable levels by week 6. Levels remain stable thereafter and increased beyond 2 years of transplant [52]. Stable, asymptomatic patients, independent of the risk of rejection or infection may be considered for dd-cfDNA assay. Studies thus far include adult transplant patients only.

TABLE 4 | Emerging biomarkers in the heart transplant field.

	Description	The state of the field	Notable studies
MicroRNAs	• small non-coding RNAs • regulate mRNA translation within pathways involved in innate and adaptive immune responses [64] • very stable in the circulation • transported within exosomes, microvesicles, and apoptotic bodies	• various miRNA panels proposed in single center studies—heterogeneity likely reflects different methodologies, patient populations studied [65–67] • not validated in large prospective studies • not available for clinical use • potential for non-invasive discrimination between AMR/ACR and potential target for therapeutic interventions	• Shah et al [68]—largest study to-date with validation in an external set of samples • proposed 12 miRNAs for ACR and 17 miRNAs for AMR monitoring with converted score 0–100 • at a threshold of ≥65, the assay has 86% sensitivity, 76% specificity, and 98% NPV for ACR, and 82% sensitivity, 84% specificity and 97% NPV for AMR
Exosomes	• small extracellular vesicles (EV) released by cells into body fluids (serum, urine, etc.) [67] • modulate immune responses through communication with surface receptors on leukocytes or intracellular delivery of immune mediators	• various exosome panels have been proposed in small and primarily single center studies [67, 69–71] • need for streamlining of the process of exosome isolation and refinement of the surface marker panels • pending validation in large prospective studies • not available for clinical use	• Castellani et al [69] -largest study to date with a training and a validation cohort • used surface marker analysis by multiplex flow cytometry • according to differential EV-marker expression, a diagnostic model was built and validated in an external cohort of patients with accuracy of the model reaching 86.5% [69]
Digital Pathology	• employs computational image analysis using machine learning methodologies [72] • aims to improve EMB grading consistency and sensitivity	• in a multi-center study, computational histological analysis of digitalized EMBs had similar diagnostic concordance as expert pathologists [72]	• Peyster et al [72]—showed that adding deeper phenotyping of biopsy tissue using quantitative multiplexed immunofluorescence techniques improves the diagnostic and prognostic performance of histologic analysis for AR. • Peyster et al [73]- a CAV prediction model was built combining clinical variables with morphological features from digitized EMB samples, and the model accurately identified patients at risk for CAV development years prior to the disease onset.
Intragraft gene expression profiling	2 types of assays • genome-wide microarray analysis of mRNA transcripts (MMDx® Heart) [74]—uses machine learning algorithms to assign samples to 4 archetypes (normal, Amr, ACR, injury); each new sample enriches the reference set, thus propagating the learning and development loop of this technique. Requires fresh EMB sample • restricted gene expression signatures (nCounter®) [75]—allow the exploration of a limited number of transcripts from formalin-fixed paraffin-embedded tissue, thus conducive to longitudinal studies	• development of MMDx®-Heart was initially based on 331 EMB samples and identified 3 archetypes: ACR, AMR and no rejection. The study reported AUCs of 0.78 (no rejection), 0.65 (ACR), and 0.81 (AMR) [76] • the ongoing 13-center INTERHEART study (NCT02670408) continues to validate and refine this system. Proposes that injury is more important prognostically than AR histological grade for the outcomes of long-term graft survival [75] • single center analysis reports 61% concordance among MMDx®, dd-cfDNA and EMB histopathology for AR; 84% agreement reported between EMB and MMDx® [77] • The MMDx® Heart is now commercially available and can be clinically applied • nCounter® is in early stages of development and validation	• the Trifecta-Heart cfDNA-MMDx study (NCT04707872) proposes to calibrate dd-cfDNA levels (using Natera's Prospera® assay) obtained at the time of a for-cause or protocol biopsy against the MMDx measurements as a new proposed gold standard
Cardiac magnetic resonance imaging	• multiparametric tissue and functional characterization -T2 mapping for myocardial edema, pre- and post-gadolinium contrast T1 mapping to quantify extracellular volume fraction [78] • assesses global cardiac structure and function and regional tissue characteristics that can capture patchy areas of AR not detected on EMB [78]	• available for clinical use • earlier studies used older spin echo sequences and showed inconsistent results in the detection of AR; with multi-parametric imaging, CMR has been shown to have good diagnostic performance in small studies and 1 single-center randomized trial [79, 80] • CMR requires high level of technical expertise and T1, T2 values can vary widely with magnet field strength, sequencing protocol used or machine specifications • lack of long-term outcomes data for a CMR-based surveillance protocol	• Anthony et al [81]—cross-sectional observational study showed CMR had sensitivity 93%, specificity 92%, NPV 99% and PPV of 62% for AR • Anthony et al [81] – single center trial that randomized 40 HT recipients at 4 weeks post-transplant to either conventional EMB-based or CMR-based surveillance. The 2 groups had similar rates of ≥2R rejection and mortality at 1yr. CMR-based surveillance led to a substantial reduction in the number of EMBs (by 94%) as well as unplanned hospitalizations

(Continued on following page)

TABLE 4 | (*Continued*) Emerging biomarkers in the heart transplant field.

Description	The state of the field	Notable studies
	• ISHLT 2022 guidelines assign Class IIb, Level of Evidence C recommendation for its use as an adjunct modality in patients with unexplained graft dysfunction and low-grade or absent histologic evidence of rejection on EMB	

ACR, acute cellular rejection; AMR, acute antibody mediated rejection; AR, acute rejection, Cav, cardiac allograft vasculopathy; CMR, cardiac magnetic resonance; EMB, endomyocardial biopsy; HT, heart transplant; ISHLT, the International Society of Heart and Lung Transplantation; MMDx, Molecular Microscope; NPV, negative predictive value; PPV, positive predictive value.

Caveats and Knowledge Gaps

Several caveats and knowledge gaps still exist in the validation process for clinical use of dd-cfDNA in lung transplantation. The major areas of uncertainty are summarized in **Table 3**.

Question 6. Is dd-cfDNA a reliable therapeutic marker to monitor treatment response for acute rejection or infection of the graft in lung transplant patients, compared with standard diagnostic methods (i.e., follow-up surveillance TBBx)?

Recommendation: While dd-cfDNA levels generally decline after treatment for acute rejection or infection is initiated, we currently do not suggest using dd-cfDNA as an indicator of treatment response.

- Level of evidence: very low
- Strength of recommendation: weak against

Supporting Evidence

In observational cohort studies, dd-cfDNA levels increased with detection of acute rejection or infection. The dd-cfDNA levels generally reduced with initiation of treatment. However, the relationship of the post-treatment dd-cfDNA kinetics and treatment response has not been addressed [52, 53, 57, 58].

Caveats and Knowledge Gaps

Carefully designed studies are needed to test if the dynamics of dd-cfDNA trends reflect response to treatment.

Question 3. Is dd-cfDNA a reliable marker to stratify prognosis of lung transplant recipients for chronic lung allograft dysfunction (CLAD), as compared to standard clinical classifiers?

Recommendations:

1. Dd-cfDNA levels and trends in the **early post-transplant period** could be used as a predictive marker for early death and/or CLAD in lung transplant patients.
   - Level of Evidence = very low
   - Level of recommendation = weak for
2. In patients with **primary graft dysfunction (PGD)**, dd-cfDNA levels could be used to predict subsequent risk of CLAD.
   - Level of Evidence = very low
   - Level of recommendation = weak for
3. For patients with **respiratory viral infections**, dd-cfDNA levels at time of infection might be used to predict subsequent risk of CLAD and/or CLAD progression.
   - Level of Evidence = very low
   - Level of recommendation = neutral

Supporting Evidence

In a study combining the GRAfT and GTD cohorts, early post-transplant average dd-cfDNA levels, computed as the mean of at least three dd-cfDNA measurements between day 14 and 90 post-transplant could predict subsequent CLAD. Patients with average dd-cfDNA in the upper tertile showed a 6.6-fold higher risk of early death and/or CLAD and 4 times higher risk of developing AMR as compared to those in the lower tertile. A 1% increase in average dd-cfDNA increased the risk of early death/CLAD by ~40% (HR 95% CI 1·1–1·5, $p = 0·015$) [61]. In a small pilot study, average dd-cfDNA levels were higher for patients who developed CLAD than for patients who did not develop CLAD [61].

From the same two cohorts, dd-cfDNA stratified PGD patients for subsequent risk of CLAD. Patients with PGD and high dd-cfDNA on day 3 of transplant showed increased odds of CLAD compared to patients with PGD and low dd-cfDNA levels [62].

The GRAfT study categorized pathogens based on their known risk of CLAD and showed that high-risk pathogens had higher dd-cfDNA levels at detection compared to low-risk pathogens. In patients with respiratory viral pathogens, dd-cfDNA ≥1% showed 2 times greater rates development of CLAD, CLAD stage progression and/or death, within 1 year of detection of viral pathogen [63].

Caveats and Knowledge Gaps

All the evidence supporting these recommendations are derived from studies performed by the same research group on two cohort of patients. There is a need for well-designed studies to test the prognostic utility of dd-cfDNA levels and trends in lung transplantation with respect to risk stratification.

CONCLUSION

This document provides current evidence on four known and upcoming biomarker assays and their use in rejection surveillance of cardiothoracic transplant recipients. The recommendations are aiming at optimizing clinical practice, patient health and post-

transplant clinical outcome as well as identifying priorities for future research. Dd-cfDNA is the biomarker closest to the clinical applicability *in lieu* of the several observational studies showing a good negative predictive power to rule out rejection. However, the recommendation in favor of its use it is still supported by weak evidence because a prospective randomized study proving the benefit of this biomarker over the standard surveillance approaches is still lacking. An important limitation of dd-cfDNA is its low specificity. However, with this limitation and its high sensitivity, dd-cfDNA can be an ideal biomarker to monitor cardiothoracic transplant patients to rule out acute graft injury. Standard cardiac biomarkers such as troponin and natriuretic peptides cannot be recommended in standard clinical practice for rejection surveillance because of scattered and contradictory data. However, both troponin and natriuretic peptides may have a role in stratifying the prognosis and in identifying patients with subclinical graft dysfunction or injury. Additional biomarkers (**Table 4**) with a potential of being useful in cardiothoracic transplantation, like cfDNA epigenetic analysis and fragmentomics, exosomes, microRNA or multimodal approaches are in the pipeline but will need additional examination before implementation in clinical practice. These upcoming approaches may improve on the low specificity of dd-cfDNA to identify acute rejection phenotypes or other transplant complications.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

KK consulted for Care-Dx; MCL received speakers fees from Care-Dx; LP received lab material from Care-Dx; SB is PI of a Eurofins sponsored study MJB received institutional grant from Eurofins.

All other authors declare no conflict of interest with the topic of current manuscript.

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European Society for Organ Transplantation (ESOT) Consensus Statement on Outcome Measures in Liver Transplantation According to Value-Based Health Care

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Liver transplantation is a highly complex, life-saving, treatment for many patients with advanced liver disease. Liver transplantation requires multidisciplinary teams, system-wide adaptations and significant investment, as well as being an expensive treatment. Several metrics have been proposed to monitor processes and outcomes, however these lack patient focus and do not capture all aspects of the process. Most of the reported outcomes do not capture those outcomes that matter to the patients. Adopting the principles of Value-Based Health Care (VBHC), may provide an opportunity to develop those metrics that matter to patients. In this article, we present a Consensus Statement on Outcome Measures in Liver Transplantation following the principles of VBHC, developed by a dedicated panel of experts under the auspices of the European Society of Organ

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Abbreviations: ATS, access to transplant score; CCI, comprehensive complication index; CET, Centre for Evidence in Transplantation; EASL, European Association for the Study of the Liver; ERCP, endoscopic retrograde cholangiopancreatography; ESOT, European Society of Organ Transplantation; ELITA, European Liver and Intestine Transplant Association; EKITA, European Kidney Transplant Association; EPITA, European Pancreas and Islet Transplant Association; ECTTA, European Cardio Thoracic Transplant Association; ETHAP, European Transplant Allied Healthcare Professionals; ICU, intensive care unit; MELD, model for end-stage liver disease; LT, liver transplantation; NIMHD, national institute minority and health disparity; NODAT, new-onset diabetes mellitus after transplantation; OPTN, Organ Procurement and Transplantation Network; PICO, Population/Intervention/Comparison/Outcome; PREMs, Patient-Reported Experience Measures; PROMs, Patient-reported outcome measures; PTLD, post-transplant lymphoproliferative disorder; QALY, quality-adjusted life year; RCT, randomized-controlled trial; UNOS, United Network for Organ Sharing; VBHC, value-based health care.

Transplantation (ESOT) Guidelines' Taskforce. The overarching goal is to provide a framework to facilitate the development of outcome measures as an initial step to apply the VMC paradigm to liver transplantation.

Keywords: liver transplantation, value-based health care, PROM, wait-list, outcomes ATS

INTRODUCTION

Liver Transplantation (LT) is a complex procedure surgically, medically, and ethically, and by necessity, a highly regulated field. It is expensive in terms of costs and resources but improves the quality and length of life of patients with end-stage liver disease [1]. LT is not a single care episode, but rather a life-long process that includes several sequential steps, from referral, to evaluation, list management, maintenance of fitness for transplantation, surgery, and life-long follow-up, which includes maintenance of patient and graft health, management of immunosuppression and, sometimes, hospitalization and additional surgeries [2]. Each of these steps requires adaptation of the recipient's everyday life and strongly impacts their quality of life and expectations.

LT is an ideal field for application of the Value-Based Health Care (VBHC) approach, but to our knowledge, this has not yet been attempted [3, 4]. A recent systematic review of quality metrics in liver transplantation identified 317 quality metrics condensed into 114 indicators. Measures were focused primarily on safety and effectiveness, but very few addressed equity and patient centeredness [5]. Furthermore, these measures were mostly process indicators. Process indicators are intended as a help to improve outcomes, but do not measure whether the desired outcome is reached. Furthermore, most studies report outcomes in terms of patient and graft survival censored at relatively short intervals after transplantation [6–8] and miss important steps of the transplant journey such as quality of life before and after transplant, death awaiting liver transplant or complications late after transplantation [9–11].

In the early years of liver transplantation, outcomes focused on short term outcomes (such as 1 year post-transplant patient survival, incidence of rejection or in-patient stay). As outcomes have improved, additional metrics have increasingly been introduced to measure the quality of liver transplantation. However, it is worth noting that, in many cases, regulatory bodies still emphasize patient and graft survival [11, 12]. This represents a simplistic, flattened, and one-dimensional description of the highly complex process of liver transplantation.

Therefore, there is a critical need to identify metrics that offer not only those that meet the patient's needs and wishes but also provide a more comprehensive measurement of the quality of the process.

Developing a culture of quality improvement means setting goals, measuring processes and outcomes, developing action plans where indicated, and assessing the impact of any change [13, 14]. The final aim should not be to equal or improve established benchmarks, but rather to develop a system that continually redefines the benchmarks to yield optimal patient care, and increased patient-level value. In this context, a paradigm change in this direction is needed to better realign clinical endpoints to patients' needs and expectations.

Such a change in paradigm can leverage on the model of VBHC. VBHC is about delivering health outcomes that truly matter to patients. Value in healthcare is defined as patient-level outcomes divided by the cost to achieve those outcomes [3]. In essence, it means delivering the best possible outcomes at the right cost and orienting the competition towards increasing the value for the patients. This definition was introduced by Michael Porter and Elizabeth Teisberg in 2006 in a publication that originated the entire field of Value-Based Health Care [13, 15].

The VBHC proposition has been applied to several conditions, hospitals and units and healthcare systems [16–20]. However, a lack of clarity regarding the definition of value has led to divergent approaches. Value, according to Porter and Teisberg, is not synonymous with lower costs or higher revenues, and is more than cost-effectiveness. The numerator of the value ratio are condition-specific outcomes that are important to patients with that condition. The denominator is the total spending for the full cycle of care. Most healthcare quality research focused on process measures, while outcomes vary dramatically and are mostly left unmeasured. VBHC differs from simple quality measurement or improvement in that it assesses the quality of the whole system from the patient's perspective [16].

The ESOT Guidelines Taskforce agreed that survival alone is a limited measure for outcome after LT and considered the need for a re-evaluation of LT outcome endpoints as a clinical and scientific priority for the Society. In particular, the Taskforce acknowledged that there is a need to look for multiple, complementary patient-centered metrics that capture the whole transplant process from a VBHC perspective; such metrics include waitlist outcomes, post-transplant complications, survival and measures of health-related quality of life. These metrics would enhance patient-level decision-making, provide evidence on LT effectiveness, benefits and complications and allow comparisons with alternative therapeutic interventions. In time, such metrics will enable benchmarking and comparison across centers and countries to assess differences and benefits of their respective processes.

Three sets of data are needed to implement a VBHC approach: clinical outcome indicators, patient-reported outcomes, and costs (or resource utilization). As a first step, the Taskforce aimed to reach a consensus on a set of clinical outcomes indicators that assess the whole process of LT from a VBHC perspective. Further work will need to focus on developing a set of patient reported outcomes and costing assessment methods allowing comparison among different healthcare systems and jurisdictions.

METHODS

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections ELITA,

TABLE 1 | Topics and questions formulated by the panel, and authors of the literature review for each topic.

Topic	Questions		Author/s
Waiting list management	In a setting with optimal potential candidate, referral and listing process, which is the best measure to evaluate the quality of waiting list management in a VBHC perspective?		Strazzabosco M
Quality of life after LT	Which is the best tool to measure health-related quality of life, when assessing benefit of liver transplantation?		Neuberger JM
Patient reported outcome and experience measures	What are the unmet needs in defining the critical PROMs and PREMs to be included in liver transplant "core" evaluation and clinical trial design?		Rowe I
Timeframe for outcomes comparison	What is the most appropriate timeframe to describe LT outcomes in a VBHC perspective?		Carbone M
Measures of early postoperative course	Which are the best metrics to describe the quality of early postoperative course?		Polak WG
Measures of late postoperative course	Which are the best metrics to describe the quality of late post-transplant course?		Polak WG
Metrics of the whole transplant journey for outcomes comparison	Which is the best single measure to evaluate the whole LT process from the VBHC perspective?	If estimates of gain in life years or reduction in years lost are not available/calculable, what is the best measure to describe the transplant process from a VBHC perspective?	Cillo U, Carbone M

Expert panel of the Consensus Statement Outcome measures in liver transplantation according to Value-Based Health Care *included: Carbone M, Neuberger JM, Rowe I, Polak WG, Forsberg A, Fondevila C, Mantovani L, Nardi A, Colli A, Rockell K, Schick L, Cristoferi L, Strazzabosco M, Cillo U.*

EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives (**Table 1**).

ESOT selected a panel of experts to use a VBHC approach to develop a proposal for a core set of metrics reflecting the whole process of LT from candidate referral and listing to transplant and post-transplant care.

Only adult, elective liver, first transplantation from deceased donors was considered. Liver transplantation of children, for fulminant hepatic failure and transplants using living donors were not considered because their complexity requires specific sets of measures.

Due to the nature and novelty of the topics treated, and substantial lack of published evidence, the analysis was not developed using the PICO process [21]. Instead, we undertook a systematic review of the published metrics in LT, to select relevant evidence and to draft "good clinical practice recommendations" according to the GRADE definition. A literature search was done by expert staff from the Centre for Evidence in Transplantation (CET) who have expertise in conducting systematic reviews and these reviews were subsequently integrated, when needed, by the working group experts.

The search strategy used was as follows:

1. value based care.mp.
2. value based medicine.mp.
3. value of life/
4. cost-benefit analysis/ec, mt, st
5. Quality-Adjusted Life Years/
6. (quality adjusted adj2 life years).ti,ab.
7. survival benefit.ti,ab.
8. Intention to Treat Analysis/ec, mt, st
9. (life expectancy adj2 gain).ti,ab.
10. QALY.ti,ab.
11. quality metric.ti,ab.
12. or/1–11
13. Models, Statistical/
14. model$.ti,ab.
15. Benchmarking/
16. decision analysis.ti,ab.
17. or/13–16
18. liver transplantation/
19. liver transplant$.ti,ab.
20. 18 or 19
21. 12 and 17 and 20
22. remove duplicates from 21

The search strategy was focused on: systematic reviews, randomised controlled trials, registry analyses, observational prospective and retrospective studies, diagnostic studies, guidelines and official reports from UNOS, and other national transplant agencies, qualitative studies.

Exclusion criteria included: any language other than English; studies published before 1990.

The Transplant Library (TL), Medline and Embase were searched on 29 June 2022. The TL includes all randomised controlled trials and systematic reviews in the field of solid organ transplantation, whether published as full text or in abstract form, sourced mainly from MEDLINE/PubMed and hand-searches of congress proceedings.

After discussion in several virtual meetings, the panel formulated eight questions, that were presented during the ESOT conference held in Prague, Czech Republic, 13–15 November 2022. The response to these questions, presented as statements, were further discussed, modified until the best possible agreement was reached, and then voted by a selected jury. The questions and the final statements are reported and discussed in this manuscript.

RESULTS

Statements will be presented prioritizing those metrics describing the whole transplant process (**Table 2**). The subsequent statements refer to metrics referring to the various transplant

TABLE 2 | Topics and statements with rates of panel agreement.

Topic	Statements	Agreement (%)
Metrics referring to liver transplant as a whole		
Metrics of the whole transplant journey for outcomes comparison	Statement 1.1 From the *patient perspective*, intention to treat (i.e., from the patient listing) *gain in life years*—preferably, *quality of life-adjusted*—enables to describe the transplant process as a whole, since it reflects all the phases of LT from patient listing to the long-term postoperative course and expresses the benefit on alternatives	100
	Statement 1.2 From the point of view of *other transplant stakeholders*, an analysis from the point of transplant may be required. In this case, *gain in quality-adjusted life years*, should be the adopted metric. *Life-years lost* compared with healthy age- and sex-matched subjects provides further information on long term outcomes	
	Statement 2 In the absence of estimates of gain in life years or reduction in years lost, outcomes (for example, mortality or graft loss) should be calculated from the point of listing (i.e., ITT survival), as ITT takes into account multiple phases, i.e., patient selection, waiting list dynamics, allocation and acceptance of organs, and transplant outcome	
Timeframe for outcomes comparison	Statement 3 From the patient's perspective, when assessing the whole transplant journey, the best timeframe for outcomes comparison should be at least 5 years and ideally 10 years, to balance urgency and utility	82
Single transplant phase metrics		
Quality of life after LT	Statement 4 Clinicians and researchers should be encouraged to use a generic instrument to measure quality of life in patients with chronic liver disease and after liver transplantation. Among the generic instruments, the EQ-5D is recommended, since it can be applied to all phases of transplantation, it is readily and freely available and validated across different countries	100
Patient reported outcome and experience measures	Statement 5 A core of validated PROMs, tailored to the sequential phases of the transplant journey, should be co-produced with public and patient involvement. This core of PROMs should be relevant to both clinical trials and routine healthcare	100
Waiting list management	Statement 6.1 In discussing the principles of waiting list management in LT, it is fundamental to underscore the importance of inclusion, diversity, equity	91
	Statement 6.2 Patient-reported experiences including managing expectations, providing appropriate education, responding to patient needs, efficient care, and maintaining communication should be assessed while patients are waiting for liver transplantation	100
	Statement 6.3 Wait list events including mortality, removal for deterioration, removal for improvement, temporary removal and removal for transplant should be recorded. These events should be subsequently processed in a competing risk analysis taking into consideration the centre case mix adjusted at the moment of listing and measuring the ability of the centre to accept higher risk patients.	91
Measures of early postoperative course	Statement 7 The metrics suggested to describe the quality of early postoperative course are early mortality and morbidity - 90 days mortality - 90 days re-transplantation rate - Length of the stay in the ICU - Length of the stay in the hospital - Readmissions rate - Surgical/radiological re-interventions rate - Clavien Dindo and the comprehensive complication index (CCI) - Vascular and biliary complications rate - Infectious complications rate - 1 year mortality	100
Measures of late postoperative course	Statement 8 The metrics suggested to describe the quality of late postoperative course are morbidity and 5 and 10 years survival ITT. When the analysis is extended to the whole transplant journey, an intention to treat approach is mandatory	100

phases following the trajectory of the patient along the liver transplant journey, starting from the time of listing for transplant. In this work we will not discuss the problem of the appropriate indications and timing for referral for LT evaluation.

Metrics Referring to the Transplant as a Whole

Question 1. Which is the best single measure to evaluate the whole LT process, from the VBHC perspective?

Statement 1.1

From the *patient perspective*, intention to treat (i.e., from the time of patient listing), *gain in life years (preferably quality of life adjusted),* best describes the transplant process as a whole, since it reflects all the phases of LT from patient listing to the long-term postoperative course.

(consensus: 100%)

Statement 1.2

From the point of view of *other transplant stakeholders*, an analysis from the point of transplant may be required. If this case, *gain in quality-adjusted life years*, should be the adopted metric. *Life-years lost* compared with healthy age- and sex-matched subjects provides further information on long term outcomes.

(consensus: 100%)

Gain in life-years from the time of transplant, estimated from candidate and graft data, might provide information about the quality-adjusted extra years of life that a given transplant procedure could be expected to provide for a given patient [22, 23]. This metric can also be useful for designing an effective organ allocation system, where offers are prioritized to candidates deemed to have a greater transplant benefit [23]. An additional advantage would be to evaluate candidate-specific treatment options, i.e., using the characteristics of a specific candidate for a range of several possible donor grafts such as grafts from ECD, non-ECD or living donors). This information, along with candidate health status and the likelihood of receiving various types of graft, could be used to make informed decisions about whether to rule out offers of certain types of donor grafts for a specific candidate.

Unmet needs: The development of models estimating the gain in life years and life-years lost would require prospective intent-to-treat studies focused on the comparison between transplant with sex- and age-matched individuals without disease. Studies on cost-effectiveness, and validation between centers and countries are also required. Whether one or two measures are appropriate to evaluate LT process as a whole, rather than a set of metrics that reflect the several layers of complexity of LT, should also be experimentally explored, as the determinant of health and HRQoL in the pre-transplant and post-transplant settings are different.

Question 2. If estimates of gain in life years or reduction in years lost are not available/calculable, what is the best measure to describe the transplant process from a VBHC perspective?

Statement 2

In the absence of estimates of gain in life years or reduction in years lost, outcomes (for example, mortality or graft loss) should be calculated from the point of listing (i.e., ITT survival), as ITT takes into account multiple phases, such as patient selection, waiting list dynamics, allocation and acceptance of organs, and transplant outcome. (See also Statement 4.)

(consensus: 100%)

From a patient perspective, mortality matters whether it happens before or after transplantation. Survival from the point at which a patient is listed for transplant, is important for clinicians, patients and regulators, although robust evidence of its value as compared with survival from transplant is still limited [24]. Emphasis on outcomes from the time of transplantation rather than from the time of listing means ignoring patients who are removed because of deterioration or death on the waiting list.

Paradoxically, transplant survival would be better if an offer of a graft is declined for an ill recipient and the death occurs on the waiting list rather than after transplantation. Furthermore, focusing outcomes from the date of listing rather than that of transplantation means shifting the focus from the procedure to the patient.

Analysis of mortality from listing can be undertaken considering LT as a time dependent therapy. However, this approach has some important drawbacks. For patients, the significance of time on the list is substantially different from time after LT: death on the list is "in competition" with LT while death after LT is not, risk factors could be different and the impact of donor characteristics on patient and graft outcomes can be assessed only for transplanted patients.

Alternatively, Time from listing to LT and Time from LT to death could be analysed separately and then results combined. In the former analysis death on the wait list, removal for deterioration (or, rarely, improvement) and LT should be analysed as competing events, patients who are still actively waiting for a transplant are censored at that time. Any periods of suspension are not included in the waiting time [25].

Thus, for transplanted patients, analysis will focus on time from LT to death or re-LT, and risk factors, including donor characteristics, can be evaluated in the classical framework of survival analysis.

Unmet needs: When analysis of time from listing to LT and time from LT to death is undertaken separately, results in terms of survival probability should be combined. Probability rules can be applied but the most appropriate approach remains an open issue.

Not all patients will be listed at a similar time with respect to their risk of death and death, whether before or after transplantation, may be due to factors unrelated to the disease or its treatment. The impact of these issues should also be considered; corrections can be made using multivariate analysis and competing risks.

Question 3. What is the most appropriate timeframe to describe LT outcomes, from a VBHC perspective?

Statement 3

From the patient's perspective, the best timeframe for outcomes comparison should be at least 5 years and ideally 10 years from the transplant to balance urgency and utility (Consensus: 82%).

While there is little doubt that, from the patient perspective, quality-adjusted long-term survival is the most relevant measure, it is important to keep in mind that, setting the time frame at 1, 3, 5 or 10 years will evaluate different aspects of the procedure and will be impacted by different risk factors. Donor factors, for example, are less important at 10 years than at 1 year. Furthermore, different healthcare professionals are often responsible for patient care at different time points of the transplant journey, thus making collection of consistent data a challenge.

Because 1 year survival exceeds 90% for most transplant indications, 1 year survival has a poor discrimination for center performance and has become more an expectation than a metric of performance. Also, a system focused on short-term outcomes (e.g., within 3 years) may lead centers to avoid higher risk recipients, a situation that undervalues the survival benefit of transplant. A system focused on long-term outcomes may incentivize centers to follow patients for longer periods. Managing the side effects of immunosuppression is key to continued patient health, with potential long-term sequelae of immunosuppression including an increased risk for malignancy, cardiovascular disease, and renal failure.

Moreover, long term outcomes will account for what matters most to patients; and these outcomes include physical function and social adaptation, return to work, mental wellbeing, and overall life satisfaction [26]. Most younger recipients will want to know life expectancy at 20 or 30 years if, for example, planning a family. A counterargument for the use of long-term follow-up is that this may be a poor metric for comparing outcomes of patients at different transplant centers as patients may choose (in countries where this is possible) not to be followed at the center where they underwent LT, and center should not be held accountable for outcomes of patients for whom they are no longer providing primary care. Furthermore, developments in the care of the transplant recipients means that extrapolation of patients grafted 20 years earlier may not be appropriate to patients about to undergo transplantation.

In summary, to provide a more comprehensive vision of the whole transplant procedure, the panel called for an extension of the outcome metrics to 5 and 10 years, but without discarding the outcome measurements currently collected at 1 and 3 years.

Unmet needs: Understanding which timeframe matters more to patients, linked to physical function, social adaptation including return to work, mental wellbeing, and overall life satisfaction should be further explored.

Single Transplant Phase Metrics

Question 4. Which is the best tool to measure health-related quality of life, when assessing the benefit of liver transplantation?

Statement 4

Clinicians and researchers should be encouraged to use a generic and validated instrument to measure quality of life in patients with chronic liver disease and after liver transplantation. Among the generic instruments, the EQ-5D is recommended, since it can be applied to all phases of transplantation, it is readily and freely available and validated across different countries.

(consensus: 100%)

There is often a mismatch between the clinician's assessment of the patient's quality of life and the patient's own assessment. Health-related quality of life is usually assessed using patient questionnaires or instruments [27–34]. These questionnaires should be relevant and acceptable to both patients and the general population and should use simple language, understandable to the patient and be culturally relevant. Questionnaires should also be simple, use as few questions as practicable, and be easily completed in a relatively short period of time. Furthermore, they should be validated in the population under evaluation, and be able to detect changes in health.

The assessments should be started at the time a patient reaches a stage when transplantation becomes an option, and repeated during assessment and at listing, and at agreed dates while awaiting and after transplantation. The European Network for Health Technology Assessment recommended that a generic HRQoL instrument is always used in clinical trials to cover a wide range of possible future uses of the HRQoL data [35]. We propose the use of EQ-5D in this setting as this is applicable in all phases of transplantation and is explicitly linked with health utility for cost-effectiveness analyses although we recognize it is neither specific for patients with liver disease nor after transplantation [36, 37].

Unmet needs: Although there are few published data, clinical experience confirms that areas of concern and their relative importance vary considerably depending on the stage of the patient's journey. For example, concerns over the risks of donated organs are much less relevant post-transplant. Furthermore, it is important to distinguish between how the patient experiences the care they receive and the interactions with the transplant center (see below) from those related directly to the medical aspects of the transplant (such as side-effects of immunosuppressive drugs). This should be taken into consideration when developing specific HRQoL instruments.

Question 5. What are the unmet needs in defining the critical PROMs and PREMs to be included in liver transplant "core" evaluation and clinical trial design?

Statement 5

A core of validated PROMs, tailored to the sequential phases of the transplant journey, should be co-produced with public and patient involvement. This core of PROMs should be relevant to both clinical trials and routine transplant follow-up.

(consensus: 100%)

There is consensus that the involvement of patients in co-producing research and in decision-making about their health and care is of critical importance [38, 39]. Patient-reported measures are the key element to patient-centered VBHC, and by focusing on measuring what matters to the patient, the value of both clinical care and research is increased. Broadly, there are two types of patient-reported measures: patient reported outcome measures (PROMs) and patient reported experience measures (PREMS). PROMs measure the individual's perception of their own outcomes in the broadest sense whereas PREMs measure perceptions on services and the experiences of care [40–42].

The nature of LT, encompassing the patient's journey from the time of registration on the waiting list to long-term post-transplant survival, highlights the need for longitudinal health related quality of life data [43]. However, most studies reported to date are most often cross-sectional analyses and pay little attention to the phase post-transplant [27, 44].

A general framework for the development of PROMs should include information from across the relevant health domains—physical, social, and mental. Furthermore, the core outcome set should include generic measures of health-related quality of life (such as EQ-5D), and disease specific tools or transplant specific tools (such as the Liver Disease QoL questionnaire), along with patient perspective measures that should include measures of symptom distress, illness perceptions and patient empowerment (such as the Brief Illness Perception Questionnaire and the Patient Empowerment Scale) [36]. Inclusion of PREMs such as being taken seriously and listened to, should also be considered to improve the patient experience of LT and compare experiences between different centers and jurisdictions [45].

Unmet needs: The most appropriate tools to measure outcomes and experiences in LT have not been fully defined. ESOT and other organizations, should encourage original research to co-produce patient reported outcome and experience measures applicable to all phases of the transplant journey to holistically assess aspects of care.

Question 6. In a setting with optimal potential candidate, referral and listing process, which is the best measure to evaluate the quality of waiting list management from a VBHC perspective?

Statement 6.1

It is of fundamental importance to underscore the importance of inclusion, diversity, equity in the access to the liver transplant waiting list.

(Consensus: 91%)

Several studies have shown there are important inequities of access to transplant, based on racial and socioeconomic disparities [46, 47]. Inequalities may be due to a number of factors and vary by jurisdiction. There is inequity of access at all stages of the journey. There is also the inequity around insurance, outcome and possibly allocation of organs. ESOT adheres to the principle of health equity, and therefore rejects any limitations driven by socio-economic and racial/ethnic disparities that impact on access to transplantation. UNOS has developed an Access to Transplant Score (ATS) that indicates the likelihood for a waitlist candidate to receive an organ and this integrates with the NIMHD (National Institute Minority and Health Disparity) framework [48].

Unmet needs: an index measuring the existence of disparities in the listing process should be developed at the European level. These should mainly focus on access to the waiting list, but there are also inequities in waiting time and chance of dying on the list. Therefore, these should be measured as well.

Statement 6.2

Patient-reported experiences including managing expectations, providing appropriate education, responding to patient needs, efficient care, and maintaining communication should be assessed while patients are waiting for liver transplantation.

(Consensus: 100%)

The VBHC model requires consideration of the patient's perspective, the clinical outcomes, and the costs. Patients may spend considerable time on the waiting list involving great uncertainty, often after a lengthy and difficult candidacy evaluation [49]. Therefore, the quality of life and the patients' experiences while on the list must be measured and managed and be an important component of the evaluation.

This aspect has not been studied systematically [50, 51]. However, in a recent qualitative study [39], five themes emerged as patient priorities while on the list:

1. *Managing expectations*: most patients feel overwhelmed and want a clear description of the path ahead and how to navigate the process and relate to their healthcare providers. Centres must be respectful of the time involved going through the listing process, which can be substantial.
2. *Providing information*: listed patients remarked that lack of adequate information is a major determinant of anxiety on the waiting list. Information should be person-centred, comprehensive, transparent, relevant, and current.
3. *Responding to patient needs*: patients value highly responsive providers who deliver timely, personalized care able to compensate for eventual inefficiencies of the system.
4. *Executing the plan of care efficiently*: avoid delays, respect the patient's time and avoid further financial burden to the patient.
5. *Maintaining effective interdisciplinary communication and coordination of care.* Patients view coordination of care as an extremely sensitive and important issue.

Unmet needs: Patient-reported experience measures (PREMs) should be co-developed in a collaboration between patients and professionals to the pre-transplant period to enable evaluation and improvement of waiting list.

Statement 6.3

Waiting list events including mortality, permanent removal because of death, deterioration or improvement, temporary removal and removal because of transplant should be recorded both at the center and national levels using a common data base and dictionary. These events should be processed in a competing risk analysis taking into consideration the centre case mix adjusted at the time of listing and measure the ability of the centre to accept higher risk patients. These events should be analysed and published by an independent group with patient and clinical and other input.

(Consensus: 91%)

The OPTN Board of Directors has recently published a briefing paper on how to enhance performance monitoring systems [52]. Although there are variations according to the jurisdiction and allocation system, ideally, a centre should make publicly available each year the number of patients who are removed from the list (because of improvement, deterioration, death) and the number of transplants done each

year, but this should not prevent listing all those in need of a transplant if they fulfil the nationally agreed criteria. From the VBHC point of view, a transplant centre should be evaluated by how efficiently and equitably it provides for the listed patients and fulfils the commitment stipulated at the time of listing. Germane to these concepts would be the adoption of an intention-to-treat analysis when evaluating the transplantation results, as proposed in several of the following statements.

Unmet needs: Development of informatics tools to easily record the above parameters is essential. In the absence of such tools, data collection and recoding become labor-intensive and impacts negatively on already overburdened transplant teams. It was strongly recommended that the data listed above be made public.

Question 7. Which are the best metrics to describe the quality of early postoperative course?

Statement 7

The metrics suggested to describe the quality of the specific fraction of early postoperative course are early mortality and morbidity.

(Consensus: 100%)

There are no single metrics available to describe the quality of early postoperative course after LT. Ninety-day survival is one of the most informative, but to better capture the early post-operative course, the panel suggested adding a few simple but comprehensive set of metrics that are easy to obtain. While some of these metrics are not directly related to the Value Based approach and are not related to the patients' experience, they remain essential to monitor and troubleshoot the process:

- 90 days mortality
- 90 days re-transplantation rate
- Length of the stay in the ICU
- Length of the stay in the hospital
- Readmissions rate (within 6 months)
- Surgical/radiological re-interventions rate (within 6 months)
- Clavien Dindo and the comprehensive complication index, CCI (within 6 months)
- Vascular and biliary complications rate (within 6 months)
- Infectious complications rate (within 6 months)
- 1 year mortality

Unmet needs: Although these metrics are available in most liver transplant centers, there is a need to harmonize their definition and expected values across the jurisdictions. Furthermore, there is a need to develop metrics for measuring the patients' satisfaction with care during the early post-transplant recovery.

Question 8. Which are the best metrics to describe the quality of late post-transplant course?

Statement 8

The metrics suggested to describe the quality of late postoperative course are morbidity and mortality at 5 and 10 years.

(Consensus: 100%)

There are no single metrics available describing the quality of the long-term course after LT. It is suggested to adopt a few simple but comprehensive set of metrics that are easy to obtain, objective, quantifiable, verifiable, and validated such as:

- 5 years risk adjusted patient survival probability from listing for adult elective first liver registrations.
- 10 years risk adjusted patient survival probability from listing for adult elective first liver transplantation.

In addition to survival, morbidities after LT impact significantly on the patient and can be captured and measured as:

- Rate of chronic ductopenic rejection
- Recurrence of initial disease (such as autoimmune, viral, alcohol, steatotic liver disease)
- Rate of chronic renal dysfunction
- Rate of *de novo* diseases (such as systemic hypertension and dyslipidemia)
- Rate of *de novo* T2DM (NODAT)
- Rate of cardiovascular events
- *De novo* malignancies

Unmet needs: There is a need to agree the definition of many of these morbidities (such as what constitutes a relevant cardiovascular event or what degree of chronic renal impairment should be recorded). Informatics tools to easily record the above data are required. In the absence of these aids, data recording becomes too labor-intensive and impacts negatively on the already overburdened transplant teams.

DISCUSSION

Monitoring performance and reporting outcomes after liver transplantation is crucial for several reasons. First, it enables patients to make well-informed decisions about the outcomes, benefits and risks of transplantation. Second, it promotes an effective utilization of resources, including that of donated organs, and provides important feedback to the health authorities. Third, it helps clinicians monitor the process and promptly address issues. Fourth, a life that is gained should also be lived and factors of concern (such as severe symptom distress) that reduces HRQoL should be addressed. Moreover, transparent reporting is necessary to promote fairness and enhance transparency. As a result, multiple metrics have been implemented to promote performance and outcomes in liver transplantation [53].

However, the process of measuring and comparing outcomes after transplantation is intricate, and a single approach or metric cannot provide a comprehensive overview. When employed appropriately, these metrics are highly valuable in promoting the effective utilization of limited resources and facilitate the sharing of best practice. However, if used improperly, such measurements can lead to erroneous or misleading conclusions, foster risk-averse behavior, and hinder innovation and research [54].

Development of adequate metrics in liver transplantation can be daunting, given the variability of clinical situations, organs, jurisdictions, technologies, case mix and predictors. Additionally, different factors, such as characteristics of the donor, recipient, and surgical aspects, may have variable impacts on survival at different points in time. Differences in outcomes can be influenced, at least in part, by variations in case mix rather than variations between or within a specific transplant center. Risk adjustment models aim to account for these variations by incorporating relevant and validated risk-factors. This approach ensures that the risk profile of patients is appropriately considered when assessing outcomes and provides a more accurate evaluation of center performance [55–57].

Publishing transplantation outcomes is positive, but simplistic interpretation and utilization of data can be more detrimental than not publishing analyses, leading to risk-averse behavior, reduced transplant benefits, discouragement of research and a lack of innovation. Furthermore, when a metric become the objective, it stops being a useful metric [58].

In the past, performance monitoring of liver transplantation focused solely on post-transplant outcomes. However, there is now a growing trend towards analyzing outcomes starting from listing, which provides a more comprehensive understanding of the transplant process. This emerging approach is still in its developmental stages, with ongoing efforts to define the most clinically relevant methods of analyzing and presenting the data. It is important to note that analyses should also consider that patients may be listed at different times in relation to their risk of death, and that deaths, whether before or after transplantation, can be caused by factors unrelated to the disease or its treatment [59].

Recognizing these concerns, ESOT brought together an international group of experts, clinicians, researchers, and patient advocates from around the world to engage in rigorous discussions and critical analysis. The aim was to explore alternative outcome measures that provide a more holistic and patient-centered understanding of the transplantation process from a VBHC perspective.

VBHC conceptual approach is rapidly diffusing in most clinical disciplines since it aggregates the different phases of the therapeutic approach in a more comprehensive view of the full therapeutic process. The aim of VBHC is to measure ethical, societal and financial values according to what really matters to the patients. VBMH metrics are engineered to capture the perspective of the patient, and therefore are less granular than the indicators discussed before. We believe that an agreement on how to measure patient-centered value in liver transplantation is urgently needed also to subsequently perform fair benchmarking analysis.

The VBHC approach supports important changes in how patients, clinicians, commissioners, and researchers measure the quality of liver transplantation. These stakeholders have different needs.

Given the absence of published evidence concerning the effectiveness of implementing a Value Based approach, our approach was generated as consensus among experts. The panel formulated eight questions that lead to eight statements. The questions and statements have been further refined during the discussion at the ESOT meeting in Prague in 2022. These questions are formulated along the journey of a patient referred for liver transplant consideration. This is a first step, as VBMH mandates to develop PROMs, PREMs and costs to fully assess the value of the care.

Much work lies ahead, especially in the areas of cost studies and quality of life research. However, we hope that our effort will lay the foundation for implementing a VBHC approach in liver transplantation, addressing the critical need for a comprehensive framework in this field.

Considering that many of the patients have some difficulty understanding health information and navigating the healthcare system, health systems will have to address health literacy [60].

Finally, it should be highlighted that the costs associated for the development and implementation of such programs are not insignificant in terms of both human resources and healthcare funding; however, the benefit in quality of care provided to patients and the subsequent cost savings from prevention of complications, and readmissions, are posed to increase overall value.

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

Involved in the conception or design of the work: UC, MS, MC, and JN. All authors contributed to the article and approved the submitted version.

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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European Society of Organ Transplantation Consensus Statement on Testing for Non-Invasive Diagnosis of Kidney Allograft Rejection

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To address the need for improved biomarkers for kidney transplant rejection, European Society of Organ Transplantation (ESOT) convened a dedicated working group comprised of experts in kidney transplant biomarkers to review literature pertaining to clinical and subclinical acute rejection to develop guidelines in the screening and diagnosis of acute rejection that were subsequently discussed and voted on during the Consensus Conference that took place in person in Prague. The findings and recommendations of the Working Group on Molecular Biomarkers of Kidney Transplant Rejection are presented in this article.

Keywords: biomarkers, kidney transplant, rejection, non-invasive, diagnostics, gene expression, cell-free DNA, urine chemokines

INTRODUCTION

The short- and long-term success of kidney transplants relies on the safe and effective prevention of allograft rejection. Monitoring the alloimmune response to the kidney graft has been done for decades by serial measurements of graft function (non-invasive measuring of serum creatinine) and immunosuppressive drug levels and employing both reactive "for-cause" and systematic "surveillance" allograft biopsies. Monitoring serum creatinine has been demonstrated to be an insensitive and lagging indicator of allograft rejection and injury [1–4] and immunosuppressive drug level monitoring may inform efficacy for groups of patients but is not suited to individual rejection monitoring outside the extremes [5]. Thus, there is a significant unmet need for a more sensitive and specific non-invasive monitoring tool for allograft rejection and the adequacy of immunosuppression that can reduce or eliminate the need for surveillance biopsies and inform the need for indicated biopsies.

The target population for improved non-invasive tests for rejection would include all patients with a functioning kidney transplant. While rates of clinical and subclinical rejection are highest in the first 2 years post-transplant, possibly due to detection bias, patients are always at risk of alloimmune graft injury if they are functionally under-immunosuppressed, regardless of the cause. The non-invasive biomarkers addressed in this review have been introduced into clinical practice around the world in various combinations and at various times throughout the transplant patient journey. The goal of this review is to provide a snapshot of the current published evidence for their use and to provide a roadmap for the future development and implementation of these technologies.

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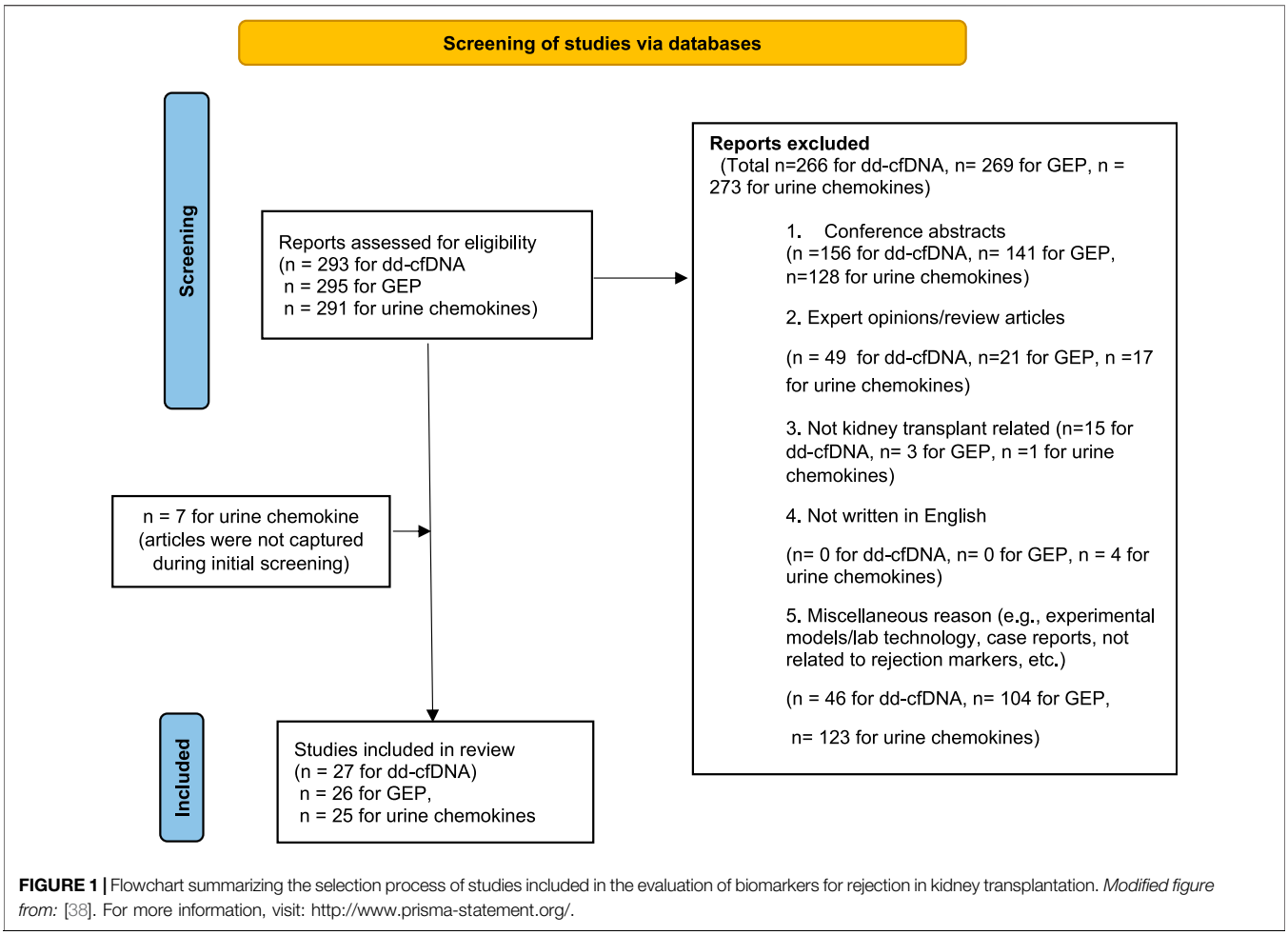

FIGURE 1 | Flowchart summarizing the selection process of studies included in the evaluation of biomarkers for rejection in kidney transplantation. *Modified figure from:* [38]. For more information, visit: http://www.prisma-statement.org/.

To address the need for evidence-based guidelines for the adoption of molecular biomarkers in kidney transplantation, ESOT convened a consensus conference, comprised of a global panel of experts to develop guidelines on key aspects of non-invasive biomarkers of rejection. Summaries of the evidence were presented to the entire group of panelists and juries. The consensus findings and recommendations of the ESOT Consensus guidelines on molecular biology testing for non-invasive diagnosis of kidney allograft rejection are presented in this document. This document, which will be updated to reflect new evidence as it becomes available, is intended for healthcare providers.

METHODS

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives. The detailed description of the methodology used is reported previously [6].

Briefly, key issues related to the topic of non-invasive biomarkers for kidney transplant rejection were identified by each working group and specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome). All PICO questions are listed in subsequent sections of the manuscript. Following the definition of the PICOs, literature searches were developed by expert staff from the CET who have expertise in conducting systematic reviews and subsequently integrated, when needed, by the steering committee experts.

A PRISMA flowchart describing the number of studies identified by the literature search and the number of studies selected for inclusion in the consensus statement appears in **Figure 1**.

A summary of the key evidence addressing each key question by the included studies was prepared in evidence **Tables 1**–**5**. The primary studies are included in these tables. Additional studies reviewed but not included in the manuscript are included in **Supplementary Appendix SA**. The workgroup proposed a recommendation for each key question based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. For evaluation of the quality of evidence according to GRADE [33], the following features were considered: study design, risk of bias,

TABLE 1 | Summary of key literature reviewed on donor-derived cell-free DNA for subclinical rejection.

Authors	Study design	Number of samples	Results
[2]	Retrospective analysis on biorepository samples, Single center	114 biopsies 13 rejections 101 no rejection	For any subAR (AUROC 0.89, PPV 0.55, NPV 0.97)
[7]	Prospective observational (*post hoc* analysis), Multicenter	428 biopsies 103 rejections 325 no rejection	For any subAR (AUROC 0.72, PPV 0.56, NPV 0.84)
[8]	Retrospective cross-sectional, Single center	37 biopsies 10 rejections 27 no rejection	For any subAR (sensitivity 0, specificity 0.89, PPV 0, NPV 0.71)

AUROC, area under the receiver operating characteristic curve; subAR, subclinical rejection; NPV, negative predictive value; PPV, positive predictive value.

TABLE 2 | Summary of key literature reviewed on donor-derived cell-free DNA for clinical acute rejection.

Authors	Study design	Number of samples	Results
[9]	Prospective observational, Multicenter	107 biopsies 27 rejections 80 no rejection	For any rejection (AUROC 0.74, PPV 0.61, NPV 0.84)
[10]	Subgroup analysis of prospective observational, Multicenter	87 patients 16 ABMR 53 no rejection	For ABMR with + DSA and dd-cfDNA >1%, (AUROC 0.86, PPV 0.81, NPV 0.83]
[2]	Retrospective analysis of biorepository samples, Single center	217 biopsies 38 rejections 179 no rejection	For any rejection for cause + SubAR (AUROC 0.87, PPV 0.52, NPV 0.95)
[11]	Prospective observational, Single center	63 biopsies 34 rejections 29 no rejection	For any rejection (AUROC 0.71, PPV 0.77, NPV 0.75)
[12]	Prospective observational, Single center	189 patients 22 rejections 395 stable samples	For any rejection (Absolute concentration of dd-cfDNA (AUROC 0.83) is better than dd-cfDNA (%) (AUROC 0.73)
[13]	Prospective cross-sectional, Multicenter (n = 2)	61 biopsies 13 ABMR 48 no rejection	For ABMR (absolute concentration AUROC 0.91 vs. dd-cfDNA (%) 0.89)
[14]	Subgroup analysis of prospective observational, Multicenter	79 patients with TCMR 1A/ borderline changes	Subjects with TCMR (1A and borderline) with high dd-cfDNA had worse clinical outcomes compared to those with low dd-cfDNA
[15]	Cross-sectional for DSA screening/Retrospective testing of dd-cfDNA on bio-banked samples, Single center	From 2 independent cohort 45/30 biopsies 25/17 ABMR 20/13 no ABMR	For ABMR with +DSA AUROC for dd-cfDNA alone 0.89/0.69 AUROC for DSA alone 0.88/0.77
[3]	Prospective observational, multicenter (ADMIRAL)	219 biopsies 113 rejections 106 no rejection	For any rejection dd-cfDNA (AUROC 0.8, PPV 0.5, NPV 0.9)
[16]	Prospective observational, Single center	208 biopsies 162 rejections by histology 46 no rejection by histology	For any rejection dd-cfDNA and MMDx (AUROC 0.80), dd-cfDNA and histology (AUROC 0.75)
[17]	Prospective observational, multicenter (TRIFECTA)	300 biopsies 120 rejections 180 no rejection	dd-cfDNA levels are strongly associated with the active molecular rejection phenotype (MMDx), particularly with AMR, mixed, and active TCMR
[18]	Prospective observational, multicenter (TRIFECTA)	367 biopsies 83 (histology test set) rejection 71 (MMDx test set) rejection	Any rejection prediction AUROC in test set by logistic regression model using both dd-cfDNA (%) and absolute concentration • 0.88 for MMDx • 0.82 for histologic rejection

AUROC, area under the receiver operating characteristic curve; DSA, donor-specific antibody; MMDx, the molecular microscope diagnostic system; NPV, negative predictive value; PPV, positive predictive value.

inconsistency, indirectness, imprecision, number of patients, effect, importance, and publication bias. The strength of recommendation was rated as 1 (strong) or 2 (weak).

Complete information including the list of consensus conference workgroup domains (and topics noted below), and process regarding consensus conference participant selection, development and refinement of consensus statements, and modified Delphi methodology including consensus polling, are previously reported in beforehand the in-person conference held in Prague, Czech Republic, 13–15 November 2022 [6].

TABLE 3 | Summary of literature review on GEP for clinical and subclinical acute rejection.

Authors	Study design	Biomarker	Number of samples	Results
[4]	Multicenter; multiple biorepository retrospective validation sets	17 gene signatures RNA seq (Tuteva)	237 46 subAR 145 No rejection	For subAR (including BL) vs. No rejection. AUROC 0.83, NPV 0.89; PPV 0.73 For AR (including BL) sets vs. No rejection AUROC 0.81–0.97 (in biorepository validation sets)
[1]	Multicenter prospective, internal validation for discovery and validation sets	57 gene signature Microarray/ qPCR (120 genes) (TruGraf)	382 143 subAR 239 stable	For subAR (including BL) vs. Stable. AUROC 0.85 NPV 0.88/PPV 0.61
[7]	Post-hoc analysis from a prospective observational	Combined TruGraf + dd-cfDNA	408 103 subAR 325 stable	For subAR (including BL) vs. Stable AUROC 0.75, NPV 0.82, PPV 0.47
[19]	Multicenter with external retrospective sample validation	23 gene signature RNA seq (Clarava)	155 For discovery set: 32 AR (cAR + subAR) 49 no rejection	For AR (mostly subAR + cAR including BL) vs. No rejection AUROC 0.74, NPV 0.88, PPV 0.70
[20]	Multicenter prospective, internal validation for discovery and validation sets	13 12-gene signature/RT-PCR fluidigm (kSORT)	558 187 AR (cAR + subAR) 371 No rejection	For AR (subAR and cAR including BL) vs. No rejection AUROC 0.95; Sen 0.92, Spec 0.93
[21]	Multicenter validation cohort	17-gene rt-PCR (kSORT)	1763 188 AR (cAR +subAR) 1575 No rejection	For AR (cAR +subAR including BL) vs. No rejection AUROC 0.51
[22]	Multicenter retrospective, internal validation for discovery and validation sets	5-gene signature RT-PCR/ RNAseq (Allomap kidney)	191 47 AR (cAR +subAR) 146 stable	For AR (cAR +subAR) vs. stable AUROC 0.78 NPV 0.9–0.95, PPV 0.23–0.48
[23]	Multicenter, prospective validation cohort	5-gene signature RT-PCR/ RNA seq (Allomap kidney)	235 66 AR 169 stable	For AR (clinical and subclinical) vs. stable Sen 0.7, Spec 0.66 NPV 0.95
[24]	Multicenter prospective, internal validation for discovery and validation sets	8-gene signature RT-PCR/RNAseq	384 186 ABMR (cAR +subAR) 248 no ABMR	For ABMR (cAR +subAR) vs. no ABMR AUROC 0.80 NPV 0.96, PPV 0.26

ABMR, antibody-mediated rejection; AR, acute rejection; AUROC, area under the receiver operating characteristic curve; BL, borderline; cAR, clinical acute rejection; subAR, subclinical acute rejection; NPV, negative predictive value; PPV, positive predictive value; Sen, sensitivity; Spec, specificity.

TABLE 4 | Summary of key literature reviewed on urine chemokines for subclinical acute rejection.

Authors	Study design	Number of samples	Results
[25]	Retrospective analysis, CXCL10, Single center	102 biopsies 30 subAR 22 normal	For scTCMR (including BL) versus normal (AUROC 0.85; OR 1.41)
[26]	Retrospective analysis, CXCL10, Single center	362 biopsies 119 subAR 243 no rejection	For subAR (including BL) versus no rejection (AUROC 0.69)
[27]	Prospective longitudinal analysis, CXCL9 and CXCL10, Single center	1722 samples 743 biopsies 50 subAR 243 no rejection	For subAR (excluding BL) versus no rejection (CXCL9 AUROC 0.57; CXCL10 AUROC 0.64)
[28]	Retrospective analysis, CXCL9 and CXCL10, Multicenter	373 biopsies 45 subAR 283 no rejection	For subAR (excluding BL) versus no rejection (multiparametric model including CXCL9 and CXLC10 AUROC 0.81)
[29]	Retrospective analysis, CXCL10, Single center	151 biopsies 23 scABMR 15 scTCMR 99 no ABMR 115 no TCMR	For scTCMR versus no rejection (scABMR AUROC 0.80; scTCMR AUROC 0.78)

AUROC, area under the ROC curve; subAR, subclinical rejection; BL, borderline rejection; scABMR, subclinical antibody-mediated rejection; scTCMR, subclinical; T cell-mediated rejection.

TABLE 5 | Summary of literature review on urine chemokines for clinical acute rejection.

Authors	Study design	Number of samples	Results
[25]	Retrospective analysis, CXCL10, Single center	102 biopsies 34 AR 22 normal	For TCMR (cAR + subAR, excluding BL) vs. normal (AUROC 0.87)
[30]	Prospective analysis, CXCL9 and CXCL10, Multicenter	337 biopsies 45 AR 228 no rejection	For AR (cAR + subAR, excluding BL) versus no rejection (CXCL9 AUROC 0.86; CXCL10 AUROC 0.77)
[31]	Retrospective analysis, CXCL9 and CXCL10, Single center	281 biopsies 78 AR 203 no rejection	For clinical AR (excluding subAR and BL) versus no rejection (CXCL9 AUROC 0.71; CXCL10 AUROC 0.76)
[27]	Prospective analysis, CXCL9 and CXCL10, Single center	1722 samples 743 biopsies 60 AR 243 no rejection	For clinical AR (excluding subAR and BL) versus no rejection (CXCL9 AUROC 0.72; CXCL10 AUROC 0.74)
[28]	Retrospective analysis, CXCL9 and CXCL10, Multicenter	373 biopsies 90 AR 283 no rejection	For AR (cAR + subAR, excluding BL) vs. no rejection (multiparametric model including CXCL9 and CXLC10 AUROC 0.85)
[29]	Retrospective analysis, CXCL10, Single center	151 biopsies 52 ABMR 36 TCMR 99 no ABMR 115 no TCMR	For scTCMR versus normal (ABMR AUROC 0.76; TCMR AUROC 0.72)
[32]	Retrospective analysis, CXCL10, Single center	182 biopsies 55 AR 98 no rejection	For late clinical AR (excluding subAR and BL) versus normal (AUROC 0.72)

ABMR, antibody-mediated rejection; AR, acute rejection; AUROC, area under the ROC curve; BL, borderline rejection; cAR, clinical acute rejection; subAR, subclinical acute rejection; TCMR, T cell-mediated rejection.

Overarching Statements From the Working Group

1. The majority of reviewed studies were conducted in adult patients; therefore, our recommendations are most applicable to the adult population. Our group acknowledged, however, that noninvasive biomarkers of rejection would be of great value in the care of pediatric kidney transplant recipients. Thus, we strongly encourage further study and development of these tests in the pediatric population. There are initial studies suggesting the potential utility of such monitoring in pediatric patients [34–36].
2. All of these diagnostic tests are not necessarily alloimmune-specific and thus, may be affected by sources of many other non-alloimmune inflammation such as infections and should be interpreted in that context.
3. Cost-benefit analyses were not considered in the forming of these statements but deserve further study.
4. All of these biomarker tests are available on more than one platform, but a paucity of head-to-head comparisons do not permit specific recommendations for one technique or specific test with a given technology (e.g., cell-free DNA) over another.
5. Most of these tests do not have validated cut-offs to interpret their output in a binary manner (high versus low-risk); therefore, the suggested threshold values should be taken with caution and their interpretation as a continuous variable may further help to translate the biological perturbation into a plausible clinical scenario.

RESULTS

Donor-Derived Cell-Free DNA (dd-cfDNA)

Question 1. In kidney transplant patients with stable graft function, is plasma dd-cfDNA measurement a reliable diagnostic tool for subclinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or surveillance biopsy)?

Recommendation 1.1 - We suggest that clinicians consider measuring serial plasma dd-cfDNA in patients with stable graft function to exclude the presence of *subclinical* antibody-mediated rejection.

Quality of Evidence - Moderate

Strength of Recommendation - Weak in Favor

Comment to Recommendation 1.1

Concomitant testing for donor-specific HLA and non-HLA antibodies along with plasma dd-cfDNA may further increase the ability to detect the presence of antibody-mediated rejection (ABMR). Screening with dd-cfDNA alone does not appear to be a reliable tool for the detection of subclinical T-cell-mediated rejection (TCMR). Combining this test with other non-invasive biomarker technologies (gene expression profiling) may improve the detection of subclinical TCMR. The optimal timing and frequency of screening have not been established.

Question 2. In kidney transplant patients with acute allograft dysfunction, is plasma dd-cfDNA measurement a reliable diagnostic tool for acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or for cause biopsy)?

Recommendation 2.1 - We recommend that clinicians measure plasma dd-cfDNA in patients with acute graft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection.

Quality of Evidence - Moderate.

Strength of Recommendation – Moderate in Favor.

Comment to Recommendation 2.1

Concomitant testing for donor specific HLA and non-HLA antibodies along with plasma dd-cfDNA may further increase the ability to detect the presence of ABMR. Low levels of dd-cfDNA do not necessarily exclude the presence of TCMR in the graft.

Analytical Considerations Regarding dd-cfDNA

Currently, the donor-derived fraction of cell-free DNA is the standard measurement. Some groups have advocated for using both the fraction of dd-cfDNA and the total quantity of dd-cfDNA to improve the detection of clinical acute rejection.

Additionally, all dd-cfDNA assays in the US are currently being run in one of several central/reference labs (currently 3 commercially available assays that vary in the technique and number of single nucleotide polymorphisms analyzed). We recommend further studies to compare the available dd-cfDNA assays head-to-head to better define their performance compared to each other.

Different methodologies involving the assay being run in individual hospital labs used in Europe may require further validation for clinical correlation.

Blood Gene Expression Profiling

Question 3. In kidney transplant patients with stable graft function, is blood gene expression profiling (GEP) a reliable diagnostic tool for subclinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or surveillance biopsy)?

Recommendation 3.1 - We do not yet recommend implementing the use of blood GEP to diagnose or exclude the presence of sub-clinical rejection.

Quality of Evidence – Low to Moderate.

Strength of Recommendation – Weak against.

Comment to Recommendation 3.1

Most of the published studies reviewed focused on using blood GEP in the setting of screening for subclinical rejection. Multiple GEP tests with differential performance were reviewed and detailed in **Table 3**. We strongly advocate the need to develop independent, prospective studies using GEP in stable patients to provide more robust evidence of its value to safely avoid surveillance biopsies.

Question 4. In kidney transplant patients with acute allograft dysfunction, is blood gene expression profiling (GEP) a reliable diagnostic tool for clinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or for cause biopsy)?

Recommendation 4.1 - We do not yet recommend the use of blood GEP to diagnose or exclude the presence of acute graft rejection in patients with acute allograft dysfunction.

Quality of Evidence – Low.

Strength of Recommendation – Weak against.

Comment to Recommendation 4.1

We strongly advocate the need to develop independent, prospective studies using GEP in the setting of graft dysfunction, to provide more robust evidence of its value to safely avoid or inform for-cause biopsies.

Analytical Considerations Regarding Gene Expression Profiling

Multiple research studies have investigated the value of blood GEP in stable patients to diagnose the presence of immune-mediated graft injury, regardless of the type of rejection. The aim of these biomarkers relies on trying to avoid unnecessary kidney allograft biopsies (for cause or for surveillance).

Blood GEP tests are all individual in their performance based on their initial derivation (cohort of patients, context of use), panel of specific genes, and locked classifier algorithm to interpret those genes. Therefore, different gene expression tests cannot be grouped together to analyze their performance.

Some studies have suggested that a combination of biomarkers (GEP with dd-cfDNA or functional cellular assays) may increase their predictive value [7], therefore such studies should be also considered and further validated.

Urinary Chemokines

Question 5. In kidney transplant patients with stable allograft function, is urine chemokine measurement a reliable diagnostic tool for subclinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or surveillance biopsy)?

Recommendation 5.1 - We suggest the monitoring of a combination of urine CXCL9 and CXCL10 in stable patients to exclude subclinical rejection (TCMR or ABMR).

Quality of Evidence – Moderate.

Strength of Recommendation – Weak in Favor.

Comment to Recommendation 5.1

Use of this test in stable patients may help avoid the need for surveillance biopsies.

Question 6. In kidney transplant patients with acute allograft dysfunction, is urinary chemokine measurement a reliable diagnostic tool for clinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or for-cause biopsy)?

Recommendation 6.1 - We recommend the measurement of urinary chemokines CXCL9 and CXCL10 to inform the presence or absence of clinical acute rejection (TCMR or ABMR) in patients with graft dysfunction.

Quality of Evidence – Moderate.

Strength of Recommendation – Moderate in Favor.

Comment to Recommendation 6.1

None.

Analytical Considerations Regarding Urine Chemokine Profiling

Major strengths for urinary chemokine-based tests are the direct link between the biomarker and the underlying pathological mechanism, the reliance on multiple measurements in some longitudinal studies, and across different populations (American, European, Asian). Additionally, urinary chemokines are highly stable in urine samples.

Similar to dd-cfDNA platforms, some limitations for urinary chemokine-based predictions include the variable cutoffs according to different measurement techniques. We recommend further study to compare these tests across different platforms and to develop standardize thresholds.

A first randomized clinical trial by P. Hirt-Minkowski et al. investigating the clinical utility of renal allograft monitoring by urine CXCL10 chemokine was published in January 2023, after the Consensus Conference was held [37]. This study did not address the diagnostic performance of urinary CXCL10 to detect allograft rejection but if biopsies triggered by a limited number of urinary CXCL10 quantifications (at week-4, -10, -22) would impact on a composite endpoint at 1 year post-transplant (death-censored graft loss, clinical rejection between month 1 and 1 year, acute rejection in 1 year surveillance biopsy, chronic active T-cell–mediated rejection in 1 year surveillance biopsy, development of *de novo* donor-specific HLA antibodies, or eGFR <25 mL/min). In this landmark study, the primary composite endpoint was not met, underlining the need for further refinement in the methods and timing of posttransplant monitoring. However, the diagnostic performance of urinary CXCL10 to detect allograft rejection defined by the Banff 2019 classification was studied in an ancillary study and confirmed the diagnostic value of uCXCL10 (ROCAUC 0.73, $p = 0.002$). We believe that this study should provide a positive signal in the field, confirming the feasibility of implementing noninvasive biomarkers and prompting new interventional studies.

SUMMARY AND NEXT STEPS

The development and evolution of non-invasive molecular biomarkers of rejection in kidney transplant patients has started and will continue to revolutionize the care and management of patients. Here we provide a thorough review of the literature supporting these different molecular tests through mid-2022. Despite the number of published studies describing the diagnostic utility of these tests, the field still lacks from adequate perspective, interventional clinical trials demonstrating the value of using these biomarkers in prospective patient management.

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

Involved in the conception or design of the work: SP, JS, CT, DA, OB, and JF. Literature screen and review: SP, JS, CT, DA, OB, and JF. Drafted the article: SP, JS, CT, DA, OB, and JF. Critically revised the article: SP, JS, CT, DA, OB, and JF. Finally approved the version to be published: SP, JS, CT, DA, OB, and JF.

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CONFLICT OF INTEREST

JF receives consulting fees from Eurofins - Transplant Genomics and grant support from Eurofins - Viracor. SP receives grant support from Eurofins - Viracor.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationship 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.2023.12115/full#supplementary-material

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Liver Transplantation for Primary Sclerosing Cholangitis (PSC) With or Without Inflammatory Bowel Disease (IBD)—A European Society of Organ Transplantation (ESOT) Consensus Statement

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Primary sclerosing cholangitis (PSC) is the classical hepatobiliary manifestation of inflammatory bowel disease (IBD) and a lead indication for liver transplantation (LT) in the western world. In this article, we present a Consensus Statement on LT practice, developed by a dedicated Guidelines' Taskforce of the European Society of Organ Transplantation (ESOT). The overarching goal is to provide practical guidance on commonly debated topics, including indications and timing of LT, management of bile duct stenosis in patients on the transplant waiting list, technical aspects of transplantation, immunosuppressive strategies post-transplant, timing and extension of intestinal resection and futility criteria for re-transplantation.

Keywords: liver transplantation, PSC, IBD, immunosuppression, retransplantation

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is an immune-mediated disorder characterized by multi-focal bile duct strictures, progressive cholestatic disease, and heightened lifetime risks of cancer. Given the absence of definitive medical therapy, liver transplantation (LT) is the only life-extending intervention for patients with advanced disease. Disease recurs in approximately one-third of recipients, leading to graft loss and need for re-transplantation.

Although a rare disease, PSC accounts for 10%–15% of liver transplant activity in Europe and North America [1]. Alongside decompensated liver disease, transplantation may be considered for intractable cholestatic pruritus, deep and persistent jaundice and recurrent bacterial cholangitis. In some centers, high-grade biliary dysplasia and early cholangiocarcinoma are also accepted as indications [2].

The dominant clinical presentation of PSC is in association with gut inflammation, with 70%–80% of patients having inflammatory bowel disease (IBD). This relationship has driven several pathogenic hypotheses, in which enteric dysbiosis, dysregulated mucosal immune responses and altered bile acid metabolism are proposed to contribute [3, 4]. Additionally, there is a growing body of evidence that the clinical course of liver disease can be affected by IBD activity; and in turn, the natural history of colitis may be affected by that of PSC [4]. Indeed, data from large volume liver programmes suggest that ongoing intestinal inflammation, an intact colon and antibiotics might influence the clinical course of PSC, both before and after LT.

Although PSC, with and without IBD, is considered a standard indication for LT, many questions remain unanswered. To address these concerns, the European Society of Organ Transplantation (ESOT) convened a dedicated working group comprised of experts in PSC, IBD and LT. The overarching goal was to develop consensus recommendations relating to the:

1. Indication, timing and allocation rules of LT in patients With PSC, with and without IBD
2. Management of bile duct stenosis on the waiting list
3. Surgical aspects of LT
4. Immunosuppressive strategies in patients with PSC-IBD
5. Indication, Timing and extension of intestinal resection (i.e., colectomy) in patients with PSC-IBD
6. Futility criteria with regards re-transplantation

In so doing, the aforementioned topics were discussed in two virtual meetings and voted on during a face-to-face Consensus Conference that took place in person in Prague, 13–15 November 2022. The rationale, literature findings and recommendations from the Working Group on *PSC and IBD in LT setting* are presented in this article.

METHODS

The consensus development process was organized by a dedicated Guidelines' Taskforce within ESOT, and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives. Detailed description of methodology is reported elsewhere [5].

Briefly, key issues related to the topic of *PSC and IBD in LT settings* were identified by the working group and specific clinical questions were formulated and agreed by the working group according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome). Following the definition of the PICOs, literature searches were developed by expert staff from the Centre for Evidence in Transplantation (CET) who have expertise in conducting systematic reviews and subsequently integrated, when needed, by the working group experts. A PRISMA flowchart describing the number of studies identified by the literature search and number of studies selected for inclusion in the consensus statement appears in **Supplementary Figures S1A–L.**

A summary of the selected studies addressing each key question is reported in **Supplementary Tables S1–S5**. The working group proposed recommendations based on the quality of evidence in relation to each question, using the GRADE approach: high quality rated as A, medium quality as B, low quality as C; very low quality as D. For evaluation of the quality of evidence according to GRADE [6], the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance and publication bias (**Table 1**). In GRADE, recommendations can be strong or weak, in favor or against an intervention. Strong recommendations suggest that all or almost all persons would choose that intervention. Weak recommendations imply that there is likely to be an important variation in the decision that informed persons are likely to make (**Table 2**).

The Delphi method was employed to reach a shared consensus among participants during the consensus conference. Complete information including the list of consensus conference workgroup domains (and topics noted below), the process regarding consensus conference participant selection, the development and refinement of consensus statements, and modified Delphi methodology including consensus polling, has been reported prior to the in-person conference held in Praque, Czech Republic, 13–15 November 2022 [5].

RESULTS

1. Indication, Timing and Allocation Rules of LT in Patients With PSC and IBD

Question: Is the MELD-based allocation scheme for organ from deceased donors a disadvantage in terms of waiting-list mortality for patients with PSC?

Recommendation 1.1: MELD should be used for prioritizing patients with PSC on the waiting list for LT. Although not disease-specific, it does not give a disadvantage in terms of waiting-list mortality compared to patients with other etiologies.

TABLE 1 | Quality of evidence (GRADE).

GRADE	Definition
High	We are very confident that the true effect lies close to that of the estimate of the effect
Moderate	We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low	Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very Low	We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

TABLE 2 | Strength of recommendation (GRADE).

Strength of recommendation	Definition
Strong	Desirable effect of intervention clearly outweigh undesirable effects, or clearly do not
Weak	Trade-offs are less certain, either because of low-quality evidence or because evidence suggests desirable and undesirable effects are closely balanced

Quality of Evidence: low

Strength of Recommendation: weak for

Consensus: 88%

Additional comment: A PSC-specific model that captures the clinical burden of PSC more holistically should be developed.

Recommendation 1.2: For PSC-specific complications not reflected by the MELD score (e.g., recurrent cholangitis and/or pruritus), exception points should be considered.

Quality of Evidence: very low

Strength of Recommendation: weak for

Consensus: 92%

The MELD score is used to predict survival in the absence of transplantation. The score has been validated for many liver diseases. MELD (or one of its derivatives) is widely used to prioritize allocation of organs. However, as with any estimation of survival, application to an individual is less precise and allocation systems allow for this in a variety of ways such as awarding additional points for various indications (such as for liver cell cancer) or having a separate category for selected conditions where MELD score does not reflect prognosis or severely impaired quality of life [7].

For patients with PSC, we recommend that transplantation should be considered, irrespective of MELD score in some patients including those with intractable severe pruritus that makes the patient's quality of life unacceptable, and/or recurrent bacterial cholangitis (at least two episodes requiring hospital admission within 1 year).

It should be noted that in many countries and under specific circumstances, individuals with PSC and documented, non-iatrogenic recurrent bacterial cholangitis, do receive additional MELD points and, thereby higher waiting list or allocation priority; even though some reports suggest that transplant candidates with PSC and recurrent cholangitis have no clear increase in mortality risk [8]. This raises the challenge of applying standardized listing procedures to the PSC population both in MELD-based and consensus-based transplant programs.

Several retrospective cohort studies across Europe and US report that PSC patients, while having significantly longer waiting time, have a lower time-dependent risk of death or removal from the waitlist in comparison with patients without PSC [8–10]. Of note, these comparisons were not age-matched (**Supplementary Table S1**).

Perhaps unsurprisingly, PSC patients with MELD Exception (ME) points have a significantly greater probability of undergoing LT than those without [10]. Moreover, the 90 days waiting list mortality in PSC patients is similar to that of individuals listed for chronic hepatitis C virus (HCV), and lower to that of alcohol-related liver disease (ALD) [11]. By contrast, PSC patients are less likely to be removed from transplant waiting lists in MELD-score based allocation programs, as compared to individuals with primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH).

A German study analyzed the temporal effect and found no difference on the WL mortality in the pre-MELD versus the post-MELD era [12]. The mean time on the waiting list increased since introduction of MELD-based allocation from 1.6 to 2.3 years but this difference failed to reach statistical significance ($p = 0.068$). No improvement in means of short-term mortality could be shown in relation to alterations of allocation policy within the MELD era (**Supplementary Table S1**).

Question: Is LT for high-grade dysplasia (HGD) in suspicious strictures in patients with PSC an acceptable indication considering the risk of cancer recurrence?

Recommendation 1.3: Liver transplantation for individuals with PSC and high-grade biliary dysplasia, as confirmed by cytology or ductal histology, and the absence of other transplant indications, can be considered on an individualized basis, taking into account local resources and policies.

Quality of Evidence: very low

Strength of Recommendation: weak for

Consensus: 92%

Additional comment: A recall policy is recommended for those on the waiting list.

High grade dysplasia is a prelude to developing cholangiocarcinoma (CCA), and LT is routinely offered in this situation in some countries, specifically where (a) screening for dysplasia is systematically performed and (b) where the organ shortage is less marked [2, 13, 14]. However, the overall mortality of patients with HGD in explanted liver is similar to those with more benign histopathology (**Supplementary Tables S2**) [15]**.** Moreover, between 20% and 57% of patients who undergo LT for HGD, are not found to have cancer on explant histology, questioning the appropriateness of transplantation in patients with pre-neoplastic changes [2, 16].

Considering that 1) the risk of HGD development in PSC patients is difficult to quantify; and 2) the donor pool is limited in many countries, blanket recommendations of LT for HGD in PSC cannot be made.

2. Management of Patients on the Waiting List

Question: Is the empirical use of prophylactic, rotating antibiotics to prevent recurrent cholangitis in patients with PSC, compared to treatment on demand, a safe approach in LT candidates?

Recommendation 2.1: Rotating antibiotics may be considered to minimize the risks of recurrent cholangitis in selected patients. It is recommended that the use of rotating antibiotics follows biliary cultures and multidisciplinary review, due to the emergent risks of multidrug resistance (MDR).

Quality of Evidence: very low

Strength of Recommendation: weak for

Consensus: 90%

Positive bile cultures (even without clinical infection) are a common finding in patients with PSC. The analysis of bile obtained from liver explants of patients with PSC resulted in positive cultures in 21 out of 36 patients whereas in none of the 14 patients with PBC [17, 18]. Moreover, overt, clinically relevant bacterial cholangitis is a recognized complication, associated with biliary strictures and need for interventional procedures [1]. Biliary infections are often polymicrobial, with *Escherichia coli* being the most frequently identified pathogen. Other pathogens include gram-negative bacteria (e.g., *Klebsiella, Pseudomonas* and *Bacteroides*) and gram-positive bacteria (e.g., *Enterococci* and *Streptococci*) [18, 19]. The selection of antibiotic therapy is generally based on targeted organisms, local epidemiology, drug-resistance, renal and liver function, and severity of infection according to local policy [1]. In addition to antibiotic treatment, current guidelines recommend dilatation of clinically relevant strictures after multidisciplinary assessment [1, 20].

Recurrent episodes of bacterial cholangitis are a widely accepted indication for LT, even in the absence of cirrhosis. Whereas, the use of long-term rotational antibiotics to prevent recurrent bacterial cholangitis (spontaneous or after biliary endoscopy), in the absence of biliary cultures, is controversial; not least given that >25% of cirrhotic patients in Europe may harbor anti-microbial resistant bacteria [21]. Thus, empirical treatment with prophylactic long-term antibiotics should be avoided whenever possible due to a potential risk of furthering antimicrobial resistance. Therefore, this option should only be considered after multidisciplinary assessment in highly selected patients.

Question: In patients with PSC awaiting liver transplant, when is endoscopic biliary treatment, compared to observation only, justified for managing benign strictures?

Recommendation 2.2: ERCP can be considered in patients with clinically relevant strictures and severe symptoms that are likely to improve following biliary intervention. Balloon dilatation should be preferred versus stenting when treating biliary strictures endoscopically in PSC.

Quality of Evidence: moderate

Strength of Recommendation: weak for

Consensus: 100%

PSC patients with an indication for endoscopic intervention should be investigated initially with a high-quality MRI/MRCP [22] and discussed at a hepato-pancreato-biliary multidisciplinary meeting before ERCP is performed [1, 23]. Indications for ERCP in PSC include presence of clinically relevant strictures, sign/symptoms of obstructive cholestasis and/or bacterial cholangitis [1, 23]. There are no studies on the potential benefit or risk of endoscopic intervention in PSC patients on the transplant waiting list.

ERCP (especially with stenting) is a major risk factor for iatrogenic bacterial cholangitis, and peri-procedural antibiotics should be routinely used (EASL-ESGE guidelines) [23]. Decision-making about endoscopic intervention in PSC patients on the LT waiting list is complex and should be individualized.

In the pre-transplant setting, it may not always be obvious to determine whether an elevated or rising serum bilirubin value is caused by loss of liver synthetic function, other factors such as drug toxicity or bile duct strictures. A pragmatic approach to endoscopic treatment on the waiting list is to treat PSC patients with the aim of relieving symptoms, particularly in those with lower MELD scores and expected long waiting times. In individuals with advanced liver disease, ERCP should be reserved for the treatment of unacceptable symptoms, when the benefit is thought to outweigh risk [24]. In waitlisted patients, who have previously been treated with repeated dilatations or stenting, further treatment during the waiting time may be justified following MDT discussion with their transplant center.

Endoscopic intervention of biliary strictures is most useful for well-defined high-grade strictures in the larger bile ducts [23]. Balloon dilatation is treatment of choice when treating biliary strictures endoscopically in PSC, and stenting for benign disease should be avoided due heightened risks of complications without additional benefit [25, 26]. Needless to say, it is always advisable that an experienced biliary endoscopist should perform ERCP in this delicate setting.

3. Technical Issues and Graft Selection

Question: In liver transplant recipients with PSC, is duct-to-duct anastomosis preferred over hepaticojejunostomy as the type of biliary anastomosis?

Recommendation 3.1: The choice of biliary anastomosis is left to operator discretion. However, duct-to-duct anastomosis is recommended as the reconstructive technique of choice whenever technically feasible.

Quality of Evidence: moderate

Strength of Recommendation: strong for

Consensus: 100%

There is a lack of literary consensus on the ideal biliary reconstruction technique in LT of patients with PSC. Historically, hepaticojejunostomy (HJ) was preferred owing to the perceived risk of complications (including recurrent or *de novo* CCA) on the biliary anastomosis in a disease that often involves the extra-hepatic bile ducts. However, the incidence of anastomotic strictures (AS) is similar between HJ and duct-to-duct anastomosis (DD), albeit with a reduced risk of ascending cholangitis with the latter [27]. Moreover, the incidence of cholangiocarcinoma in the remnant BD system, and the 1 year incidence of biliary leaks and anastomotic strictures, does not appear to be different between patient groups stratified by anastomosis type [28]. Perhaps most striking, acute cholangitis episodes within the first year and non-AS (NAS) beyond the first post-transplant year, appear to be more frequent in the HJ group [29–32].

Apart from the above outlined outcomes, duct-to-duct reconstruction confers certain advantages as compared to HJ. It maintains a more 'normal' bile duct anatomy, preserves sphincter of Oddi function, and provides easier endoscopic access to the biliary tree if and when needed. This is of particular relevance in PSC, since 10%–30% of the patients may develop recurrent disease during the first 5–10 post-transplant years [33].

Question: Is the use of extended criteria donors (ECD) acceptable in liver transplantation for PSC?

Recommendation 3.2: Extended criteria liver grafts should be used with caution, considering the risk-benefit balance, given heightened risks of post-transplant biliary complications.

Quality of Evidence: weak

Strength of Recommendation: strong for

Consensus: 80%

Extended criteria grafts, in particular those with high grade steatosis (i.e., >30% macro-steatosis) and grafts from older donors (i.e., >55 years old), represent risk factor for post-transplant complications, including recurrent biliary disease [33–35]. The use of livers donated after circulatory death (DCD) has also been associated with heightened risks of ischemic type biliary strictures [28, 36]. The number of studies is low, and existing reports are heterogeneous in terms of graft types studied and classifications applied. Within DCD groupings, there are differences in procurement protocol, graft quality, and the risks of ischemic damage to bile ducts depending on whether normothermic regional perfusion was utilized. Furthermore, the use of machine perfusion after organ retrieval has been shown to reduce the incidence of NAS, but no study has reported specific outcomes in LT for PSC [37].

4. Immunosuppressive Strategies

Questions: What is the optimal immunosuppression regimen for adult patients transplanted for PSC?

Recommendation 4.1: The optimal immunosuppression regimen must be tailored to the needs of the individual and depends on many factors, in particular the heightened risks of rejection in PSC.

Quality of Evidence: high

Strength of Recommendation: strong for

Consensus: 100%

Recommendation 4.2: As acute rejection is associated with PSC recurrence, it is recommended that patients transplanted for PSC should start on a triple-immunosuppression regimen based on tacrolimus, an anti-proliferative agent and corticosteroids.
As acute cellular rejection may develop also late after transplantation, consideration should be given to maintaining such patients on dual or triple therapy long term.

Quality of Evidence: moderate

Strength of Recommendation: weak for

Consensus: 100%

Recommendation 4.3: We recommend against empirical protocol switching from a tacrolimus-to cyclosporin-based regimen. In transplantation for immune-mediated liver diseases like PSC, the merits of cyclosporin vs. tacrolimus use must be counterbalanced with risks of allograft rejection and acute kidney injury.

Quality of Evidence: low

Strength of Recommendation: weak for

Consensus: 100%

Despite a wide armamentarium of available immunosuppressive therapy, there is no evidence-based accepted immunosuppressive strategy in PSC recipients [38, 39]. This should ideally be tailored to the complication more often encountered in PSC such as early and late acute rejection and recurrent disease [40].

There are many studies evaluating the impact of different immunosuppressive regimens on a variety of outcomes, although very few are randomized, prospective and long term. Additionally, very few studies take into account variations in dose or cumulative levels of medications, and changes in regimen over time. Therefore, any conclusions drawn from studies looking at outcomes related to immunosuppression will need to be cautious and limited.

Cyclosporin (CyA) has shown a marginal benefit on recurrent PSC (rPSC) compared to tacrolimus (Tac). However, this has been attributed to a "era" effect rather than a pharmacological one [33]. Considering that early and late acute rejection has been widely associated with rPSC [41], the priority in patients transplanted for PSC should be to avoid early acute rejection

through a triple-immunosuppression regimen (ideally Tac-based) and late acute rejection on dual therapy [42, 43].

The inferiority of azathioprine (AZT) over mycophenolate mofetil (MMF) on overall survival has been suggested in some studies [44], although not confirmed in follow-up studies [40, 45].

The impact of immunosuppressive regimen on the IBD activity add another layer of complexity to manage PSC patients and this will be discussed in the next paragraph.

5. Management of IBD Before and After Liver Transplant for PSC

Question: What is the optimum (safety/efficacy) therapeutic approach for maintaining remission in IBD associated with PSC pre-, peri- and post-LT?

Recommendation 5.1A: In patients on antimetabolites, Azathioprine is favoured over mycophenolate post-LT, as maintenance therapy for PSC-associated colitis

Quality of Evidence: moderate

Strength of Recommendation: strong for

Consensus: 93%

5.1B: Anti-TNFα therapy should be used with caution in patients with a history of bacterial acute cholangitis

Quality of Evidence: moderate

Strength of Recommendation: strong for

Consensus: 100%

5.1C: Anti-TNFα therapy may be administered post-LT alongside CNI, provided that antimetabolites (AZT/MMF) have been stopped.

Quality of Evidence: Very low;

Strength of Recommendation: strong for

Consensus: 100%

No randomized controlled clinical trials, specifically to attenuate IBD activity in PSC have been found. Thus, clinical data are limited to largely retrospective case series and observational cohort studies. Persistent inflammatory activity pre-transplant can affect IBD behavior post LT, with a 3-fold greater risk of acute colitis "flares." Among transplant recipients, the cumulative probability of deterioration in colitis activity at 10 years is estimated to range between 25.5% and 40%, despite ongoing use of anti-rejection/immunosuppression [46–48]. All efforts to attain mucosal healing in PSC should be pursued, particularly for patients with evidence of progressive liver disease over time that will ultimately require re-LT. This is particularly relevant given that a) PSC is invariably a progressive liver disease, b) LT is the only life-extending intervention for PSC patients, and c) ongoing IBD activity is associated with a heightened risk of peri- and post-transplant complications including hepatic artery thrombosis, rPSC and overall rates of graft loss.

European and American guidelines [1, 23] recommend that 5-ASAs may be used in the pre- and post-transplantation period for the induction and maintenance of remission in IBD associated with PSC. Corticosteroids may be used for the induction of remission in PSC-associated IBD, and as a bridge to escalating treatment.

Thiopurines, principally azathioprine (AZT), can be used to maintain remission from IBD pre- and post-transplantation, and does not adversely affect post-operative outcomes nor the risks of PSC-associated cancers [48–51]. Whilst differences in ciclosporin vs. tacrolimus have been suggested, they may reflect an era effect in transplant practice, which is less apparent for azathioprine vs. mycophenolate treatment paradigms.

Given its comparative safety profile and limited off-target effects, retrospective studies favoring the anti-a4b7 agent, vedolizumab, have also been assessed [52]. In a multicenter cohort of 16 and 14 PSC patients with Crohn's disease and UC, respectively, with a median follow-up of 9 months, clinical remission was evident in 29% (PSC-UC) and 55% (PSC-Crohn's disease) of patients following 30 weeks of therapy. A systematic review of vedolizumab use among liver transplant recipients (eight studies) indicates greater response rates than pre-transplant studies, with 20/27 patients reporting clinical improvement over a mean follow-up of 5–20 months. However, seven/31 patients experienced an infectious event after a mean-time vedolizumab exposure of 11.4 months [52].

The two most commonly used anti-TNFα agents are infliximab and adalimumab. Safety outcomes in relation to biologics mostly concern opportunistic infections, particularly when used in combination with other immunosuppressive agents [46, 53–55]. Pre-transplant data also indicates a sevenfold heightened risk of developing acute cholangitis with anti-TNFα agents (compared to no anti-TNFα treatment) [56]. Pragmatically, there is rationale from a safety point of view to minimize immunosuppressive burden among transplant recipients commencing anti-TNFα therapy, whilst balancing the risks of allograft rejection and recurrent disease. For instance, this may include cessation of corticosteroids and antimetabolites agents in patients who are being treated with calcineurin inhibitors and anti-TNFα therapy simultaneously. At present, there is no published data studying the safety and efficacy of newer biological agents post-transplant such as those directed toward Janus Kinase and/or IL12/23.

Several retrospective studies have shown the use of tacrolimus was associated with progression of IBD and increased risk of *de novo* IBD post-transplant [57]. In the absence of robust evidence, we cannot provide any recommendation on the CNI regimen concerning IBD activity.

Question: Which individuals with PSC-associated colitis should undergo (sub/total) colectomy?

Recommendation 5.2: We recommend (sub/total) colectomy in the following situations, among patients who are fit for surgery:

5.2A) Resectable colorectal cancer/neoplasia

Quality of Evidence: high

Strength of Recommendation: strong

Consensus: 100%

5.2B) High grade colonic dysplasia

Quality of Evidence: high

Strength of Recommendation: strong

Consensus: 100%

5.2C) Low grade dysplastic lesions with high-risk features (e.g., flat/invisible lesions) or multi-focal (synchronous or metachronous) low-grade dysplastic lesions

Quality of Evidence: low

Strength of Recommendation: strong

Consensus: 93%

5.2D) Fulminant colitis

Quality of Evidence: high

Strength of Recommendation: strong

Consensus: 100%

5.2E) Active colitis-refractory to medical therapy

Quality of Evidence: high

Strength of Recommendation: strong

Consensus: 100%

5.2F) Evidence of progressive liver disease (albeit well-compensated) and persistent colitis despite 5ASAs, AZTs (thiopurines) and a single biological agent

Quality of Evidence: low

Strength of Recommendation: strong

Consensus: 93%

Patients with PSC-associated ulcerative colitis harbor heightened lifetime risks of colonic dysplasia and colorectal cancer (CRC), as compared to their age- and sex-matched counterparts with UC alone, and against the general population [58–62]. Moreover, the majority of cancers tend to develop in the proximal colon [63, 64]. Of note, colorectal cancer is among the leading causes of death in patients with PSC-IBD [58, 59]. Risks persist after LT [65, 66], with an estimated CRC incidence rate of 5.8–13.5 per 1,000 patient years [47].

The risk of progression of low-grade dysplasia (LGD) in PSC-associated colitis is not fully quantified. It is likely that progression occurs within the first year of initial detection of LGD, and that flat lesions possess the greatest risk [67], similar to the background IBD population [68]. Thus, international guidelines prompt consideration of surgery (colectomy) with curative intent in patients with colitis and flat LGD, any degree of HGD, and in those with overt neoplasia that is deemed resectable provided patient fitness/comorbidities allow [69, 70].

In addition to CRC risk, colitis activity refractory to medical treatment is the commonest indication for colonic resection in PSC patients [58, 71–73]. It is generally accepted that the definition of fulminant colitis is similar in PSC-associated colitis and in UC alone—the indication for colectomy herein is rarely debated [74, 75]. However, for patients with steroid-dependent or steroid refractory chronic colitis, there is lack of consensus as to what stage colectomy should be performed.

As PSC is an invariably progressive disease, with LT being the only life-extending intervention, there is premise for adopting a lower threshold with regards colonic resection in these patients compared to those with IBD alone. In fact, colitis refractory to single (maximum two) biological agents warrants referral to (or at least discussion with) colorectal surgery. This is relevant given 1) the risks of colonic resection in patients with cirrhosis and portal hypertension, 2) the risks of multivisceral surgery (colectomy at the time of LT), and 3) the impact of persistent colitis activity on peri-/post-transplant complications (e.g., hepatic artery thrombosis) [76, 77].

Question: What is the optimal timing of (sub/total) colectomy for non-oncology indication?

Recommendation 5.3: We recommend that subtotal colectomy for non-oncology indication is performed for patients who have an indication (see recommendations 5.2E above) prior to the onset of advanced liver disease. This is to specifically minimize future risks of native liver decompensation (in patients who develop cirrhosis), post-LT recurrent disease, and graft loss post-LT

Quality of Evidence: moderate

Strength of Recommendation: strong for

Consensus: 93%

There are no comparative data stratifying the benefits vs. risks of colectomy according to the extent of ductal disease involvement, liver disease stage or the risk of disease recurrence. Nevertheless, data from chronic liver disease cohorts (including patients with PSC) highlight significant peri- and post-operative mortality following colectomy among patients with advanced liver disease compared to those with earlier stages (detailed in later sections, below) [78, 79].

Early studies showed that patients with more aggressive PSC liver disease requiring LT had a milder clinical course of IBD, with less need of colectomy pre-transplant [80, 81]. Reciprocally, patients in need of colectomy due to severe colitis can manifest less severe features of PSC liver disease [82].

A systematic review and metanalysis of seven studies post-colectomy, estimated a 2.11% per year overall mortality risk among patients with PSC, unstratified for indication and severity of liver disease [83]. Two studies directly compared colectomy vs. no colectomy groups and showed no difference in overall mortality across all evaluated time points (15.3% vs. 11.8% at 3 years in one study; and 17.4% vs. 20.4% over a median follow-up time of 5.9 years in another) [84, 85]. However, risk-stratified survival analysis of matched patient groups, who met

indications for colectomy and underwent resection, versus those who met indications but did not have surgery, has not been performed.

The impact of colectomy on PSC-prognosis has been reported from a study of 45 PSC-IBD patients in whom colectomy did not affect liver function [84]. Other small studies, not primarily designed to investigate the effect of colectomy on PSC-prognosis, concluded that colectomy had no impact on liver-related prognosis [86–88]. However, emerging data from the pediatric literature indicates that late-onset colitis (>6 months following PSC diagnosis) is associated with higher rates of clinically significant portal hypertension [5/11 (45%) vs. 3/26 (12%); $p = 0.007$] and LT [5/11 (45%) vs. 2/26 (8%]; $p = 0.02$) over a median follow-up duration of 54 months [89]. Moreover, nationwide data from Sweden (N = 2,594) shows that very early colectomy (prior to, or close to the onset of PSC) is associated with a lower risk of LT/death (hazard ratio: 0.71, 0.53–0.95), with a 5 and 10 years incidence of 14.0% and 25.5%, respectively. This was as compared to 20.7% and 33.0% among those without colectomy [85].

At present, there are no data to support routine pre-vs. post-transplant colectomy timings, with regards the safety and efficacy of the colonic resection procedure itself. However, patients with advanced liver disease (i.e., cirrhosis) carry a greater risk of morbidity and mortality following any operation.

Presently, there are no data to support the empirical use of transjugular intrahepatic portosystemic shunts (TIPSS) to mitigate peri-/post-operative risk among patients with cirrhosis. In fact, data from a single retrospective study showed a heightened risk of complications among PSC patients undergoing TIPSS prior to colectomy (greater proportion with wound infections and wound dehiscence, longer hospital stays: 5 days vs. 8 days, and higher readmission rates) [90].

There is limited literature available comparing outcomes related to pre-vs. post-liver transplant colectomy, or to suggest the optimal timing of colonic resection post-transplant. Poritz et al. suggest that patients with PSC who require colectomy may undergo simultaneous LT and total abdominal colectomy [71], and other investigators have described this approach across their own respective practices [33, 57, 65].

Question: How does the type of colectomy (i.e., restorative vs. non-restorative/ileal pouch-anal anastomosis vs. ileostomy alone) affect liver outcomes?

Recommendation 5.4: When colectomy is indicated, it is imperative to provide patients with comprehensive counseling regarding their choice of restorative surgery. Patients should be empowered to weigh the benefits of avoiding a stoma against the increased risks associated with ileal pouch-anal anastomosis, including graft loss, non-anastomotic biliary stricture, and hepatic artery thrombosis. Additionally, patients should be informed about potential implications on their quality of life, as well as the heightened risks of acute pouchitis and pouch failure.

Quality of Evidence: moderate

Strength of Recommendation: strong for

Consensus: 86%

Data linking the type of colonic resection and liver-related outcomes are largely descriptive, with few comparative studies. Whilst the failure rate of ileal pouch-anal anastomosis (IPAA) and ileo-rectal anastomosis (IRA) in PSC-IBD may be no different to that of UC alone [91], the cumulative incidence of acute pouchitis (31% vs. 14% at 10 years), overall pouch related dysfunction (Oresland score: 7.7 vs. 5.4) and poor nocturnal pouch function is significantly greater in patients with PSC [92, 93]. Additionally, patients with large duct PSC and an IPAA exhibit a markedly lower quality of life compared to individuals with UC alone and an IPAA.

Epidemiological data from the Netherlands show how patients that undergo colectomy and retain a permanent ileostomy are at a significantly lower risk of needing a liver transplant/dying over time [HR 0.47 (0.24–0.93)] compared to patients without colectomy. In turn, sensitivity analysis shows no beneficial effect for colectomy with a pouch (HR 0.95, 0.62–1.44) [94] (No full publication, data in abstract version).

Very early studies suggest that approximately 50% of patients who undergo colonic resection may be at risk of developing ileostomal varices [95]. However, contemporary data are lacking, and there is no validating evidence to indicate such high risks in non-cirrhotic PSC.

In the post-transplant setting, there appears to be a significant difference in the incidence of graft loss between patient groups with an IPAA, end-ileostomy and those without a colectomy, with data from one large-volume center (n = 240) showing 10 years graft survival rates of 70%, 95% and 88%, respectively, p = 0.038 [96]. These differences were seen to persist on sub-analysis of patients undergoing colonic resection pre-transplant. With regards graft-related complications, the rate of hepatic artery thrombosis was also elevated in the IPAA group by more than 4-fold compared to the end ileostomy group; whereas end-ileostomy appeared to have a protective effect including against non-anastomotic biliary stricturing disease.

In conclusion, colectomy and retention of an end ileostomy is associated with lower risks of: 1) disease progression in the native liver compared to those having a restorative IPAA; 2) graft loss; 3) non-anastomotic biliary stricturing; 4)hepatic artery thrombosis compared to IPAA and no colectomy. Patients undergoing colectomy should be counselled about the risks of IPAA with regards to quality of life, acute pouchitis, pouch failure and liver/graft-related outcomes.

6. Post Transplant Course

Question: Are there criteria of futility for re-LT in case of rPSC?

Recommendation 6.1: Patients with recurrent PSC and graft failure can be offered re-transplant, if expected patient's survival is more than 50% at 5 years, taking in consideration local waiting list mortality and surgical issues.

Quality of Evidence: very low

Strength of Recommendation: strong for

Consensus: 100%

Re-transplantation in rPSC is controversial, because of the historical lower patient and graft survival rates compared with primary transplantation, due to surgical challenges and septic complications. This raises ethical concerns on utility and equity in the use of a scarce resource (liver organ) for a disease that will tend to recur, sometimes more than once.

Several studies have explored the impact of rPSC on patient survival showing conflicting results [97–103]. This might be related to the different study design and study limitations, e.g., small sample size, short follow-up time, single vs. combined endpoints used, selection bias in patient selection. In some studies, the evidence of recurrence was not included as time-varying covariate, therefore disregarding the impact of survived time until rPSC development on the overall.

A recent analysis of the ELTR data, on 1,549 patients undergoing LT for PSC over a period of 35 years (1980–2015), reported graft survival (including re-transplants) at 1, 5, 10 and 20 years of 80%, 70%, 60% and 41%, respectively. This survival rate is far superior to the expectation of at least 50% at 5 years that has been proposed by the transplant community as a minimum threshold to avoid futility [104]. The rate of rPSC was 17%, including re-transplants, after a median of 5.1 years. Authors reported a negative impact of rPSC on patient survival (HR = 2.3) independent of other transplant related co-variates. Patients with rPSC underwent significantly more re-transplants than those without rPSC (OR 3.6). Notably, patients affected by rPSC did benefit from re-transplantation, showing a patient survival similar to that of patients without rPSC but re-transplanted for other causes. Moreover, in patients with and without rPSC, 5 years graft survival for second graft was noted to be 77% vs. 79%, with no difference in patient survival.

Similar results come from the analysis of the UNOS/OPTN database of 5,080 PSC patients who received LT in the US [105]. Recipients of re-LT for rPSC were more likely to be in the ICU or on mechanical ventilation at LT, and they also had a greater degree of hepatic and renal dysfunction. However, their outcomes were similar at 5 years. Furthermore, the majority of wait-list deaths from rPSC occurred within 6 months, highlighting the risk of not receiving re-LT. Putting together these data, considering the favorable post-re-LT outcomes and the high proportion of waitlist mortalities occurring soon after relisting, support the consideration of re-LT in patients with rPSC.

Patients who undergo a second liver transplant for rPSC have similar graft and patient survival than those transplanted for other causes.

An important caveat to this statement though is that the patients included in this analysis were likely highly selected to undergo re-LT for their favorable pre-LT characteristics

While these data are based on the largest multicenter study on rPSC post-transplant, granular patient data, such as imaging and biopsy, were only available for a minority (approximately one-third of all the transplant center included in the ELTR and not available in the UNOS/OPTN database). Conclusions are limited by several factors inherent with retrospective review of a large administrative database, including missing, incomplete, or potentially inaccurate data.

At the time being, based on a pure needs and outcomes standpoint, it seems reasonable to continue offering re-transplant to patients with rPSC until further prospective studies demonstrate otherwise.

LIVER TRANSPLANTATION FOR CHILDREN WITH PSC AND IBD

Although PSC in adults shares many features with the same condition in children, some clinicopathological features may differ at pediatric age, including rate of progression, severity of pruritus, or development of biliary strictures and malignancies. In pediatrics, the diagnosis typically occurs in the second decade, and most children do not require a LT in childhood. Alongside, the risk of cholangiocarcinoma is very low before 18 years of age. The pediatric studies on PSC are scarce and their quality of evidence remains limited [106–109]. Furthermore, the balance between the existing data and clinical impact of recommended interventions could vary at different ages. For instance, re-transplantation is usually not controversial for children with recurrent PSC in a failing graft. Similarly, suggesting colectomy with a permanent ileal-pouch has very different social implications in children compared to the adults. For these reasons the recommendations produced for the adult patients have been largely supported by the pediatric co-authors when applicable but the guidance from this document should be tailored to the individual patients following multidisciplinary input and discussion.

SUMMARY AND NEXT STEPS

No therapies have proved to cure PSC or slow down disease progression and most patients ultimately require LT. Transplantation faces several challenges in PSC, from the fairness of the extra-MELD indications, the donor selection and the technical issues, to the disease recurrence with risk of graft loss. The association between IBD and recurrence, underscores the interplay between the bowel and the liver in PSC patients.

The systematic literature review undertaken for these recommendations, highlighted for many of the topics a low-quality level of evidence and statements were often based on clinical expertise. Prospective clinical studies on the debated topics are urgently needed.

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

Literature screen and review: MC, ED, AB, P-DL, JN, PT, LC, DD, LD'A, BF, and UB. Drafted the article: MC, AD, CM, ED, AB, P-DL, JN, PT, LB, and SN. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

MC has received grant support unrelated to this study from Genetics spa, PSC Pediatric Foundation, AMAF, EpaC and AIRCS. He also received speakers/consultancy/advisory fees for GlaxoSmithKline, Dr. Falk Pharma, Cymabay, Advanz Pharma, Albireo, Ipsen, Mayoly Spindler, Perspectum, Echosens, Kowa, and Mirun. PT receives institutional support from Birmingham NIHR BRC. Unrelated to this study PT has received grant support from the Wellcome Trust, Innovate UK, the Medical Research Foundation, GlaxoSmithKline (GSK), Guts UK, PSC Support, LifeArc, NIHR, Intercept Pharma, Dr. Falk Pharma, Gilead Sciences, and Bristol Myers Squibb. He has also received speaker fees from Intercept and Dr. Falk, and advisory board/consultancy fees from Intercept, Cymabay, Pliant Pharma, Dr. Falk, Albireo, Ipsen and GlaxoSmithKine. HNH consultancies for Arrowhead, Takeda, GLG, Albireo, Alnylam and Mirum. LC received speakers fee from Advanz and Echosens. LD'A received consultancy fees from: Albireo, Mirum, Alexion, Astra Zeneca, Selecta, Vivet, Spark, Genespire, and Tome, in fields unrelated to the current topic. DD received consultancy fees from: Mirum, Vertex, Orphalan, Univar, Alexion, in fields unrelated to this study. UB received consultancy fees or grant support from: Mirum, Albireo, Alexion, Nestle and Vivet in fields unrelated to this study.

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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.2023.11729/full#supplementary-material

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GLOSSARY

AGA	American Gastroenterological Association
AIH	autoimmune hepatitis
ALD	alcohol-related liver disease
AS	anastomotic strictures
ASA	aminosalicylic acid
AZT	azathioprine
CET	Centre for Evidence in Transplantation
CCA	cholangio-carcinoma
CYA	cyclosporin
DD	duct-to-duct anastomosis
EASL	European Association for the Study of the Liver
ERCP	endoscopic retrograde cholangiopancreaticography
ESOT	European Society of Organ Transplantation
ELITA	European Liver and Intestine Transplant Association
EKITA	European Kidney Transplant Association
EPITA	European Pancreas and Islet Transplant Association
ECD	extended criteria donors
ECTTA	European Cardio Thoracic Transplant Association
ETHAP	European Transplant Allied Healthcare Professionals
HCV	Hepatitis C virus
HD	hepaticojejunostomy
HGD	high grade dysplasia
IBD	Inflammatory Bowel Disease
IPAA	ileal pouch-anal anastomosis
LT	liver transplantation
MDR	multidrug resistance
ME	MELD Exception
MELD	model for end-stage liver disease
MMF	mycophenolate mofetil
MRC	magnetic resonance cholangiography
MRCP	magnetic resonance cholangiopancreatography
MRE	magnetic resonance elastography
NAS	non-anastomotic strictures
OPTN	Organ Procurement and Transplantation Network
PICO	Population/Intervention/Comparison/Outcome
PBC	primary biliary cholangitis
PSC	Primary Sclerosing Cholangitis
RCT	randomised-controlled trial
rPSC	recurrent primary sclerosing cholangitis
TAC	tacrolimus
TIPS	transjugular intrahepatic portosystemic shunt
TNF	tumour necrosing factor
UC	ulcerative colitis
UDCA	ursodeoxycholic acid
UNOS	United Network for Organ Sharing

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European Society of Organ Transplantation (ESOT) Consensus Report on Downstaging, Bridging and Immunotherapy in Liver Transplantation for Hepatocellular Carcinoma

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Liver transplantation offers the best chance of cure for most patients with non-metastatic hepatocellular carcinoma (HCC). Although not all patients with HCC are eligible for liver transplantation at diagnosis, some can be downstaged using locoregional treatments such as ablation and transarterial chemoembolization. These aforementioned treatments are being applied as bridging therapies to keep patients within transplant criteria and to avoid them from dropping out of the waiting list while awaiting a liver transplant. Moreover, immunotherapy might have great potential to support downstaging and bridging therapies. To address the contemporary status of downstaging, bridging, and immunotherapy in liver transplantation for HCC, European Society of Organ Transplantation (ESOT) convened a dedicated working group comprised of experts in the treatment of HCC to review literature and to develop guidelines pertaining to this cause that were subsequently discussed and voted during the Transplant Learning Journey (TLJ) 3.0 Consensus Conference that took place in person in Prague. The findings and recommendations of the working group on Downstaging, Bridging and Immunotherapy in Liver Transplantation for Hepatocellular Carcinoma are presented in this article.

Keywords: hepatocellular carcinoma, liver transplantation, downstaging, bridging, immunotherapy

INTRODUCTION

Liver transplantation offers the best chance of cure for most patients with non-metastatic hepatocellular carcinoma (HCC). After their introduction in 1996, the Milan Criteria (a single lesion of ≤5 cm or 2–3 lesions of ≤3 cm) became the standard for patient eligibility for transplantation [1]. In later years, several expended selection criteria were introduced. Of these, the University of California San Francisco (UCSF) criteria (a single lesion of ≤6.5 cm or 2–3 lesions ≤4.5 cm with a total diameter ≤8 cm), the Up-to-seven criteria (the sum of the size of the largest tumor [in cm] and the number of tumors should not exceed 7), and the French AFP model (a score calculated based on a combination of AFP level, tumor size, and number which should not exceed 2) have been most widely accepted [2–4]. Post-transplant survival rates for patients transplanted within these established criteria exceed 70% at 5 years and 60% at 10 years [2, 3, 5–7]. To keep patients within these criteria while awaiting transplant and to avoid them from dropping out of the waiting list, bridging therapies such as ablation and transarterial chemoembolization (TACE) are being applied. Similarly, these treatments are used to downstage patients from outside established HCC transplant criteria to within these criteria, allowing them to become eligible for liver transplantation. When successful, downstaged patients can achieve equally meaningful post-transplant survival outcomes exceeding 65% at 5 years and 50% at 10 years [5, 8–10].

Although still in development and only recently added as part of the first-line treatment of patients with advanced HCC, immunotherapy too offers great potential in furthering the treatment of HCC [11]. Evidence for immunotherapy in neoadjuvant settings is already accumulating from early phase trials in various solid tumor types and also in HCC few studies have shown promising results, reporting major pathological response (≥70% necrosis) in 20%–42% of resected patients after receipt of neoadjuvant immunotherapy [12–15].

To address the contemporary status of downstaging, bridging, and the role of immunotherapy in both these strategies in the specific context of liver transplantation for HCC, ESOT convened a consensus conference, comprised of a global panel of expert hepatologists, transplant surgeons, and oncologists to develop guidelines on key aspects of Downstaging, Bridging and Immunotherapy in Liver Transplantation for Hepatocellular Carcinoma. The consensus findings and recommendations of these ESOT Consensus guidelines are presented in this document and are intended for healthcare providers.

METHODS

The consensus development process was governed by a dedicated ESOT Guidelines Taskforce with support from its sections, and specifically for this work the European Liver and Intestine Transplant Association (ELITA), European Transplant Allied Healthcare Professionals (ETHAP), Education Committee, Young Professionals in Transplantation (YPT), Transplant International editorial board members and patient representatives. The detailed description of methodology used is reported previously [16].

Briefly, key issues related to Downstaging, Bridging and Immunotherapy in Liver Transplantation for HCC were identified by the working group and specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome) [17]. All PICO questions are listed in **Table 1** and further specified in the **Supplementary Material**. Following the definition of the PICOs, literature searches were developed (**Supplementary Material**). In some, support was provided by expert staff from the Centre for Evidence in Transplantation (CET) who have expertise in conducting systematic reviews. Search strategies differed based on the type of question and whether CET was involved or not and were conducted between 14 July 2022 and 31 October 2022.

A summary of the evidence addressing each key question by the included studies was prepared in evidence **Supplementary Tables S1–S10** (**Supplementary Material**). The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. For evaluation of the quality of evidence according to GRADE the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance and publication bias [18]. Strength of recommendation was rated as 1 (strong) or 2 (weak).

Complete information including the list of consensus conference workgroup domains and process regarding consensus conference participant selection, development and refinement of consensus statements, are previously reported, in beforehand of the in-person conference held in Prague, Czech Republic, 13–15 November 2022 [16].

RESULTS

1. Should all Eligible Patients Be Transplanted After Successful Downstaging?

Currently, given the scarcity of graft resources and competing indications for liver transplantation, patients beyond conventional pre-defined criteria are often not transplanted. Despite achieving successful downstaging to within accepted criteria, patients are not always offered the option of liver transplantation. The question remains whether they should.

Recommendation 1.1: All HCC patients achieving a successful downstaging to pre-defined transplantable criteria should be considered for liver transplantation as the benefit in terms of both recurrence-free survival and overall survival of this approach is significantly higher than any other non-transplant strategy.

TABLE 1 | Population, Intervention, Comparator and Outcome (PICO) questions.

1. Should all eligible patients be transplanted after successful downstaging?
2. Should all patients outside transplant criteria (all comers) be considered for downstaging?
3. Should Patients with Complete Response of HCC Macrovascular Invasion be considered for Liver Transplantation?
4. Does bridging therapy improve post-transplant survival?
5. Does bridging therapy decrease waitlist dropout?
6. Does the type of response to bridging therapy have an impact on post-transplant survival?
7. What locoregional therapy results into best short-term disease-control in HCC patients without extrahepatic disease?
8. Are patients on immunotherapy prior to liver transplantation at risk for rejection?
9. What is the best way to assess response to immunotherapy?
10. What is the safety of combined treatment with locoregional therapy and immunotherapy in the setting of transplantation?

Quality of Evidence: High.

Strength of Recommendation: Strong for.

Unmet needs: There are no specific unmet needs. Nonetheless, additional high-quality evidence could help refine, expand and/or strengthen a future recommendation on the topic.

One single reference, a 2020 randomized controlled trial by Mazzaferro et al., met the pre-defined PICO criteria and was included for review (**Supplementary Table S1**) [10]. This study analyzed 74 patients from nine different Italian centers and showed that after an effective and sustained downstaging of tumors originally beyond Milan criteria, liver transplantation improved tumor event-free survival and overall survival compared with non-transplantation therapies [10]. Data supporting that successfully downstaged patients should be considered for liver transplantation.

2. Should all Patients Outside Transplant Criteria (All Comers) Be Considered for Downstaging?

Many patients with HCC are diagnosed at an advanced stage, falling beyond accepted transplant criteria. However, if the overall tumor burden were to decrease, they could potentially reach a stage for which liver transplantation is usually indicated. Whether this should be actively pursued, treating patients with the goal of lowering their tumor burden so that liver transplantation might become possible, regardless of their initial stage, is still up for debate.

Recommendation 2.1: All patients beyond transplant criteria, without extra-hepatic disease or macrovascular invasion, should be considered for downstaging as long as potentially eligible for transplantation, as the original HCC state has not demonstrated to significantly hamper post-transplant survival.

Quality of Evidence: Low.

Strength of Recommendation: Strong for.

Unmet needs: There are no specific unmet needs. Nonetheless, additional higher quality evidence could help refine, expand and/or strengthen a future recommendation on the topic.

After reviewing 413 references, six observational studies were found to meet the PICO criteria (**Supplementary Table S2**) [5, 8, 19–21]. All these six studies showed no impact of the original HCC state on post-transplant survival. Although some studies showed a trend towards decreased disease-free survival in patients with advanced HCC (based on size and number) compared to those with less advanced HCC before downstaging, none reached significance [5, 8, 20]. In addition, one study based on waitlist alpha-fetoprotein (AFP) changes even suggested the opposite, utilizing the United States (US) Scientific Registry of Transplant Recipients (SRTR) and including 60 highly selected patients. In the cohort of patients demonstrating a waitlist AFP decrease below 400 ng/mL, those with high original AFP >1,000 ng/mL showed a trend towards better post-transplant survival compared to those with original AFP between 400 and 700, and between 700 and 100 ng/mL (100% vs. ~75% vs. ~55%, $p = 0.072$) [19]. Altogether, the identified studies support the use of downstaging in all patients with HCC beyond conventional criteria (all comers) as long as potentially eligible for transplantation, as the post-transplant survival in case of successful downstaging is not negatively influenced by the original HCC state. Of note, data suggest that a combination of morphological and biological (AFP) criteria should be used to assess the success of downstaging in all comer patients [22]. Also, enough time should be left between a successful downstaging and transplantation (e.g., >6 months) to decrease the risk of post-transplant recurrence [22].

Note: The higher the burden of disease (based on morphology and/or biology), the lower the likelihood to achieve successful downstaging.

Quality of Evidence: Moderate.

Although the original HCC state has no demonstrated impact on post-transplant survival, several studies showed that patients with advanced HCC are more likely to fail downstaging strategies, confirming the role of downstaging as a selection tool. To illustrate, two studies including 209 and 326 patients reported rates of successful downstaging to within Milan criteria at 39.1% and 38.2% for patients originally beyond UCSF criteria, and at 58% and 45.2% for patients originally between Milan and UCSF ($p = 0.042$, $p = 0.001$) [20, 23]. However, as downstaging and palliation involve similar locoregional and systemic treatments, it can generally be argued that it is to the patients' benefit to keep them in a downstaging strategy.

3. Should Patients With Complete Response of HCC Macrovascular Invasion Be Considered for Liver Transplantation?

Macrovascular invasion has historically been a contraindication for liver transplantation in patients with HCC. Although difficult to treat, some patients with macrovascular invasion manage to achieve complete radiologic response after locoregional or systemic treatment. Whether these patients should be considered for liver transplantation is still to be answered.

Recommendation 3.1: There is insufficient evidence to recommend or not recommend liver transplantation for patients with HCC macrovascular invasion with complete response to therapy.

Quality of Evidence: Low.

Strength of Recommendation: N/A.

Unmet needs: Outcomes for patients with HCC and macrovascular invasion transplanted after complete response by pre-operative therapy are missing. Therefore, future studies should focus on neoadjuvant locoregional or systemic therapies and sustained (~6 months) complete response. In this effort, differences in type of portal vein tumor thrombus (Vp1-Vp4) should also be compared.

Of the 85 references found, seven studies met all pre-defined PICO criteria. After reviewing their references, one more study was identified for inclusion, bringing the total to eight studies for further review (**Supplementary Table S3**) [24–30]. Although several studies demonstrated a 5 years overall survival rate of more than 50% in patients who received downstaging treatments before transplantation, most studies also reported high recurrence rates [24–30]. The largest included study, by Yu et al., analyzed 176 patients with portal vein tumor thrombus (PVTT) type 1–2 and showed a 5 years overall survival of 78.3% in patients with type 1 PVTT compared to 51.6% for those with type 2 PVTT (p = 0.005) [28]. However, recurrence-free survival was about 46% in both groups. Moreover, no subgroup analysis was performed for patients who achieved complete response after pre-operative therapy. This subgroup analysis was also lacking in most of the other included studies [24, 25, 29, 30]. The two studies that did report on outcomes for patients with radiologic (near-to) complete response, by Soin et al. (n = 25) and Serenari et al. (n = 5), showed a 5 years overall survival of 57% and 60%, and a recurrence rate of 24% and 60%, respectively [26, 27]. Consequently, due to insufficient evidence in the contemporary literature, no clear recommendation can be made on whether or not patients with HCC and macrovascular invasion should be considered for transplantation after complete radiologic response. If pursued, this strategy should be carried out within specific clinical trial settings.

4. Does Bridging Therapy Improve Post-Transplant Survival?

Bridging therapy is commonly used to keep patients with HCC within established transplant criteria. However, it is uncertain whether this also results in improved post-transplant survival and should therefore be standard practice for every patient on the transplant waiting list.

Recommendation 4.1: There are some studies that suggest a positive effect of bridging therapy on long-term post-transplant survival. Therefore, bridging therapy should be considered in patients if feasible.

Quality of Evidence: Low.

Strength of Recommendation: Strong for.

Unmet needs: There are no specific unmet needs. Nonetheless, additional higher quality evidence could help refine, expand and/or strengthen a future recommendation on the topic.

After screening 989 references, eight studies were selected for full review. One was a systematic review and meta-analysis (the studies analyzed herein were not separately reinstated for full review), the remaining seven were observational studies (**Supplementary Table S4**) [31–38]. Some of the identified studies showed significantly better long-term post-transplant survival outcomes in patients treated with bridging therapy [33, 35, 37, 38]. The largest of these studies, by Xing and Kim, looked at 14.511 transplanted patients within Milan criteria pre-transplant (3.889 with bridging, 10.622 without) and showed a 1, 3, and 5 years post-transplant survival of 95%, 85%, 80% in bridged patients versus 94%, 83%, 78% in patients without bridging (p < 0.001) [37]. In the multivariable analysis, bridging therapy remained associated with a significantly better post-transplant survival with a hazard ratio (HR) of 2.28 (95% CI 1.39–3.14; p = 0.003). Bauschke et al. showed in their cohort of 70 patients, all within Milan criteria, that the survival benefit persists even after 10 years post-transplant (95% bridged vs. 73% without bridging, p = 0.014) [33]. Another study analysing patients classified as within Milan criteria pre-transplant showed that the positive effect of bridging therapy on post-transplant survival even seems to last in a setting of recurrence, where the median survival of recurred bridged patients was 75.9 months versus 53.1 months in patients without bridging treatment (p = 0.001) [35]. Looking specifically at patients within UCSF criteria, two studies were evaluated, one with 134 patients and another with 39 patients, but both failed to report any statistical difference in survival between bridged and non-bridged patients.

5. Does Bridging Therapy Decrease Waitlist Dropout?

It is widely believed that bridging therapy is effective in keeping patients within established transplant criteria, however, whether it actually results in reduced waitlist dropout has yet to be confirmed.

Recommendation 5.1: Due to inherent confounding in the indication to bridge, evidence in the current literature is insufficient to identify whether or not bridging therapy decreases waitlist dropout. Therefore, no recommendation can be made.

Quality of Evidence: Low.

Strength of Recommendation: N/A.

Unmet needs: To determine whether bridging therapy actually results in a reduction in waitlist dropout, avoiding the currently inherited confounding in the indication to bridge, a randomized controlled trial would be required. However, with the current assumption that bridging therapy, already standard practice, is

effective in keeping patients within transplant criteria, such a trial is considered ethically unjustifiable.

A total of 634 references were identified, of which six observational studies and one systematic review and meta-analysis met the pre-defined PICO criteria (the studies analyzed in the systematic review were not separately reinstated for review) (**Supplementary Table S5**) [31, 34, 36, 38–41]. Considering the most common transplant criteria (Milan, UCSF, ETC), none of the identified studies showed a decrease in overall or disease-specific waitlist dropout for patients who received bridging treatment compared to those without bridging treatment [31, 34, 36, 38–41]. Although not statistically significant, some of the studies did show a longer waitlist time in the group of patients who received bridging therapy [34, 36, 39, 40]. When specifically focussing on progression-related waitlist dropout, one study—evaluating 265 patients within Milan criteria—showed a statistically significantly lower dropout rate in the bridged patient population (2.58%) versus patients without bridging therapy (8.18%) [38]. However, the all-cause waitlist dropout in this study was higher in the bridged patient group (28.4% vs. 14.5% without bridging). Another study, a 2018 meta-analysis by Kulik and others, evaluating 257 cirrhotic patients classified as T2 HCC (patients within Milan criteria), reported no difference in progression-related waitlist dropout between groups treated with and without bridging treatment (relative risk [RR] 0.32; 95% confidence interval 0.06–1.85) [31]. Whether the type of bridging therapy plays a role in waitlist dropout was evaluated in the study by Györi et al., where they analyzed 84 patients within Milan criteria [34]. A transarterial chemoembolization (TACE)-based group (n = 48) was compared with a percutaneous ethanol injection (PEI)/radiofrequency ablation (RFA) group (n = 32) and a control group consisting of patients without bridging treatment (n = 22). They found no difference in all-cause waitlist dropout between groups: 41.7% TACE-based vs. 31.2% PEI/RFA vs. 36.4% control (p = 0.65) [34]. However, a serious limitation in all these retrospective studies, is the inextricable involvement of selection bias in the indication for bridging. Consequently, bridged and non-bridged populations consistently include non-comparable groups of patients and therefore ineluctably mask any effect that bridging therapy might have on waiting list dropout. Thus, precluding the effect of bridging on waitlist dropout from being inferred.

6. Does the Type of Response to Bridging Therapy Have an Impact on Post-Transplant Survival?

Bridging therapies are used in several patients within conventional transplant criteria to delay tumor progression and to minimize the risk of de-listing while on the waiting-list (dropout). Despite the strong belief that the type of response to bridging is able of influencing the rate of post-transplant tumor recurrence, this, and the weight that tumor response may have on post-transplant survival, have yet to be determined.

Recommendation 6.1: The aim of all bridging treatments carried out on the waiting-list should be to achieve a complete pathological response as this has shown to be associated with both improved recurrence-free and overall survival. Since there is no radiological imaging yet able of accurately predicting post-transplant complete pathologic response, sustained radiologic response may be considered as the best surrogate to pursue in the pre-transplant setting.

Quality of Evidence: Low.

Strength of Recommendation: Strong for.

Unmet needs: There are no specific unmet needs. Nonetheless, additional higher quality evidence could help refine, expand and/or strengthen a future recommendation on the topic.

Given the high rate of overestimation of treatment response of radiology over pathology, the literature review focused on pathologic responses only. After the identification of 423 references, nine references were included for further review (**Supplementary Table S6**) [35, 42–49]. All but one study analyzed outcomes achieved after both bridging and downstaging therapies, with TACE being the most commonly used treatment modality. In all studies, patients with complete pathologic response at explant pathology showed better overall survival and recurrence-free survival rates compared with those without complete pathological response [35, 42–49]. Allard et al. found that the favorable prognostic effect of response induced by TACE on explant pathology in 189 patients was confirmed not just for complete necrosis but also for "near to complete responses" (>90%), suggesting a "nearly all - or nothing" rule [48]. This data was later confirmed by the largest single-center US experience (n = 501) published by Agopian in the same year, updated in 2020 in a multicentric fashion including 3,439 patients undergoing liver transplantation from 2002 to 2013 in 20 US centers and all receiving bridging and/or downstaging therapies pre-transplant (with 802 patients showing complete pathological response) [43, 49]. All data supporting the need to pursue a complete (or close to complete) radiological tumor response in patients with HCC listed for liver transplantation.

7. What Locoregional Therapy Results Into Best Short-Term Disease-Control in HCC Patients Without Extrahepatic Disease?

Many different types of locoregional therapy for HCC exist. In the context of liver transplantation, locoregional therapy is used in the attempt to effectively control the patient's tumor burden until a suitable liver donor becomes available for transplantation. Consequently, adequate short-term disease control is desired. What type of locoregional therapy best achieves this remains to be determined.

Recommendation 7.1: Specifically for waitlisted patients, no recommendation can be made due to the absence of unconfounded evidence. Therefore, the type of locoregional therapy should be selected according to patient and center characteristics using multidisciplinary assessment. Although data outside a transplant setting cannot be translated directly to waitlisted patients, they can provide guidance in determining which treatment might be advisable for different patients (**Table 2**).

TABLE 2 | Guidance document for determining the best locoregional treatment approach for short-term disease control in patients with HCC based on randomized controlled trials of locoregional treatment in a non-transplant setting.

Lesion number	Lesion size	Supporting statements
1	3–5 cm	1. When feasible, liver resection, preferably by laparoscopic route and segmental extension, should be considered Level of evidence: Moderate Level of recommendation: Weak for 2. When technically feasible RFA or MWA are the preferred second line therapies and are equally effective in obtaining short-term tumor control. When ablation is not obtained or not expected to be obtained, TACE is the preferred therapy Level of evidence: Moderate Level of recommendation: Weak for 3. Intention to treat with combined RFA/MWA and TACE may result in superior short term tumor control compared to TACE or RFA alone and can be used on indication Level of evidence: Low Level of recommendation: Weak for 4. Alternatives to TACE or RFA/MWA, including radio-embolization or SIRT, SBRT, proton-beam radiation therapy or brachytherapy have shown non-inferior or improved short term tumor control in preliminary trials and should preferably be used in a research setting Level of evidence: Low Level of recommendation: Weak for
≤3	≤3 cm	1. RFA or MWA is the preferred first line therapy and are equally effective in obtaining short-term tumor control Level of evidence: Moderate Level of recommendation: Strong for 2. Intention-to-treat with combined ablation therapy and TACE does not impact short term tumor control Level of evidence: Low Level of recommendation: Weak for
≥1	≥5	1. Liver resection, if feasible and indicated, is associated to the higher probability to obtain a complete response on the single HCC Level of evidence: Low Level of recommendation: Weak for 2. Downstaging therapy with TACE is preferred over bland embolization or chemo infusion alone Level of evidence: Low Level of recommendation: Weak for 3. Intention to treat with combined RFA/MWA and TACE may result in superior short term tumor control than TACE alone and can be used on indication. Level of evidence: Low Level of recommendation: Weak for 4. Alternatives to TACE, including radio-embolization or SIRT, SBRT, proton-beam radiation therapy or brachytherapy have shown non-inferior or slightly improved short term tumor control in preliminary trials and should preferably be used in a research setting Level of evidence: Low Level of recommendation: Weak for

Quality of Evidence: Low.

Strength of Recommendation: N/A.

Unmet needs: To determine what locoregional therapy results into best short-term disease-control in waitlisted HCC patients, avoiding both selection bias and the many patient-related confounders, randomized controlled trials would be required. However, given many patient-related and treatment-related confounders determine whether certain types of locoregional therapies can be applied to selected patients with HCC, accruing enough patients in such trials will be extremely difficult.

As treatment allocation in clinical practice is subjected to both confounding factors and selection bias, only randomized controlled trials (RCTs) on the application of locoregional therapies outside a transplant setting were included. This approach allows for the least biased comparison between therapeutic modalities. Of the 2,944 unique references found, 40 RCTs comparing at least two treatment modalities were included for further review (**Supplementary Table S7**) [50–88]. Treatment comparisons were grouped according to lesion size and number combinations.

RCTs on uninodular lesions with size up to 3 cm (BLCL 0, A; within Milan) have compared: radiofrequency ablation (RFA) to

percutaneous ethanol injection (PEI) [52, 57, 68, 74, 77], RFA to percutaneous laser ablation (PLA) [80], RFA to percutaneous acetic acid injection (PAAI) [57, 64], RFA to cryoablation [55], RFA to microwave ablation (MWA) [50, 58, 59, 69, 73, 76], and RFA to RFA combinatorial approaches [61, 65, 70, 71, 78, 82]. RFA appeared to induce higher frequencies of radiological complete responses (rCR) and improved 1 year local recurrence (LR) rate compared to PEI and PLA. Compared to PAAI, RFA induced similar rCR. However, 3 years LR rate was improved in RFA-treated versus PAAI-treated patients (RR = 0.41, 95% CI: 023–0.91) [57]. Cryoablation has been shown to have equal rCR, 1 year LR rate, 1-year overall survival, and 1 year disease-free survival as RFA, albeit in a single RCT [55]. In a meta-analysis on RCTs among RFA- and MWA-treated lesions no difference in radiological complete response rates was observed (RR: 1.01, 95% CI: 0.99–1.02) [67]. Moreover, 1 year disease-free and overall survival rates were similar. No difference in adverse events (Aes) could be observed between RFA and MWA-treated patients. RCTs on combination of RFA with TACE [65, 70, 82] or other therapeutic regimen (PEI [71], Iodine-125 [78], Interferon alpha [61]) did not show or report any difference in rCR in these tumor lesions compared to RFA only.

RCTs comparing RFA to PEI [52, 74], RFA to PLA [80], RFA to PAAI [64], RFA to cryoablation [55], and RFA to MWA [50, 58, 59, 69, 73, 76] have included uninodular lesions, ranging 3–5 cm as well. As RFA and MWA in these trials have shown to be clinically effective one might suggest that these techniques are preferred as first line regimen. Yet, locoregional ablative therapies tend to become less effective if tumor lesion size increases.

In case of increased tumor burden, intra-arterial therapies or radiotherapy provide an alternative. Different RCTs on uninodular lesions ranging 3–5 cm (BCLC A; within Milan) and uni-/multinodular lesions ≥5 cm (BCLC A, outside Milan; BCLC B, outside Milan, resp.) have compared: TACE to transarterial or "bland" embolization (TAE) [54, 60, 81, 84], TACE/RFA to TACE combined with RFA [51, 56, 65, 66, 70, 72, 86], TACE to transarterial radio-embolization (TARE) [53, 62, 83, 88], TACE to transarterial ethanol ablation (TAEA) [75], TACE to transarterial chemo-infusion (TACI) [63], and TACE to radiotherapy [79, 85]. Hyperselective TACE (tend to) induced higher frequencies of rCR or radiological partial response (rPR) compared to "bland" embolization. 1 year disease-free and overall survival was either non-significantly different among the groups or tended to be increased in TACE-treated patients. When combining TACE with ablative therapies, combination regimen appeared to induce higher rCR (i.e., + PEI [66], + RFA [86], and + cryoablation [51]), 1 year disease-free survival [66], and 1-year overall survival [51, 86], although studied in relatively small cohorts. RCTs comparing TACE to TARE have shown conflicting results. Whereas Raoul et al. reported no difference in rCR/PR when using Iodine-131 radioembolisation [53], other trials have shown a trend to higher radiological response rates in Yttrium-90 (Y-90) radioembolization cohorts compared to TACE [83, 88]. Moreover, Salem et al. have observed that Y-90 appeared to have lower 1 year LR rate [62]. Generally, treatment-related or grade ≥3 AEs were either equal or reduced in favor of TARE. Conformably, in the prospective, multi-center, non-randomized MERITS-LT trial both TACE and Y90-TARE showed equal efficacy in downstaging towards liver transplantation [87]. Though not statistically significant, explanted livers of TARE-treated patients demonstrated higher frequencies of tumor necrosis (30.8% vs. 20.5%) and lower frequencies microvascular invasion (7.7% vs. 20.5%) hinting towards improved local tumor control. Nowadays, TARE has been accepted as an effective alternative in case TACE is contraindicated (e.g., portal thrombosis). To this end, no clear benefit of TAEA, TACI, or radiotherapy (i.e., proton-beam, brachytherapy) over TACE in RCTs was observed. Yet, recent prospective cohort studies strongly hint to safe and superior efficacy of stereotactic body radiotherapy over TACE as bridge to transplant [89, 90]. Any conclusive results on these therapies are expected from ongoing phase III RCTs (i.e., NCT03960008).

Although this data provides valuable insight in the potential of each locoregional treatment in a non-transplant setting, their results cannot directly be translated to waitlisted patients. Therefore, no recommendations can be made. Nonetheless, these comparisons can provide guidance in determining the kind of treatment to pursue (**Table 2**).

8. Are Patients on Immunotherapy Prior to Liver Transplantation at Risk for Rejection?

Immunotherapy has recently become part of the standard treatment for advanced unresectable HCC who are not amenable to curative or locoregional therapy. Due to its promising results, interest has emerged in the use of immunotherapy in a neoadjuvant setting. Whether patients receiving immunotherapy prior to liver transplantation are at risk for rejection has yet to be determined.

Recommendation 8.1: Due to insufficient evidence, no meaningful recommendation can be made.

Quality of Evidence: Low.

Strength of Recommendation: N/A.

Unmet needs: (1) Further investigations are needed to explore the safety and long-term oncologic outcomes in the pre-transplant setting. (2) Patient selection for immune checkpoint inhibitors (ICI), minimal washout period between the last drug dose and transplantation, observation period, biomarkers are unmet clinical needs that require investigation.

Of the 1,560 references identified, nine studies on liver transplantation in patients previously treated with immune checkpoint inhibitors were included, representing 27 cases (**Supplementary Table S8**) [91–99]. The first case reported resulted in fatal hepatic necrosis at day 8th and patient loss [91]. The ICI was given within 4 weeks before transplantation. A minimum washout period (4 weeks) prior to transplantation given the half-life of 27 days was proposed. Subsequent reports have shown successful results [92, 94–99]. In total, four cases of severe rejection were reported with two successful re-transplantations [91, 93, 97, 98]. Since drug type, pre-

transplant treatment and dosage, tumor burden, and response vary from case to case, further investigations are needed to explore the safety and long term oncologic outcomes in a pre-transplant setting.

9. What is the Best Way to Assess Response to Immunotherapy?

To optimize the use of immunotherapy treatment in patients with HCC and to be able to evaluate its effect in a (neo)adjuvant setting, it is imperative that tumor response after immunotherapy can be adequately assessed. However, the best way to do this has yet to be determined.

Recommendation 9.1: There is insufficient evidence to make any meaningful recommendation on how best to assess response to immunotherapy for HCC.

Quality of Evidence: Low.

Strength of Recommendation: N/A.

Unmet needs: (1) Improved imaging techniques and biomarkers are needed to define response ahead of pathologic assessment and oncologic outcomes. (2) Explant analysis of specimens should be done prospectively with careful radiology-pathology correlation.

After an extensive review of 6,800 references, seven studies were selected for inclusion (**Supplementary Table S9**) [11, 13–15, 100–102]. Radiologic evaluation of response after immunotherapy is primarily derived from the recent trials on immunotherapy within advanced HCC where survival benefit was associated with objective response and significant reduction in tumor burden [11, 100–102]. In these studies, the objective response rate by mRECIST ranged from 22% to 34%, whereas complete response was reported in 2.2%–5.5% of the cases [11, 100–102]. Unfortunately, these studies lack confirmation of actual response through pathological assessment. Three recent trials that published on the use of neoadjuvant therapy prior to resection in HCC did report on both response seen on imaging and determined by pathologic assessment. Complete pathologic response ranged from 8% to 25% and major pathologic response (>70% necrosis) was seen in 20%–42%, while pre-operative imaging according to RECIST 1.1 reported partial and complete response in only 8%–15% and 0%, respectively [13–15]. Although data on imaging-pathology response correlations in a transplantation setting are lacking, encouraging pathologic response rates have been reported. In a study of 9 patients who underwent ICI in combination with locoregional therapy, downstaging was successful in 4/5 patients and major pathologic response (>70% necrosis) was noted in 6/9 patients [96]. Improved imaging techniques and biomarkers are needed to define response ahead of pathologic assessment and oncologic outcomes. Given the high rate of explants exceeding Milan criteria post transplantation, significant limitations occur with the current contrast enhanced computed tomography (CT) and magnetic resonance imaging techniques (MRI) in predicting treatment response [87]. In addition, with the use of immunotherapies, the immunologic changes within the tumor and tumor microenvironment may impact the relation between the degree of pathologic and radiographic response [14]. Moreover, the vasoconstrictive and antiangiogenic effects of the drugs may induce a false positive assessment of response by mRECIST [103, 104].

10. What Is the Safety of Combined Treatment With Locoregional Therapy and Immunotherapy in the Setting of Transplantation?

A combined treatment of immunotherapy and locoregional therapy may be more effective than each treatment separately. However, it remains to be seen whether such combined treatment approach is safe in the context of transplantation.

Recommendation 10.1: Since there is no data in the context of pre- or post-liver transplantation, no recommendation can be made.

Quality of Evidence: N/A.

Strength of Recommendation: N/A.

Unmet needs: Further investigations that explore the safety and long-term oncologic outcomes in the pre- and post-transplant setting are needed.

Since no data was found on combined treatment with locoregional and immunotherapy in the setting of transplantation, data outside transplant setting was assessed. In this context, a total of 450 references were identified, whereas 14 were eventually included for further review (**Supplementary Table S10**) [105–119]. Two of these were systematic reviews and meta-analyses [105, 106]. The first, including 19 studies and comparing TACE or RFA with immunotherapy, did not evaluate safety profiles [105]. The second, including four studies comparing TACE with dendritic cells therapy, reported that patients in the TACE-DC-CIK group were more likely to suffer a fever than the ones in the control group ($p = 0.001$). In the five prospective studies, one randomized controlled trial and four non-randomized trials, no safety difference between arms was reported [110–115]. However, the small sample sizes limited the robustness of their conclusion. Finally, of the seven non-randomized retrospective studies, five focused on early-death or severe complications with none of the studies reporting any major complication or death associated with the treatment evaluated [108, 117–120]. In the remaining two retrospective studies safety was not reported [107, 109]. Although these data provide valuable insight into the safety and long-term oncologic outcomes of combined treatments of locoregional therapy and immunotherapy in a non-transplant setting, they cannot be extrapolated to a transplant/waitlist-setting. Therefore, no recommendation can be made.

SUMMARY AND FUTURE CONSIDERATIONS

The Transplant Learning Journey (TLJ) 3.0 consensus conference resulted in several recommendations pertaining to Downstaging, Bridging and Immunotherapy in Liver Transplantation for HCC. Starting with downstaging. Though not always successful, downstaging should always be aimed for regardless of disease burden as the original HCC state has demonstrated little impact on post-transplant survival. Moreover, as downstaging and palliation involve similar locoregional and systemic treatments, it can generally be argued that it is to the patients' benefit to keep them in a downstaging strategy. If successful downstaging has been achieved, patients should always be considered for liver transplantation as the benefit in terms of both recurrence-free and overall survival of this approach is significantly higher than any other non-transplant strategy. Although liver transplantation for patients with macrovascular invasion has been shown to be feasible, recurrence rates are generally high, necessitating further investigation to determine whether patients with HCC and macrovascular invasion should be considered for liver transplantation if complete radiologic response has been achieved. In the context of bridging, some studies suggest a positive effect of bridging therapy on long-term post-transplant survival and therefore should be considered if feasible. When applied, the aim should be to attain complete response, as a complete pathological response has shown to be associated with improved recurrence-free and overall survival. Since radiological imaging is not able to accurately predict post-transplant complete pathologic response, sustained radiologic response may be considered as the best surrogate to pursue in the pre-transplant setting. Unfortunately, whether or not bridging therapy decreases waitlist dropout cannot be determined from the contemporary literature due to inherent confounding in the indication to bridge. In terms of the type of bridging therapy to use, selection should be made according to patient and center characteristics using multidisciplinary assessment. Finally, although immunotherapy has shown promising results, further investigations are needed to explore its safety (rejection) and long-term oncologic outcomes in a pre-transplant setting, as well as which patients to select, the minimal washout period between the last drug dose and transplantation, and the optimal duration of observance. The same holds for immunotherapy use in a pre- or post-transplant setting when combined with locoregional treatments. To support research in these areas, improved imaging techniques and biomarkers are needed to define immunotherapeutic response ahead of pathologic assessment and oncologic outcomes.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Involved in the conception or design of the work: MC, DS, YR, RA, SB, UC, CF, MR, GS, PT, and CT. Literature screen and review: MC, DS, YR, RA, SB, UC, CF, MR, GS, PT, and CT. Drafted the article: MC, DS, YR, RA, SB, UC, CF, MR, GS, PT, and CT. Critically revised the article: MC, DS, YR, RA, SB, UC, CF, MR, GS, PT, and CT. Finally approved the version to be published: MC, DS, YR, RA, SB, UC, CF, MR, GS, PT, and CT. 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.2023.11648/full#supplementary-material

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GLOSSARY

AE adverse events

AFP alpha-fetoprotein

CET Centre for Evidence in Transplantation

CT computed tomography

DFS disease-free survival

ELITA European Liver and Intestine Transplant Association

ESOT European Society of Organ Transplantation

ETHAP European Transplant Allied Healthcare Professionals

GRADE Grading of Recommendations Assessment, Development and Evaluation

HCC hepatocellular carcinoma

HR hazard ratio

LR local recurrence

LT liver transplantation

MRI magnetic resonance imaging

MWA microwave ablation

N/A not applicable

OS overall survival

PAAI percutaneous acetic acid injection

PEI percutaneous ethanol injection

PICO Population, Intervention, Comparator and Outcome

PLA percutaneous laser ablation

ICI immune checkpoint inhibitors

PVTT portal vein tumor thrombus

rCR radiological complete response

RCT randomized controlled trial

RFA radiofrequency ablation

RFS recurrence-free survival

rPR radiological partial response

RR relative risk

SRTR Scientific Registry of Transplant Recipients

TACE transarterial chemoembolization

TACI transarterial chemo-infusion

TAE transarterial embolization

TAEA transarterial ethanol ablation

TARE transarterial radio-embolization

TLJ Transplant Learning Journey

UCSF University of California San Francisco

US United States

Y-90 Yttrium-90

YPT Young Professionals in Transplantation

ESOT | Transplant International

GUIDELINES
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European Society for Organ Transplantation Consensus Statement on Biomarkers in Liver Transplantation

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Currently, one-year survival following liver transplantation (LT) exceeds 90% in large international registries, and LT is considered definitive treatment for patients with end-stage liver disease and liver cancer. Recurrence of disease, including hepatocellular carcinoma (HCC), significantly hampers post-LT outcomes. An optimal approach to immunosuppression (IS), including safe weaning, may benefit patients by mitigating the effect on recurrent diseases, as well as reducing adverse events associated with over-/under-IS, including chronic kidney disease (CKD). Prediction of these outcome measures—disease recurrence, CKD, and immune status—has long been based on relatively inaccurate clinical models. To address the utility of new biomarkers in predicting these outcomes in the post-LT setting, the European Society of Organ Transplantation (ESOT) and International Liver Transplant Society (ILTS) convened a working group of experts to review literature pertaining to primary disease recurrence, development of CKD, and safe weaning of IS. Summaries of evidence were presented to the group of panelists and juries to develop guidelines, which were discussed and voted in-person at the Consensus Conference in Prague November 2022. The consensus findings and recommendations of the Liver Working Group on new biomarkers in LT, clinical applicability, and future needs are presented in this article.

Keywords: liver transplantation, biomarkers, chronic kidney disease, hepatocellular carcinoma, rejection, recurrent primary diseases

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INTRODUCTION

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections, which include ELITA, EKITA, EPITA, ECTTA, ETHAP, the Education Committee, YPT, Transplant International editorial board members and patient representatives. A detailed description of methodology used has been reported previously [1].

Briefly, key issues related to biomarkers in liver transplantation (LT) were identified by the Liver Working Group. Biomarkers were defined as characteristics that may be objectively measured and evaluated to serve as indicators of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention. Specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome). The four PICO questions related to disease recurrence, hepatocellular carcinoma (HCC) recurrence, chronic kidney disease (CKD), and weaning of immunosuppression (IS) are listed in **Tables 1–4**. Following the definition of PICOs, literature searches were conducted by expert staff from the CET (Centre for Evidence in Transplantation), who have expertise in conducting systematic reviews, and subsequently integrated, as needed, by the steering committee experts.

The Working Group proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C, and very low quality of evidence as D. For evaluation of the quality of evidence according to GRADE, the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance, and publication bias. Strength of recommendation was rated as 1 (strong) or 2 (weak).

RECURRENCE OF LIVER DISEASES AFTER LIVER TRANSPLANTATION

Autoimmune diseases, such as primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), and autoimmune hepatitis (AIH), represent about 8% of indications for LT [2], with 5-year patient survival rates surpassing 85% [3]. Disease recurrence, which is primarily responsible for impaired graft survival, is seen in 8.6%–27% of patients transplanted for PSC [4, 5], 10.9%–42.3% for PBC [6], and 7%–42% for AIH [7]. The diagnosis of recurrent disease is based on a combination of clinical, biological, and histological criteria and is often challenging [8].

Several studies are based on histology and even encourage performing protocol liver biopsy, which can facilitate the diagnosis of disease recurrence in the absence of biochemical and immunological abnormalities [9]. For recurrent PBC (rPBC), the gold standard for diagnosis is histological findings, including bile duct destruction by epithelioid granulomas, lymphocyte cholangitis, ductular proliferation, lymphocytic aggregates, and bile duct paucity. Elevated alkaline phosphatase and anti-mitochondrial antibody (AMA) levels are unreliable diagnostic markers [10]. For recurrent PSC (rPCS), diagnosis is made when cholangiographic imaging and liver biopsy findings similar to those described in native livers with PSC are observed in the context of mild cholestasis [11]. Pre-LT immunoglobulin G (IgG) level, high transaminase levels, severe inflammatory activity or plasma cell infiltration in the liver explant,

TABLE 1 | Literature Search Request for the 4 PICO questions in Liver Transplantation. Recurrent disease in liver transplantation.

Topics and research questions	Can biomarkers be used to diagnose recurrent liver diseases (MASH, alcohol resumption, autoimmune diseases) after liver transplantation?
Population(s) e.g.:	
• Type of transplant(s)	Liver Transplantation
• Age (pediatric/adult)	Adult
• Condition	Patients with and without elevated liver enzymes
Intervention	Use of biomarkers to diagnose recurrent diseases
Comparators (Where appropriate)	Diagnosis of recurrent disease based on liver biopsy +/- imaging data
Outcomes	- Recurrent disease in the graft (MASH, ASH, AIH, PBC)
Exclusion criteria (optional)	1. Journal with IF <2 2. Papers without a clear ethical approval 3. Systemic review and metanalysis 4. Conference abstracts
Search period	2000-day of the research
Types of studies	Randomized controlled trials Registry analyses Observational studies
Language	English
Comments/context/suggested keywords	- Liver transplantation - Recurrent liver disease - MASH - Alcohol relapse - Autoimmune diseases (autoimmune hepatitis, primary biliary cholangitis/primary sclerosing cholangitis recurrence)

TABLE 2 | Literature Search Request for the 4 PICO questions in Liver Transplantation. Recurrent HCC in liver transplantation.

Topics and research questions	Can biomarkers be used to predict HCC recurrence
Population(s) e.g.:	
• Type of transplant(s)	Liver transplant recipients undergoing LT due to HCC disease
• Age (pediatric/adult)	Adult
• Condition	HCC
Intervention	Use of biomarkers to predict HCC recurrence and thereby improve posttransplant monitoring
Comparators (Where appropriate)	Prediction of HCC recurrence based on classical models (up to seven Model, Milan criteria, Retreat Model)
Outcomes	- HCC recurrence - Cost of post-transplant monitoring - HCC recurrence free survival - Post-transplant survival
Exclusion criteria (optional)	1. Journal with IF <2 2. Papers without a clear ethical approval 3. Systemic review and metanalysis 4. Conference abstracts
Search period	2010–2022
Types of studies	Randomized controlled trials Diagnostic studies Observational studies
Language	English
Comments/context/suggested keywords. Please give as much detail as possible	- Evaluation of biomarkers (conventional and new ones) - Molecular biomarkers: gene expression, microRNAs, proteomics, metabolomics, cell free DNA, cell free methylated DNA, cell free RNA. - Non-invasive biomarkers in different sample types: peripheral blood mononuclear cells, plasma, serum - Biomarkers using liver graft tissue - Specificity, sensitivity, positive predictive value, negative predictive value - Gold standard, controls, study endpoints

concomitant autoimmune disease, recipient age <42 years, and donor-recipient sex mismatch have been associated with higher risk of recurrent AIH (rAIH) [12, 13]. Post-transplant auto-antibodies, such as anti-nuclear (ANA), anti-smooth muscle antibodies (ASMA), and anti-LKM at high titer, are also predictive of rAIH [14], even though they also appear in 64% of patients transplanted for non-autoimmune liver diseases and are therefore not specific [15]. Similar to the pre-LT setting, rAIH is characterized by elevated transaminases, hyper-gammaglobulinemia, and increased IgG. The gold standard for diagnosing disease recurrence remains histology, with typical features including lymphoplasmocytic interface hepatitis, lobular hepatitis, and portal plasmocytic infiltration [16, 17].

Metabolic dysfunction associated steatohepatitis (MASH) is one of the most frequent liver diseases in the United States and Europe [18], and its prevalence varies from 7% to 30% among metabolic dysfunction-associated steatotic liver disease (MASLD) patients [19]. MASH has become the second indication for LT after alcohol-related liver disease in the United States (US), and it currently represents 8.4% of LT indications in Europe [20]. In terms of post-transplant outcomes, 10-year graft survival of 62% has been described, similar to non-MASH patients [21]. On the contrary, another study from the United States described post-LT graft survival that was significantly lower compared to PSC, PBC, and AIH indications [22]. Pre-transplant factors, such as metabolic syndrome, insulin resistance, and arterial hypertension, are not reliable predictors of disease recurrence. Rather, high pre- and post-LT body mass index and increased post-LT triglyceride levels were significant predictors [23]. Simliar to autoimmune liver diseases, liver biopsy remains the most reliable method for assessing rMASH and its severity after LT [24, 25].

For all of the aforementioned diseases, liver biopsy remains the gold standard for the diagnosis of primary disease recurrence. As such, identification of more reliable biomarkers is urgently needed.

Methods

MEDLINE and EMBASE databases were used to search for relevant articles (**Table 1**). The following search terms were used in the MEDLINE database: liver transplantation/recurrent liver disease/MASH/autoimmune diseases (autoimmune hepatitis, primary biliary cholangitis)/primary sclerosing cholangitis recurrence. A manual search was also conducted of the reference lists in the review articles. The study inclusion period was 2000–2022. Prospective, observational, and diagnostic studies and reviews were included. Specific exclusion criteria were (i) studies including LT for cryptogenic disease, even though autoimmune liver diseases or MASH were diagnosed on follow up; (ii) studies including clinical parameters such as hypertension; body mass index; or classic biological parameters, such as liver enzymes, bilirubin, alkaline phosphatase, AMA, ANA, ASMA, IgG, serum glucose, HbA1c, cholesterol, and/or triglycerides. The flowchart summarizing the literature search is reflected in **Figure 1**.

TABLE 3 | Literature Search Request for the 4 PICO questions in Liver Transplantation. Immunosuppression weaning in Liver Transplantation.

Topics and research questions	Can biomarkers be used to safely wean IS (minimization and eventually full withdrawal)?
Population(s) e.g.:	
• Type of transplant(s)	Liver transplant recipients receiving maintenance immunosuppression.
• Age (adult)	Adult
• Condition	Maintenance IS
Intervention	Use of biomarkers to guide IS minimization and withdrawal
Comparators (Where appropriate)	IS minimization and withdrawal based on classical clinical approach (risk factors associated with rejection, time from LT, trough levels)
Outcomes	- weaning IS without rejection - time to minimal/no immunosuppression - adverse events associated with IS (Diabetes, AHT, CVD, *de novo* cancer), subclinical graft injury - acute rejection
Exclusion criteria (optional)	1. Journal with IF <2 2. Systemic review and metanalysis 3. Conference abstracts 4. Studies with less than 25 patients
Search period	January 2005- May 2022
Types of studies	Randomized controlled trials Diagnostic studies Observational studies
Language	English
Comments/context/suggested keywords	- Evaluation of biomarkers (conventional and new ones) - Molecular biomarkers: gene expression, microRNAs, proteomics, metabolomics, cell free DNA, cell free methylated DNA, cell free RNA. - Non-invasive biomarkers in different sample types: peripheral blood mononuclear cells, plasma, serum - Biomarkers using liver graft tissue - Specificity, sensitivity, positive predictive value, negative predictive value - Gold standard, controls, study endpoints

Results

A total of 127 articles were found on recurrent primary diseases, and 11 studies were selected (**Supplementary Table S1**): 3 studies for AIH, 4 for PBC, 2 for PSC, and 2 for MASH. The aims of the studies were to evaluate risk factors for disease recurrence. Eight out of 9 studies reflected the role of human leukocyte antigen (HLA) as risk factor for recurrent autoimmune diseases. The study of Gonzalez-Koch et al. demonstrated that HLA-DR3 or HLA-DR4 with HLA-DR3 were more important risk factors for rAIH than HLA-DR4, even though the difference was not statistically significant [26]. Another study identified HLA-DR3 phenotype in the recipient and/or donor as a risk factor for rAIH [17]. More recently, high-level HLA-DR mismatch was associated with an increased risk of rAIH [27]. Concerning rPBC, in Sanchez's study, only donor alleles A1, B57, B58, DR44, DR57, and DR58 and recipient allele B48 were found more frequently in patients with disease recurrence, but there was no significant association for HLA mismatches between donor and recipient [28]. On the other hand, Guy et al. found an increased mismatch of donor DR3 and recipient DR4 in patients with rPBC [29], and another study reported that HLA-A, -B, and -DR mismatches were risk factors for disease recurrence [30]. The study from Carbone et al. found that risk of rPBC was greatest for rs62270414 genotype for IL12A locus [31].

Regarding rPSC, in one study which had all HLA data available for all donors and recipients, HLA-DRB1*08 allele was detected in either donor or recipient with rPSC [32]. On the other hand, in the study by Bajer et al., HLA-DRB1*07 in the donor represented a potential risk factor for rPSC [33]. For rNASH, G-allele in position rs738409 of patatin-like phospholipase domain-containing protein 3 (PNPLA3) presence in the recipient was associated with an increased hepatic concentration of triglycerides and with rMASH, though liver biopsy to confirm the diagnosis was only available in a minority of patients and recurrent disease diagnosis was based on biological and clinical criteria [34]. A more recent study found 16 metabolites associated with rMASH compared to MASLD. The most differentially expressed chemical class was phosphatidylcholines, with 10 of these lipids significantly decreased in the MASH cohort. The remaining metabolites consisted of AAs, sterols, phosphatidylethanolamines, and phingomyelins [35].

The summary of the evidence addressing the recurrent diseases in LT key question by included studies is shown in **Table 5**.

Recommendation

Additional studies are needed before any recommendation can be issued regarding the application of biomarkers to reliably predict and/or diagnose disease recurrence after liver transplantation.

Quality of evidence: Very Low.

Grade of recommendation: Strong for.

Discussion and Next Steps

Post-LT recurrence of the initial disease process is heterogeneous in presentation and severity. Due to its impact on long-term outcomes, it is important to identify new biomarkers for early identification.

Among the 9 studies selected for autoimmune diseases, the majority had a small sample size, with only two studies including more than 100 patients. The small cohorts can be explained by the rarity of these recurrent diseases. Eight studies supported specific

TABLE 4 | Literature Search Request for the 4 PICO questions in Liver Transplantation. Chronic kidney disease development in liver transplantation.

Topics and research questions	Can biomarkers be used to predict chronic kidney disease (CKD) in liver transplant recipients
Population(s) e.g.:	
• Type of transplant(s)	Liver transplant recipients receiving maintenance immunosuppression
• Age (pediatric/adult)	Adult
• Condition	Maintenance immunosuppression
Intervention	Use of biomarkers to predict future development of CKD and progression to end stage renal disease (ESRD)
Comparators (Where appropriate)	CKD prediction based on classical clinical approach (risk factors associated with CKD such as diabetes, hypertension, age, pre-LT kidney function, trough levels of calcineurin inhibitors. . .)
Outcomes	- Development of CKD stage III (<60 mL/min eGFR) - Progression through different stages of CKD (I to V) - Development of ESRD (CKD stage V), need for hemodialysis, need for kidney transplantation - Patient/graft survival in relation to CKD stage
Exclusion criteria (optional)	1. Journal with IF <2 2. Papers without a clear ethical approval 3. Systemic review and metanalysis 4. Conference abstracts
Search period	January 2005- May 2022
Types of studies	Randomized controlled trials Diagnostic studies Observational studies
Language	English
Comments/context/suggested keywords. Please give as much detail as possible	- Evaluation of biomarkers (conventional and new ones) - Non-invasive biomarkers in different sample types: mainly plasma, serum, urine, DNA (genetic predictors) - Predictive models (clinical alone, biomarker alone, clinical + biomarker) - Specificity, sensitivity, positive predictive value, negative predictive value - Would include endpoints of GFR: serum creatinine-based estimated (eGFR) using MDRD, CKD-EPI; addition of cystatin-C to these equations; measured GFR using inulin, iothalamate, iohexol, or even radionuclide renal scans

HLA or donor-recipient HLA mismatches as risk factors for disease recurrence. However, given the small number of patients included and the differences in disease diagnosis (per protocol versus clinically indicated liver biopsy), the correlation between HLA and recurrent autoimmune diseases should be further investigated, and strong recommendations cannot be made. One study evaluated genetic loci associated with rPBC. Though the study was well-conducted on a relatively large cohort of patients, it remains singular, and more data are needed.

Regarding rMASH, metabolomic analysis was shown in one study to be a promising tool. Further studies are needed, however, as the study included a small number of observations and analyzed many variables, thereby increasing the potential for errors.

Overall, given the low number of studies addressing this issue and their retrospective nature, the small number of patients included, heterogeneous inclusion criteria and results, and incomplete datasets in some instances, no strong recommendations regarding the use of specific biomarkers to detect post-LT recurrence of primary liver disease can be made. Prospective studies must be conducted to establish the role of biomarkers in predicting and diagnosing these processes.

RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER LIVER TRANSPLANTATION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with an incidence that is predicted to increase in the coming decades [36]. Unfortunately, mortality associated with HCC remains high. In fact, treatment strategies are only curative for early-stage tumors. Among these, LT is considered the best treatment option for BCLC A stage one patients selected according to Milan criteria (MC) [37]. Although application of MC led to a significant decrease in recurrence rates, recurrence still occurs in some that fulfill the criteria and, more importantly, leaves out a significant proportion who might be cured by LT despite being outside MC [38]. Several models have been proposed to expand LT HCC inclusion criteria, usually based on morphological features, simple biological markers (e.g., alpha-fetoprotein—AFP), explant pathology, and/or response to locoregional therapy (LRT). Depending on the time frame they are applied (pre-vs. post-LT), they might be used to predict recurrence and help in the selection process and/or to adapt post-LT strategies. These models have shown to adequately predict recurrence risk, yet they continue to lack molecular factors reflecting the biological complexity of HCC and remain only partially predictive in this regard [38, 39].

Indeed, there are many known genetic mutations and other molecular alterations occurring in HCC tumors, and multiple studies report associations between molecular biomarkers and tumor-specific post-LT outcomes (i.e., presence, timing, and location and/or extent of HCC recurrence) [40]. Biomarkers that have been assessed in human tissue appear useful for the classification of HCC into subclasses indicative of disease aggressiveness and prognosis. While theoretically promising, drawbacks associated with such assays and biologically based classification systems include lack of prospective, well-

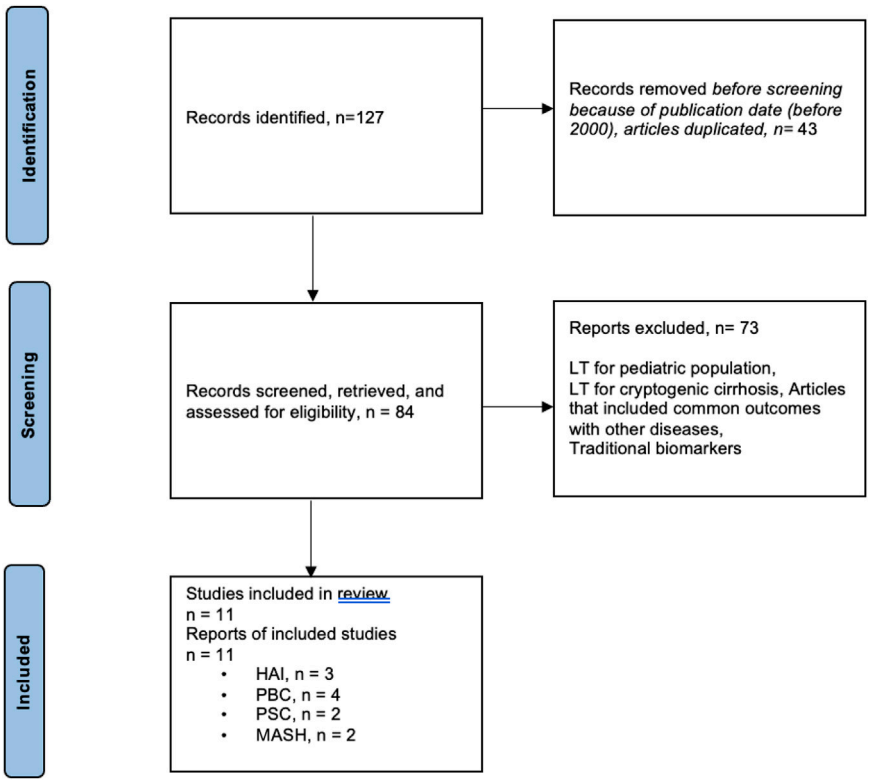

FIGURE 1 | Flowchart summarizing the selection process of studies included in the evaluation of biomarkers for recurrent diseases in liver transplantation.

powered studies that definitively establish their ability to accurately predict post-LT HCC recurrence and/or survival [41, 42]. As well, molecular assays relying on tissue are invasive and often require the actual liver explant for their assessment, severely (if not altogether) limiting their utility in pre-LT patient stratification and selection and optimization of liver allograft utilization (primary goals). "Liquid biopsy" is promising tool in this regard, as it represents a minimally invasive approach to analyzing tumor components (cells or small pieces of DNA, RNA, or other molecules released by tumor cells) without need for tissue [43–45]. Liquid biopsy is dynamic and may be assessed at different peri- and post-LT time points [46].

Methods

The specific question that was made for literature review was (**Table 2**): Can circulating tumor biomarkers be used to predict HCC recurrence? The study population included adult liver transplant recipients undergoing LT due to HCC related liver disease. The intervention was the analysis of whether circulating tumor cells or components could accurately predict HCC recurrence and thereby improve posttransplant monitoring, while the comparator was the use of classical models (up-to-seven, Milan criteria, RETREAT Model). Outcomes assessed included HCC recurrence, cost of post-transplant monitoring, HCC recurrence-free survival, and overall post-transplant survival.

The initial literature search was performed by the CET, followed by the inclusion of additional articles extracted from the bibliographies. The study period was 2010–2022. Inclusion criteria were English language studies published on adult patients (18 years and older) analyzing the association between circulating tumor biomarkers and post-LT HCC recurrence. Exclusion criteria included evaluation of traditional serum biomarkers (AFP, serum C-reactive protein, des-gamma-carboxy prothrombin, bilirubin, lipid profile, and protein induced by vitamin K absence or antagonist-II) as well as tissue-based biomarkers. Randomized clinical trials, diagnostic, and observational studies were included.

Results

The literature search produced a total of 111 articles. Excluding publications arising prior to 2010, those written in a language other than English, congress publications, articles addressing traditional biomarkers or biomarkers evaluated in explanted tissue, and studies in which detecting HCC recurrence was not the objective, a total of 15 studies related to liquid biopsy were included. The PRISMA flowchart describing the number of studies identified by the literature search and number of studies selected for inclusion in the consensus statement appears in **Figure 2**.

According to the results of the literature search, few studies evaluating the utility of liquid biopsy for the assessment of HCC

TABLE 5 | Summary of evidence for biomarkers in recurrent diseases after LT.

Number of studies			No. of patients	Factors that may decrease the certainty of the evidence					Quality of evidence (GRADE)
RCT	Observational comparative	Observational non-comparative		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Index Test 1: AIH recurrence									
0	0	3	133	serious	not serious	not serious	serious	Likely	Very Low (D)
Index Test 2: PBC recurrence									
0	4	0	502	serious	serious	very serious	serious	Likely	Very Low (D)
Index Test 3: PSC recurrence									
0	1	1	116	serious	serious	serious	serious	Likely	Very Low (D)
Index Test 4: MASH recurrence									
0	0	2	274	serious	serious	very serious	serious	Likely	Very Low (D)

Effect estimates from comparative studies: This is a qualitive (not quantitative) evaluation of the effect estimate/size derived from comparative studies. Examples are shown above on such assessments. Limitations: Make a judgement on the risk of bias across studies for an individual outcome. It is possible to consider the size of a study, its risk of bias and the impact it would have on the summarized effect. Inconsistency: Evaluate the difference in the magnitude of effects across studies. Widely differing estimates of the effects indicate inconsistency. Indirectness: Make a global judgement on how dissimilar the research evidence is to the clinical question at hand (in terms of population, interventions, and outcomes s studies). Imprecision: Consider the optimal information size (or the total number of events for binary outcomes and the number of participants in continuous outcomes) across all studies. Results may also be imprecise when the confidence intervals (CI) of all the studies or of the largest studies include no effect and clinically meaningful benefits or harms. Publication bias can be suspected when the body of evidence consists of only small positive studies or when studies are reported in trial registries but not published. Statistical evaluation of publication bias is not possible in this case.

tumor biology in the LT setting, including risk for recurrence, have been published to date (**Supplementary Table S2**). Risk that negative results or studies have not been reported remains. Studies that have been published to date have focused on exosomal miRNA (2 studies) [47, 48], circulating messenger and micro-RNA (mRNA and miRNA, respectively) (7 studies) [49–55], and circulating tumor cells (CTCs) (6 studies) [53, 56–60].

Assessment of HCC CTCs in the LT setting has been reported in eight studies, though only six evaluate their prognostic value and relevance to post-LT outcomes. Studies evaluate CTCs at different peri-operative time points (both prior to and following LT) and include relatively small patient cohorts largely recruited in Asia. Data remain conflicting regarding the utility of isolated CTC measurements (pre-LT only, for example) in predicting HCC recurrence [56–58], while dynamic CTC assessment, including evaluation of CTC subtypes, may increase CTC prognostic capacity [53, 59, 60]. In general, while preliminary evidence appears to support a role for CTCs in HCC prognostication in LT candidates and recipients, larger prospective studies recruiting more patients in more geographical regions are needed before any recommendations regarding their use can be made.

Micro RNAs are short, non-coding RNAs that post-transcriptionally regulate gene expression by binding with mRNA; circulating levels of both have been measured in HCC LT recipients in the context of two and five studies, respectively. The preliminary results they report suggest potential associations between HCC recurrence and circulating pre-LT mRNAs encoding different proteins (albumin, h-TERT, AFP) as well as between HCC recurrence and circulating post-LT miRNAs [49–51, 54, 55]. Exosomal RNAs and circulating free DNA have also been evaluated in preliminary clinical studies and variably associated with post-LT recurrence and survival [47, 48].

The summary of the evidence addressing the HCC key question by the included studies is shown in **Table 6**.

Recommendation

In summary, on the question "Can biomarkers be used to predict HCC recurrence following liver transplantation," and based on the low quality of evidence, the following recommendation was issued: While preliminary studies suggest a role for molecular biomarkers measured in liquid biopsy (circulating tumor cells, in particular) in prediction of HCC recurrence, additional studies are needed before any recommendation can be issued regarding their application in clinical practice, either as predictive factors to select patients for liver transplantation or to guide post-transplant management.

Quality of Evidence Low (C).

Strength of Recommendation Weak for.

Discussion and Next Steps

HCC is one of the most common cancers worldwide and one of the most frequent indications for LT. Despite careful selection using MC, HCC recurs in some patients who meet criteria, and other patients are left out who could potentially benefit from this therapy. Currently, there are models mostly based on clinical variables and traditional biomarkers that predict recurrence and thus help with patient selection [38, 39]. Because many of the genetic alterations in HCC are now known and some have been associated with post-transplant outcomes, we aimed at determining the role of the new biomarkers in predicting HCC recurrence. The purpose of the present review was to evaluate the evidence for new biomarkers, and to determine their potential role in patient selection as well as recurrence surveillance. Our findings indicate that while there is potential to better select HCC patients, the evidence remains low, and these biomarkers cannot be recommended in clinical practice until more evidence is gathered.

Aside from the clear objective of improving candidate selection when applied in the pre-LT setting, a role for HCC molecular biomarkers in directing post-LT patient management is also discussed. Post-LT strategies that might be applied in high-

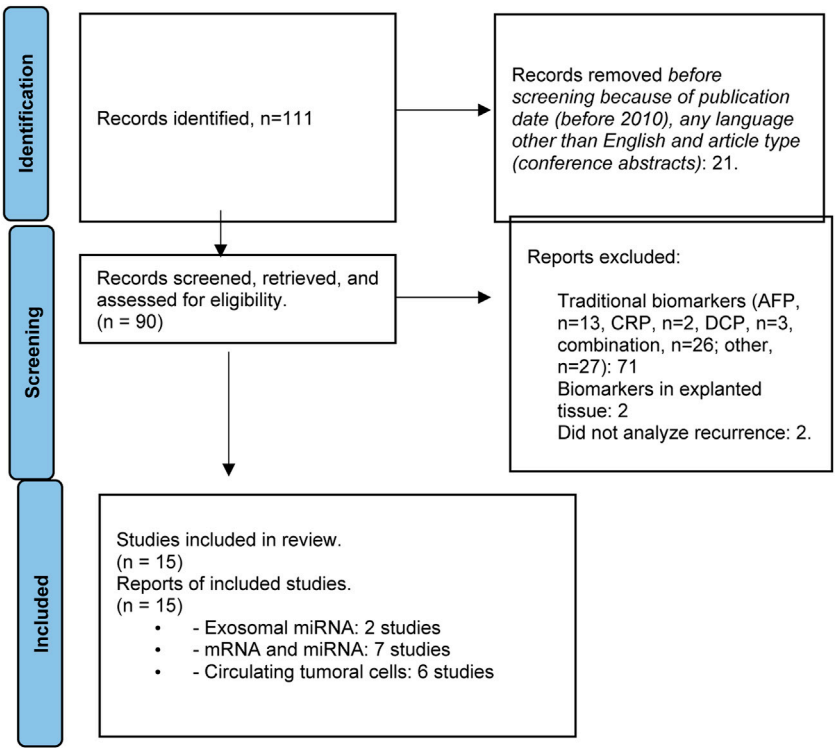

FIGURE 2 | Flowchart summarizing the selection process of studies included in the evaluation of biomarkers for HCC recurrence.

risk patients include implementation of adjuvant systemic therapy/-ies and/or HCC surveillance protocols, though neither has been shown to be of clear clinical benefit [61]. As well, it is important to note that serial liquid biopsies performed during post-LT follow-up may create the complex and potentially distressing situation whereby HCC recurrence is "detected" (likely present) yet not located or visualized on cross-sectional imaging. How often such cases will arise and how best to proceed when they are encountered, with options including watchful waiting vs. "blind" administration of systemic therapy (both of which are associated with certain drawbacks for patients and clinicians) remain uncertain.

IMMUNOSUPPRESSION WEANING IN LIVER TRANSPLANTATION

Outcomes following LT have significantly improved over the past three decades, and the use of modern immunosuppressant agents has been an important factor in this regard [62]. Unfortunately, the need for long-term IS is associated with serious complications and increases the chances of toxicities, rates of opportunistic infection, and malignancy [62–64]. For example, the use of CNIs increases the incidence of chronic kidney disease (CKD) in LT recipients [65]. Therefore, the establishment of long-term graft tolerance without ongoing need for IS is a primary goal in transplantation. However, we currently lack the tools necessary to identify patients who may benefit from IS minimization and withdrawal or to even identify those patients who are at risk of acute rejection (AR) upon IS reduction. Recent literature has described a variety of molecular, cellular, and histological markers originating from the peripheral blood and allograft that may help predict post-LT patients who can successfully be weaned off IS or who might be at risk of AR upon IS reduction. Although graft biopsy is an invasive technique and current practices are more interested in non-/minimally invasive techniques for patient stratification, we included graft biopsy-based biomarkers in our analysis, to assess if they offer any superior outcome compared to the recent "liquid biopsy" technique.

Methods

For the third PICO question, "Can biomarkers be used to safely wean IS (minimization and/or full withdrawal)?", the study population was again adult liver transplant recipients undergoing IS minimization or withdrawal (**Table 3**). The population also consisted of patients who were assessed for markers for acute graft injury following LT. The outcome of the study was evaluation of non-invasive and invasive biomarkers from peripheral blood mononuclear cells, plasma, serum, and liver graft tissue. Molecular biomarkers of interest included gene expression, miRNAs, proteomics, metabolomics, cell-free DNA (cfDNA), cell-free methylated DNA, and cell-free RNA. The flowchart summarizing the literature search is reflected in **Figure 3**.

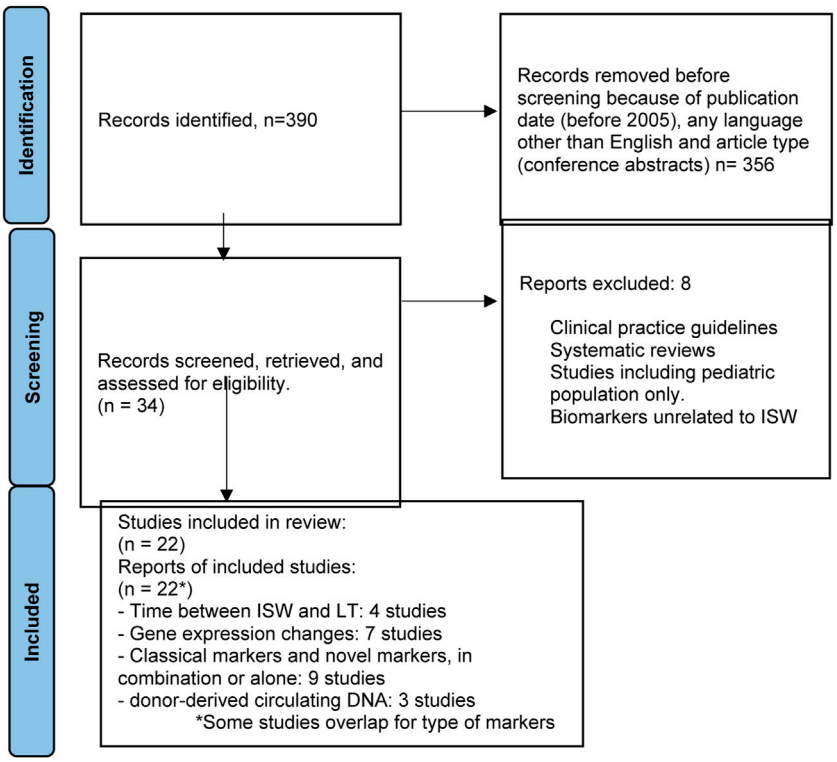

FIGURE 3 | Flowchart summarizing the selection process of studies included in the evaluation of biomarkers for immunosuppression minimization and/or full withdrawal.

Results

Supplementary Table S3 summarizes the studies assessing the role of biomarkers in safe IS minimization or withdrawal [66–87]. A positive association was observed in three studies between time from LT to IS withdrawal (ISW) among non-viral patients [66]. However, this remains conflicting, as North American studies have not observed this finding [87]. *De novo* donor specific antibody development was found to be associated with ISW [69]. Intra-tissue gene expression and immune cell infiltrations have been observed to have an association with induction and achievement of ISW. These, however, are invasive biomarkers and do not constitute effective biomarkers, and the studies supporting their use are potentially biased, as they were performed on relatively small numbers of patients.

Serum miRNA signatures were analyzed as biomarkers predicting development of operational tolerance (OT), with miR-483-3p and miR-885-5p signatures found to be positively associated with OT [70]. In transplantation, the contribution of donor-derived cfDNA has been an important indicator of graft injury post-transplantation [5, 84, 86]. Methylation-induced alterations in the released DNA were identified using droplet-digital PCR (ddPCR) to determine acute injury [82]. ddPCR was also used to identify genomic SNPs between donor and recipient to give a better indication of injury [86]. The donor-derived cfDNA (dd-cfDNA) had serial elevation of dd-cfDNA between injury and rejection and could identify pre-clinical graft injury in the context of normal liver function tests compared to rejection [83]. While these studies indicate the prospects of a non-invasive biomarker, independent validation and replication is needed using larger cohorts of LT patients from a variety of geographical and racial background to identify the benefit of their use.

The summary of the evidence addressing the IS minimization/withdrawal key question by the included studies is shown in **Tables 7–9**.

Recommendation

Based on the moderate quality of evidence available, the following recommendation was issued: We suggest that biomarker assays may be able to help to guide ISW by monitoring liver injury. The use of longitudinal evaluations using non-invasive markers may lead to better stratification of patients for ISW.

Quality of evidence: Moderate.

Strength of recommendation: Weak for.

Discussion and Next Steps

The prognostic and diagnostic value of invasive and non-invasive biomarkers to optimize IS and evaluate graft injury has been widely explored in LT. However, despite a decade of research, no LT biomarkers are currently available for use in clinical practice. Large multicenter clinical trials have generated vast amounts of data and information at various molecular levels, demonstrating a promising opportunity for cell-free biomarkers to be introduced into clinical care. Findings have not yet been translated into routine clinical use, due to small sample sizes, and the lack of proper control groups or independent validations.

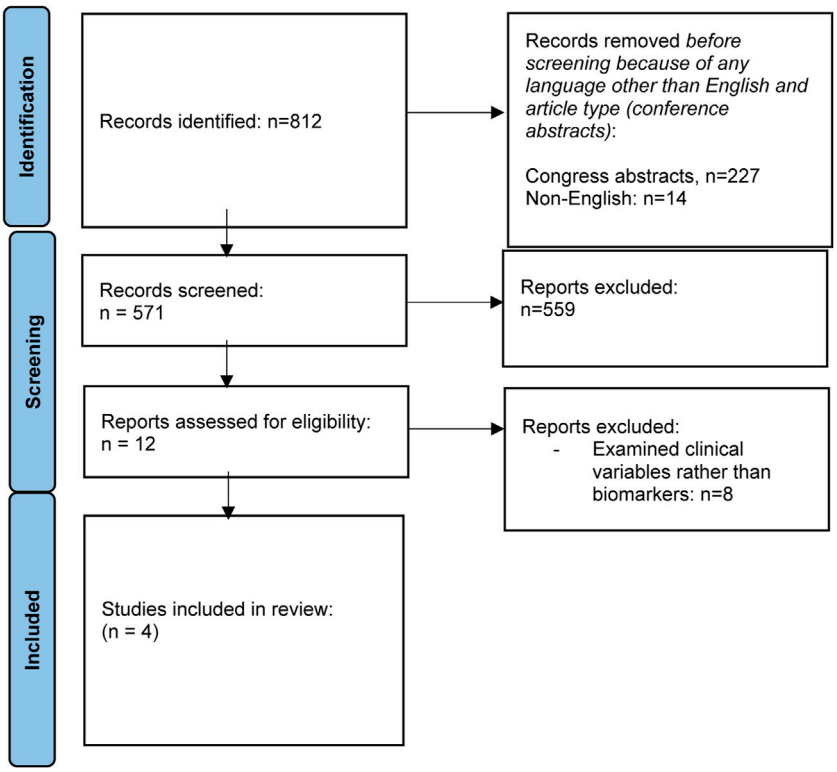

FIGURE 4 | Flowchart summarizing the selection process of studies included in the evaluation of biomarkers for CKD after liver transplantation.

TABLE 6 | Summary of evidence for biomarkers in HCC after LT.

Number of studies			No. of patients	Factors that may decrease the certainty of the evidence					Quality of evidence (GRADE)
RCT	Observational comparative	Observational non-comparative		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Index Test 1: HCC recurrence									
0	4	10 (retrospective)	1,018	serious	serious	serious	serious	Likely	Very Low (D)
Index Test 2: Cost of posttransplant monitoring									
0	0	0							Very Low (D)
Index Test 3: HCC recurrence free survival									
0	2	6 (retrospective)	353	serious	serious	serious	serious	Likely	Very Low (D)
Index Test 4: Post-transplant patient survival									
0	0	3 (retrospective)	194	serious	serious	very serious	serious	Likely	Very Low (D)

Inconsistency: Evaluate the difference in the magnitude of effects across studies. Widely differing estimates of the effects indicate inconsistency. Indirectness: Make a global judgement on how dissimilar the research evidence is to the clinical question at hand (in terms of population, interventions, and outcomes across studies).
Imprecision: Consider the optimal information size (or the total number of events for binary outcomes and the number of participants in continuous outcomes) across all studies. Results may also be imprecise when the confidence intervals (CI) of all the studies or of the largest studies include no effect and clinically meaningful benefits or harms.
Publication bias can be suspected when the body of evidence consists of only small positive studies or when studies are reported in trial registries but not published. Statistical evaluation of publication bias is not possible in this case.

CHRONIC KIDNEY DISEASE (CKD) IN LIVER TRANSPLANT RECIPIENTS

An estimated 40% of liver transplant recipients develop stage 3 CKD, and about 18% will develop end-stage renal disease within 5 years of LT, both of which are associated with increased risk of death [88, 89]. One of the primary culprits of renal deterioration post-transplant is calcineurin inhibitors. Although early reductions in CNIs within 1 year of transplant are associated with improvements in long-term renal function, reduced dosing of CNIs are also associated with higher rates of AR [90]. As such, identifying biomarkers

TABLE 7 | GRADE approach-based summary of the quality of evidence for the development of operational tolerance or risk of injury upon weaning of immunosuppression.

Number of studies			No. of patients	Factors that may decrease the certainty of the evidence					Quality of evidence (GRADE)
RCT	Observational comparative	Observational non-comparative		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Index Test 1: Time between ISW and LT in non-viral patients									
0	3	0	163	not serious	not serious	serious[a]	not serious	None	Low (C)
Index Test 2: Combination of non-invasive PBMC GEX: FGL2/IFNG ratio and invasive baseline intrahepatic FOXP3/IFNG ratio at transplant									
0	0	1	14	serious[b]	not serious	not serious	not serious	None	Very Low (D)
Index Test 3: dnDSA during IS minimization									
0	2	0	130	not serious	not serious	serious[c]	not serious	None	Very Low (D)
Index Test 4: serum miRNA profile of hsa-miR-483-3p and hsa-miR-885-5p									
0	1	0	64	not serious	not serious	not serious	not serious	None	Low (C)
Index Test 5: Association between portal vein infiltrates and elapsed time post-ISW									
0	0	1	18	serious[b]	serious[d]	not serious	not serious	None	Very Low (D)
Index Test 6: Association of intrahepatic GEX of select genes[e] and elapsed time post-ISW									
0	0	1	18	serious[b]	serious[d]	not serious	not serious	None	Very Low (D)
Index Test 7: *Ex vivo* cytokine production by PBMCs									
0	1	0	24	serious[b]	serious[f]	not serious	not serious	None	Very Low (D)
Index Test 8: Peripheral blood Vδ1/Vδ 2 T cell ratio quantification									
0	2	0	34	serious[b]	not serious	not serious	not serious	None	Low (C)
Index Test 9: Gender									
0	1	0	98	serious[b]	not serious	not serious	not serious	None	Low (C)
Index Test 10: Intrahepatic gene expression[g]									
0	1	0	75	serious[b]	serious[h]	not serious	not serious	None	Low (C)
Index Test 11: Serum hepcidin and ferritin									
0	1	0	80	serious[b]	not serious	not serious	not serious	None	Low (C)
Index Test 12: T-cell production of IFN-γ									
0	1	0	24	serious[b]	not serious	not serious	not serious	None	Low (C)

[a]*While 4 studies report the benefit of a longer time duration between LT and ISW commencement, some studies did not find it significant in their patient cohort. Moreover, 2 of the studies use the same patient population.*

[b]*Only one study with low sample size was included.*

[c]*The cut-off for DSA MFI is not truly defined and different studies have used different MFI cut-offs, depending on the variability of mismatched HLA loci.*

[d]*The invasive nature of the identified biomarker and post-ISW biopsy as indicators of graft acceptance are not efficient biomarkers for ISW-associated graft injury.*

[e]*Genes of interest: FOXP3, CXCL10, CXCL9, UBD, IRF1, STAT1, IL32, CD52, CD68, STAT1, GPNMB, S1PR1, RGS5, ENPP2, MSL3, OPN3, PAK2, CDH5, SELP.*

[f]*While the study demonstrates an increase in cytokine production, the isolation and culturing of PBMCs* ex vivo *will add complexity and is an indirect indicator of OT.*

[g]*5-gene (*CDHR2, MIF, PEBP1, SOCS1, TFRC*) signature and iron metabolism genes,* HAMP *and* TFRC *(FDR = 0, FC > |2|), and* FTHL12 *and* FTHL8.

[h]*Invasive biomarker and hence an indirect indicator of rejection.*

for predicting CKD in liver transplant recipients would help select patients for early CNIs dose reductions and other nephroprotective interventions.

Methods

The search on the topic question "Can biomarkers be used to predict chronic kidney disease (CKD) in liver transplant recipients" is summarized in **Table 4**. Adult LT recipients under maintenance IS were the focus of the literature search. Outcome measures included (i) development of CKD stage III (<60 mL/min eGFR), (ii) progression through different stages of CKD (I to V); (iii) development of ESRD (CKD stage V), need for hemodialysis, need for kidney transplantation; and (iv) patient/graft survival in relation to CKD stage. The flowchart summarizing the literature search is reflected in **Figure 4**.

Results

Most of the literature assessing variables associated with post-LT CKD examines clinical variables, rather than biomarkers. Only four studies were identified that assess the role of biomarkers in predicting post-transplant CKD [91–94]. **Supplementary Table S4** summarizes baseline characteristics of the studies reviewed. PRESERVE used a discovery and validation cohort to develop a predictive model for post-LT CKD, incorporating beta-2 microglobulin (B2MG) and CD40 antigen [91]. Cullaro et al demonstrated that post-LT urinary neutrophil gelatinase-associated lipocalin (uNGAL) may be helpful in predicting post-LT CKD, particularly when combined with clinical variables [92]. Levitsky et al used proteomic testing to identify several proteins of interest, which may be associated with post-LT CKD [93], while Milongo et al found no association between the pre-LT urinary peptidome and CKD 6 months post-LT [94].

The summary of the evidence addressing the prediction of CKD among stable LT recipients is shown in **Table 10**.

Recommendation

Based on the very low quality of evidence available, the following recommendation was issued: We suggest that biomarker assays

TABLE 8 | GRADE approach-based summary of the quality of evidence for the identification of subclinical graft injury and acute injury markers during IS.

Number of studies			No. of patients	Factors that may decrease the certainty of the evidence					Quality of evidence (GRADE)
RCT	Observational comparative	Observational non-comparative		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Index Test 1: Intrahepatic 11-gene marker for probable TCMR									
0	1	0	341	not serious	not serious	not serious	not serious	None	Low (C)
Index Test 2: Combination of ALT with liver stiffness measurement or DSAs									
0	0	1	185	serious[a]	serious[b]	not serious	not serious	None	Very Low (D)
Index Test 3: Combination of ALT with class II DSAs									
0	0	1	157	serious[a]	serious[b]	not serious	not serious	None	Very Low (D)
Index Test 4: serum miRNA profile of hsa-miR-483-3p and hsa-miR-885-5p									
0	1	0	130	not serious	not serious	not serious	not serious	None	Low (C)
Index Test 5: Galectin-1									
0	1	0	45	serious[a]	not serious	not serious[c]	not serious	None	Very Low (D)

[a]*Only one study with low sample size was included.*
[b]*Study indicates the indirect stratification of patients with a medium to moderate injury.*
[c]*Presentation of the data in the original article is a bit convoluted with the convention of naming of groups and sample size.*

TABLE 9 | GRADE approach-based summary of the quality of evidence for the genomic markers of acute injury post-liver transplantation.

Number of studies			No. of patients	Factors that may decrease the certainty of the evidence					Quality of evidence (GRADE)
RCT	Observational comparative	Observational non-comparative		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Index Test 1: detection of donor-derived cell-free DNA (dd-cf-DNA) using next-generation sequencing									
0	1	0	219	not serious	not serious	not serious	not serious	None	Moderate (B)
Index Test 2: detection of pre-identified donor DNA polymorphisms in dd-cf-DNA using droplet digital PCR									
0	2	0	185	not serious	serious[a]	not serious	not serious	None	Low (C)
Index Test 3: serum Diagnostic signature of miR-122 + miR210									
0	1	0	30	serious[b]	not serious	not serious	not serious	None	Low (C)
Index Test 4: plasma signature of miR-181a-5p									
0	1	0	145	not serious	not serious	not serious	not serious	None	Low (C)
Index Test 5: hepatocyte-specific methylated *PTK2B* as marker of dd-cf-DNA									
0	1	0	51	serious[b]	not serious	not serious	not serious	None	Low (C)

[a]*The two studies that have been identified using different cut-off values, thus reducing the potential assay adaptation.*
[b]*Only one study with low sample size was included.*

may be able to help predict chronic kidney disease after liver transplantation.

Quality of evidence: Very Low.

Strength of recommendation: weak for.

Discussion and Next Steps

Given the high prevalence of CKD in post-LT patients, early identification of patients at risk for developing CKD is crucial for targeting interventions to prevent renal deterioration. Biomarkers such as uGAL, B2MG, CD40 antigen, and others may be helpful in the early identification of LT who are prone to developing CKD. However, the available data is insufficient for recommending a specific clinical protocol for using biomarkers to guide reno-protective interventions in post-LT patients. It is also unclear if collecting these biomarkers pre-transplant or post-transplant is more predictive of post-transplant CKD development. The limited number of studies assessing biomarkers for post-LT CKD mostly utilizes small single-center cohorts without independent validation cohorts, making their findings difficult to generalize to the broader LT population. Rather than utilizing single biomarker, it is possible that a combination of multiple biomarkers and clinical variables is the optimal strategy for predicting post-LT CKD. Further studies are needed to validate biomarkers for CKD prior to incorporating into post-LT clinical management including targeting patients for early CNIs reductions.

SUMMARY

LT is a complex medico-surgical process, the consequences of which are lifelong for recipients. While surgical and infectious

TABLE 10 | Summary of the evidence addressing the prediction of CKD.

Paper	Summary	Quality of evidence (GRADE)
Levitsky 2020	"PRESERVE" Analytic approach: Used discovery cohort to develop prediction model for GFR deterioration using 16 proteins in samples collected after LT; validated prediction model using validation cohort Results: Developed predictive model using proteins including Beta-2 microglobulin (B2MG) and CD40 antigen; model had area under the curve (AUC) of 0.814 in discovery cohort and 0.801 in validation cohort year 1 GFR deterioration Limitations: single sample collection timepoint; hepatitis C virus infection status included in predictive model (not a biomarker)	Moderate (B)
Cullaro 2018	Analytic approach: receiver operating characteristic curves used to determine Urinary neutrophil gelatinase-associated lipocalin (uNGAL) cutoffs that maximized sensitivity/specificity Results: uNGAL at 24 h, 24-hour post-LT renal function, initial calcineurin inhibitor use, and age were independent predictors of CKD; AUC for uNGAL24h for CKD at 4 years was 0.65; when all the above variables combined in model-AUC 0.84 at 4 years post-LT Limitations: single center; no validation cohort; incorporated non-biomarkers into predictive model (i.e., age, calcineurin inhibitor use, etc)	Low (C)
Levitsky 2011	Analytic approach: retrospective identification of clinical characteristics associated with CKD in post-LT patients; proteomic testing in two independent cohorts (test and validation) Results: Age, cyclosporine use, and pre-LT GFR independently associated with new onset CKD; 10 proteins associated with new CKD in proteomic evaluations when GFR inputted as a continuous variable including: Cyc, alpha-1-microglobulin, beta-2-microglobulin, TFF3, FABP, factor VII, apolipoprotein H, apolipoprotein CIII, chromogranin A, and CD40 (notably NGAL was not associated with CKD) Limitations: not a prospective study; single sample collection timepoint; single center	Low (C)
Milongo 2015	Analytic approach: prospective study; pre-transplant urine samples collected for peptidome analysis and association with GFR<60 mL/min 6 months post-LT Results: Assessed thousands of peptides in the urinary peptidome, none associated with CKD at 6 months; Viral hepatitis sole independent predictor for CKD Limitations: small sample; single center; single sample collection timepoint	Very low (D)

TABLE 11 | Summary of research questions and recommendations.

Topic	Research question	Recommendation	Quality of evidence	Grade
Recurrent diseases	Can biomarkers be used to diagnose recurrent liver diseases after LT?	Additional studies are needed before a recommendation can be issued regarding the application of biomarkers to reliably predict and/or diagnosis disease recurrence after LT.	Very low	Strong for
Recurrent HCC	Can biomarkers be used to predict HCC recurrence?	While preliminary studies suggest a role for molecular biomarkers measured in liquid biopsy (circulating tumor cells, in particular), in prediction of HCC recurrence, additional studies are needed before any recommendation can be issued regarding their application in clinical practice, either as predictive factors to select patients for LT or to guide post-LT management	Low	Weak for
Immunosuppression weaning	Can biomarkers be used to safely wean immunosuppression?	Biomarker assays may be able to help guide immunosuppression weaning by monitoring liver injury. The use of longitudinal evaluations using non-invasive markers may lead to better stratification of patients for this purpose	Moderate	Weak for
Chronic kidney disease	Can biomarkers be used to predict chronic kidney disease in LT recipients?	Biomarker assays may be able to help predict chronic kidney disease after LT.	Very low	Weak for

complications commonly arise in the early post-LT period, the majority of more remote complications are related to disease recurrence and adverse effects of ongoing IS therapy (cancers, cardiovascular disease, and CKD, in particular). Traditionally, non-specific and oftentimes invasive monitoring has been needed to detect recurrent or *de novo* disease processes as well as to direct interventions, including the active reduction of IS therapy. In recent years, however, the focus of the transplant community at large has shifted to identifying more non-invasive biomarkers, in order to objectively measure and even predict the appearance of adverse events in transplant recipients.

Table 11 summarizes the specific research questions and recommendations formulated by this Working Group regarding the use of biomarkers in post-LT patient care. For studies evaluating use of biomarkers in predicting or detecting disease recurrence, including HCC, methodologies and findings are rather inconsistent, and evidence remains low. For these relatively rare post-LT events, future studies will require simultaneously recruiting patients at

multiple centers and likely in different countries, in order to accrue a sufficient number to evaluate biomarker efficacy. For more routine post-LT care, use of biomarkers to tailor IS management appears helpful, but clear recommendations can still not be given regarding which specific marker or set of markers to use. In the future, larger studies including more diverse post-LT patient populations are needed to validate the utility of makers that have shown promise in preliminary clinical trials.

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

Involved in the conception or design of the work: MB, EM, AH, JL, and VM. Literature screen and review: HE, AL, NW, and HZ. Drafted the article: MB, EM, AH, JL, VM, HE, AL, NW, and HZ. Critically revised the article: all contributing authors. Finally approved the version to be published: all contributing authors.

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CONFLICT OF INTEREST

JL is advisor for Eurofins and advisor/speaker for Mallinckrodt.

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11358/full#supplementary-material

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GLOSSARY

AIH	Autoimmune Hepatitis
AFP	alpha-fetoprotein
AMA	anti-mitochondrial antibody
ANA	anti-nuclear antibodies
AR	acute rejection
ASMA	anti-smooth muscle antibodies
B2MG	beta-2 microglobulin
CET	Centre for Evidence in Transplantation
cfDNA	cell free DNA
CKD	chronic kidney disease
CNIs	calcineurin inhibitors
CRP	Serum C-reactive protein
CTCs	circulating tumor cells
DCP	des-gamma-carboxy prothrombin
ESOT	European Society of Organ Transplantation
HCC	hepatocellular carcinoma
HLA	human leukocyte antigen
IgG	immunoglobulin G
ILTS	International Liver Transplant Society
IS	immunosuppression
ISW	immunosuppression withdrawal
LDLT	live donor liver transplantation
LRT	locoregional therapy
LT	liver transplantation
MASH	metabolic dysfunction-associated steatohepatitis
MC	Milan criteria
PNPLA3	patatin-like phospholipase domain-containing protein 3
PICO	Population, Intervention, Comparator and Outcome
PBC	Primary biliary cholangitis
PSC	Primary sclerosing cholangitis
rAIH	recurrent AIH
rPBC	recurrent PBC
rPCS	recurrent PSC
US	United States
uNGAL	urinary neutrophil gelatinase-associated lipocalin.

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The Clinical Utility of Post-Transplant Monitoring of Donor-Specific Antibodies in Stable Renal Transplant Recipients: A Consensus Report With Guideline Statements for Clinical Practice

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Solid phase immunoassays improved the detection and determination of the antigen-specificity of donor-specific antibodies (DSA) to human leukocyte antigens (HLA). The widespread use of SPI in kidney transplantation also introduced new clinical dilemmas, such as whether patients should be monitored for DSA pre- or post-transplantation. Pretransplant screening through SPI has become standard practice and DSA are readily determined in case of suspected rejection. However, DSA monitoring in recipients with stable graft function has not been universally established as standard of care. This may be related to uncertainty regarding the clinical utility of DSA monitoring as a screening tool. This consensus report aims to appraise the clinical utility of DSA monitoring in recipients

Abbreviations: aABMR, Active antibody mediated rejection; ABMR, Antibody mediated rejection; aTCMR, Acute T-cell mediated rejection; caABMR, Chronic active antibody mediated rejection; cABMR, Chronic antibody mediated rejection; CDC, Complement-dependent cytotoxicity assay; COMMIT, Consensus On Managing Modifiable risk In Transplantation workgroup; CTS, Collaborative Transplant Study; dnDSA, *de novo* donor-specific antibody; DSA, Donor-specific antibody; eGFR, Estimated glomerular filtration rate; ESOT, European Society for Organ Transplantation; GRADE, Grades of Recommendation Assessment, Development and Evaluation; HLA, Human leukocyte antigen; IgG, Immunoglobulin G; IVIG, Intravenous Immunoglobulins; KTR, Kidney transplant recipients; MFI, Mean fluorescence intensity; MHC, Major histocompatibility complex; MVI, Microvascular inflammation; PP, Plasmapheresis; TCMR, T-cell mediated rejection; TLJ, Transplantation Learning Journey; RCT, Randomized controlled trial; SPI, Solid phase immunoassay; STAR, North-American Sensitization in Transplantation: Assessment of Risk workgroup; Subclinical DSA, Donor-specific antibody that has been noted in patients who otherwise do not show any sign of clinical dysfunction of the allograft, such as significantly increased proteinuria or decreased eGFR; QALY, Quality-adjusted life year.

without overt signs of graft dysfunction, using the Wilson & Junger criteria for assessing the validity of a screening practice. To assess the evidence on DSA monitoring, the European Society for Organ Transplantation (ESOT) convened a dedicated workgroup, comprised of experts in transplantation nephrology and immunology, to review relevant literature. Guidelines and statements were developed during a consensus conference by Delphi methodology that took place in person in November 2022 in Prague. The findings and recommendations of the workgroup on subclinical DSA monitoring are presented in this article.

Keywords: DSA, donor-specific HLA antibodies, biomarker, guidelines, subclinical rejection, monitoring

INTRODUCTION

The introduction of the complement-dependent cytotoxicity assay (CDC) in 1969 was the first step towards addressing the deleterious consequences of the humoral immune response and antibody-mediated rejection (ABMR) [1]. Means to investigate these entities were further expanded in later years by the introduction of novel techniques, amongst others, flow-cytometry and solid phase immunoassays (SPI). The use of the sensitive SPI also introduced new dilemmas, such as how to interpret SPI results in case of a negative pretransplant CDC-crossmatch or whether patients should be monitored for the incidence of donor-specific antibodies (DSA) to human leukocyte antigens (HLA) post-transplantation. A consensus meeting in 2013 concluded that pretransplant screening for potential DSA via single-antigen bead (SAB) SPI could be of benefit in risk stratification [2]. As a result, organ allocation organizations have mandated pretransplant screening of HLA antibodies through SAB-SPI as immunological risk stratification in order to define non-acceptable HLA antigens [3]. A recent position paper on pretransplant immunologic risk stratification adds further arguments for this screening practice [4]. Post-transplant monitoring of DSA in patients with graft dysfunction seems to be equally standard practice in case of clinical suspicion of ABMR [5, 6]. However, standardized monitoring of DSA in kidney transplant recipients (KTR) without signs of overt transplant dysfunction, so called *subclinical DSA*, has not universally taken hold as standard of care in most transplant centers. This is likely related to uncertainty regarding the clinical utility of standardized monitoring for subclinical DSA. The main aim of subclinical DSA monitoring is to identify patients who are at greater risk for rejection, either incipient or future, which makes it a form of (transplant) population screening. For such a strategy to have clinical utility, diagnostic and therapeutic ramifications need to be defined in case a patient is identified through screening and these consequences should lead to improved graft and patient outcomes. This may relate to earlier diagnosis and treatment of underlying subclinical rejection, but perhaps also to adaptation of maintenance treatment strategies to prevent future rejection. Additionally, cost-effectiveness of such practices should be considered. While DSA monitoring in stable patients has been recommended in previous guidelines, potential benefits of its consequences were largely unknown, especially in regards to treatment of underlying subclinical rejection [2]. This could possibly explain why some centers were hesitant to implement such strategies. However, uncertainties regarding effective therapeutic ramifications may counteract potential benefits of early detection. This limits potential further improvements in long-term allograft survival from an ontological reductionist view on alloimmunity. In the wake of new developments in this field over the past decade, this consensus report aims to appraise the clinical utility of regular standardized post-transplant monitoring of DSA in stable KTR. We will utilize the criteria for successful screening as developed by Wilson & Jungner in 1964, to ensure that all relevant aspects are reviewed [7] (**Table 1**). Additionally, potential knowledge gaps are identified and future research objectives stated.

To formulate this consensus statement, the European Society for Organ Transplantation (ESOT) convened a consensus conference, comprised of a European panel of experts in transplantation nephrology and immunology. The aim of this conference was to develop guidelines on DSA monitoring. The panel and juries were presented with summaries of evidence. Consensus statements and recommendations, and the Wilson & Jungner criteria they reflect, are summarized in **Table 2**. This document, which will be updated to reflect new evidence as it becomes available, is intended for healthcare providers.

METHODS

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives. The detailed description of methodology used is reported previously [8]. Briefly, key issues were identified by each workgroup and specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome). All PICO questions are listed in **Table 3**. Following the definition of the PICOs, literature searches were developed by expert staff from the Centre for Evidence in Transplantation, who have expertise in conducting systematic reviews and subsequently integrated, when needed, by the steering committee experts. The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. For evaluation of the quality of evidence according

TABLE 1 | Wilson & Jungner's principles of screening.

1. The condition sought should be an important health problem
2. The natural history of the condition, including development from latent to declared disease, should be adequately understood
3. There should be a recognizable latent or early symptomatic stage
4. There should be a suitable test or examination
5. The test should be acceptable to the population
6. There should be an agreed policy on whom to treat as patients
7. There should be an accepted treatment for patients with recognized disease
8. Facilities for diagnosis and treatment should be available
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole
10. Case-finding should be a continuing process and not a "once and for all" project

to GRADE the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance and publication bias. Strength of recommendation was rated as 1 (strong) or 2 (weak).

The Delphi method was applied to arrive at a group opinion during the consensus conference. Complete information, including the list of consensus conference workgroup domains and topics, and the process regarding consensus conference participant selection, development and refinement of consensus statements, and modified Delphi methodology including consensus polling, were determined before the conference held in Prague, Czech Republic, November 13–15, 2022, as previously reported [8] **Table 3**.

Efforts Should Be Made to Prevent Late Renal Allograft Loss, of Which One of the Leading Causes Is ABMR. (1A)

For a successful screening strategy it is important that the disease is relevant and constitutes an significant health problem. Breakthroughs in maintenance immunosuppression during the latter part of the past century drastically increased kidney graft survival rates [9]. This was, however, realized mainly through increases in graft survival over the first year. Comparably less progress has been made in improving graft attrition rates beyond the first year during this era. However, more recent European analysis of collaborative transplant study (CTS) data showed that improvement of long-term graft survival since 2000 was greater than short-term advancement, independently of changing donor and recipient characteristics, likely reflecting the evolutions in posttransplant monitoring and management [10, 11]. An important limiting factor to prolong long-term death-censored graft survival is the development of antibody-mediated rejection (ABMR), in which DSA play an important role [12]. This entity is recognized as a major cause for overall death-censored renal allograft loss in recent decades [13, 14]. The Banff '19 pathology classification recognizes three forms of ABMR in renal allografts: active, chronic active and chronic ABMR [5]. Even though there is incidental empiric evidence for reversal in the case of (hyper)active forms of ABMR [15], all forms of ABMR infer a great risk for graft failure [16]. A recent analysis attributed around a third of all allograft loss to ABMR, particularly contributing to late allograft failure [17]. Therefore, it seems undisputable that diminishing the rate of allograft loss due to ABMR is an important health issue in kidney transplantation and we recommend that efforts to further improve long-term graft survival should explore new openings to steer away from the current diagnostic and therapeutic nihilistic view on chronic rejection.

Clinicians Should Note That DSA Are Associated With a High Risk of Rejection, Primarily ABMR, and Subsequent Allograft Loss. (1A)

Epidemiological Associations Between HLA-DSA and Allograft Outcome

For screening to be successful, one should have an understanding of how underlying pathological processes can develop into overt graft dysfunction.

In case of ABMR, the screening marker itself seems to be implicated in the underlying pathological process [6, 12]. This is

TABLE 2 | Summary of statements and recommendations.

Recommendations	GRADE level	W&J criterium
Efforts should be made to prevent late renal allograft loss, of which one of the leading causes is ABMR.	1A	1
Clinicians should note that DSA are associated with a high risk of rejection, primarily ABMR, and subsequent allograft loss	1A	2
DSA can signal for underlying microscopic injury, indicative of subclinical rejection (ABMR and TCMR), which can be identified through allograft biopsy	1C	3
Upon detection of *de novo* DSA, the pathogenicity and the impact on prognosis is currently best assessed by doing a biopsy	1C	3
Efforts should be made to standardize testing and reporting of DSA, including information on MFI, their plausibility and possible cross-reactive antigens/epitopes	1B	4,5,8
Whilst post-transplant monitoring of preformed DSA in patients with stable graft function might be helpful, additional clinical and laboratory parameters should also be considered when deciding if a biopsy should be performed	2C	4,5,8
DSA MFI levels or complement binding ability (C1q, C4d, C3d) should not influence decision-making regarding whether a biopsy in patients with subclinical dnDSA should be performed	2C	4,5,8
We recommend optimization of maintenance therapy, including addressing non-adherence, in patients who develop subclinical dnDSA. Additional treatment should only be considered after performing an allograft biopsy	1C	6–7
Cost-effectiveness of DSA monitoring in patients with stable graft function depends on incidence rate of dnDSA and importantly on size effect of treatment	2D	9
Monitoring for dnDSA during functional graft life is a continuous process and should not change upon detection of dnDSA.	2C	10
The optimal DSA monitoring scheme has not been established, but a routine approach would be antibody monitoring at three to six months post-transplant and annually thereafter	2C	10

TABLE 3 | Overarching questions & PICOs.

W&J criterium	Overarching question	PICO(s)
1	Does late rejection pose a health problem?	In renal transplant recipients (P), is late rejection (I) a significant contributor to allograft attrition rates compared to other factors (C)?
2	Do we understand the natural history of rejection sufficiently?	In renal transplant recipients with rejection (P), are DSA (I) a significant independent causative contributor to development of the rejection process (O) compared to those without DSA (C)? In renal transplant recipient with rejection (P), are other factors (I) determined as significant independent cause for the development of the rejection process (O) compared to those without those factors (C)?
3	Are we able to identify latent rejection through DSA screening before overt dysfunction occurs?	In renal transplant recipients (P), is development of dnDSA or prevalence of preformed DSA (I) associated with subclinical rejection (O) compared to those without DSA (C)? In renal transplant recipients with subclinical DSA (P), can allograft biopsy guided by DSA development/evolution (I) identify subclinical rejection in an earlier pathological stage (O) compared to biopsies in the event of more overt dysfunction (C)?
4,5,8	Are current DSA testing methods suitable for DSA screening and can certain DSA characteristics be used to further guide allograft biopsy decision making	In renal transplant recipients are current DSA assessment methods sufficient to reliably detect anti-HLA antibodies and their donor specificity? In renal transplant recipients with subclinical DSA (P), can DSA characteristics (MFI, class, IgG subclass, complement binding ability) (I), reliably be used to identify patients without rejection (O) compared to allograft biopsy (C)?
6–7	Is treatment for patients with subclinical DSA or subclinical rejection defined?	In renal transplant recipients with subclinical DSA who have not yet been biopsied (P), is treatment of any kind (I) compared to no treatment (C) beneficial for transplant outcome (O) (allograft loss, clinical rejection risk)? In renal transplant recipients with rejection (ABMR or TCMR) (P), is treatment in the subclinical phase (I) more beneficial to transplant outcome (O) (allograft loss/kidney function) compared to treatment in case of overt dysfunction (C)?
9	Is there any evidence of cost-effectiveness of standardized DSA monitoring and treatment of found cases?	In renal transplant recipients (P), has monitoring of DSA (I) been shown to be cost-effective compared to no monitoring of DSA (C)?
10	How frequent and until what time should DSA monitoring be conducted?	Is the incidence rate as a function of time post-transplant defined? In renal transplant recipients who have developed dnDSA (P), is development of additional dnDSA (I) associated with worse transplant outcome (O), compared to no additional dnDSA (C)? In renal transplant recipients who have developed dnDSA (P), is disappearance of the dnDSA (I) associated with better transplant outcomes (O) compared to persistence (C)? In renal transplant recipients (P), are clear risk categories (I) defined for the risk of development of dnDSA (O) compared to those without those risks (C)? In renal transplant recipients (P), are certain monitoring frequencies (annually, biannually, etc.) (I) associated with better transplant outcomes (O) compared to other monitoring frequencies (C)?

apparent with pretransplant DSA, considering the high risk of hyperacute rejection if transplantation proceeds despite a positive CDC-crossmatch. Modern practice precludes such transplantation with pretransplant listing of non-acceptable HLA antigens, or with measures such as paired kidney exchange programs or desensitization in the living donation setting. In contrast, CDC-crossmatch negative pretransplant DSA, which are identified through SPI only, are not necessarily a contraindication to transplantation in patients faced with no alternatives beyond dialysis [3]. However, these DSA still convey increased risk for ABMR and allograft loss according to a meta-analysis by Mohan et al. [18] Recent analysis of CTS data indicated that nearly 15% of recipients of deceased donor kidneys with crossmatch negative pretransplant DSA progressed to allograft failure within the first year post-transplant [19]. This figure was even higher in retransplant patients. In regards to dnDSA, a large meta-analysis by Sharma et al. [20] implicated development of dnDSA as a severe risk factor for notably cellular rejection, acute ABMR (aABMR), chronic ABMR (cABMR), and allograft loss. Moreover, CTS data showed that 20% of patients who developed dnDSA in the first post-transplant year progressed to allograft failure within the next five [19]. A recent randomized trial corroborated these results [21].

Pathogenesis of HLA-DSA and Plausible Causality With Subsequent Rejection

The genesis of DSA after transplantation is a complex process. B-cells can initiate and subsequently differentiated plasma cells (as well as B-cells) can maintain production of these antibodies as a result of sensitization of the adaptive immune system.

Sensitization could be related to a period of underexposure, either due to non-adherence or iatrogenic reduction of immunosuppression [22–28]. Additionally, poor HLA matching [28–31] and previous episodes of T-cell mediated rejection (TCMR) [29, 30, 32–34] have been associated with DSA development. Other risk factors pertain to certain recipient characteristics such as age or ethnicity [35, 36]. The association of previous TCMR and dnDSA development is hypothesized to be explained by sensitization of the B-cell compartment through inflammation induced by T-cell alloimmunity, especially T-follicular helper cells [37–39]. The role of T-cells in a process which could ultimately lead to ABMR seems to question the dichotomous view on rejection (i.e., either TCMR or ABMR as separate entities). Perhaps a more contemporary view on rejection is that it is a heterogenous spectrum with different histological and clinical manifestations [40].

While the process of sensitization leading to DSA formation is complex and multifactorial, the risks DSA convey are clear. Still, this does not necessarily infer a causal relationship. Though the pathogenicity of HLA-DSA was extensively studied in recent years and a recent thorough literature review by Callemeyn et al. [40] attempted to untangle association from causation. This review assessed the possible causal relationship between HLA-DSA and microvascular inflammation (MVI), a histopathological hallmark of ABMR, through the Bradford-Hill criteria, which can be used as guide for causal inference in epidemiological research. These criteria include: strength of effect size and reproducibility, experimental evidence *in vitro* and *in vivo*, temporality between HLA-DSA appearance and graft injury, biological gradient, and coherence and analogy [40, 41]. Callemeyn et al; [40] illustrates that most criteria are met. However, more investigations are warranted to demonstrate a clear biological gradient between antibody titre and occurrence of ABMR or graft failure; [42]. Yet, recent studies by Viglietti et al; [43, 44] showed that treatment of ABMR through plasmapheresis (PP), intravenous immunoglobulins (IVIG) and rituximab is associated with a significant decrease in DSA MFI and capacity to bind C1q. Interestingly, these reductions in DSA properties were significantly associated with improved graft survival in patients with ABMR. However, treatment effects on more chronic or late ABMR are variable [15]. Furthermore, the histological presentation of ABMR including MVI is not always specific for antibody involvement, as other causes could appear clinicopathologically similar. Nonetheless, there seems to be clear preclinical and clinical evidence of a pathogenic relation between HLA-DSA and ABMR.

Mechanisms of HLA-DSA-Induced Allograft Damage to Explain Phenotypic Variability

Despite this strong relationship, not all recipients with preformed DSA or dnDSA seem to progress to ABMR or graft failure [16, 29, 45]. Multiple mechanisms have been proposed to explain this variation in effect of HLA-DSA on graft outcomes. A recent comprehensive review has summarized HLA-DSA attributes and discussed mechanisms of HLA-DSA-induced effector functions in mediating allograft damage [46]. These effector functions may be Fc-dependent, such as the impact of antibody glycosylation status on complement activation and recruitment of cytotoxic NK-cells and macrophages [47, 48]. Regarding Fc-independent mechanisms, recent studies describe intracellular signalling downstream of HLA-antibody binding to endothelial cells that promote upregulation of adhesion molecules, proliferation and activation of endothelial cells, induction of dendritic cells and $CD4^+$ T-cell maturation [46, 49, 50]. HLA-antibody ligation of the HLA-molecule of endothelial cells can also lead to anaphylatoxin production that can result in more monocyte recruitment. Recruitment is also mediated by the cellular expression of anaphylatoxin receptors on $CD4^+$ and $CD8^+$ T cells, and myeloid cells [51, 52]. Lastly, regulatory T and B-cell populations may play a pivotal role in suppressing the deleterious effects of DSA on the graft. Recent research indicates that these cell lines impart tolerogenic effects through impairment of the T-follicular helper cell – B-cell interaction and that these regulatory cells were significantly reduced in frequency in patients with DSA who developed ABMR, as compared to patients with DSA but absent ABMR [38, 39, 53].

Relationship Between HLA-DSA Properties and Allograft Injury Phenotypes

Several studies have shown that high titre HLA-DSA reflected by high MFI levels, and inflammatory isotype switching toward IgG1 and IgG3, and thus their capacity to bind C1q or C3d are associated with significantly increased microvascular inflammation and C4d deposition [54, 55]. Although considered classically non-inflammatory, the IgG4 isotype has been associated with subclinical graft rejection, including ABMR, in several studies in kidney and other solid organ transplants [55, 56]. Subclinical ABMR was shown to lead to significantly more transplant glomerulopathy and accelerated graft loss when compared to subclinical TCMR [57]. In addition to subclinical ABMR, HLA-DSA have been shown to be significantly associated with kidney graft fibrosis and subsequent accelerated graft loss [58]. The relationship between HLA-DSA and graft fibrosis was independent of previous ABMR episodes. Thus, HLA-DSA, even detected at low strength/MFI, are associated with subclinical damage and fibrosis independent of clinical ABMR occurrence [58, 59].

HLA-DSA Independent Mechanisms of Microvascular Inflammation

Lastly, it must be mentioned that not all patients with MVI, indicative of injury attributed as being "antibody-mediated" by current Banff criteria, have detectable levels of HLA-DSA. The histopathologic entity of MVI without detectable HLA-DSA by definition suggests that factors other than HLA-DSA may mediate MVI, such as non-HLA antibodies [60–63]. Antibody-*in*dependent pathways may include NK-cell alloimmunity through a "missing-self" mechanism [64, 65] or direct allorecognition by monocytes [66, 67]. Other causes may not even be related directly to alloimmunity, such as recurrent complement-mediated renal disease, ischemia/reperfusion injury, or viral endothelial infection.

The above presented body of evidence illustrates that there are likely multiple individual pathways, not all of which are fully understood, that eventually lead to varying levels of microscopic injury that is currently defined as MVI and ABMR, which may need some clarification [6, 68]. Nonetheless, regardless of the incompletely understood natural history of ABMR and MVI, there is a large amount of preclinical and clinical evidence warranting strong support for the notion that anti-HLA-DSA are significantly associated with, and predictive of rejection and clinicians should be aware of this [5, 12, 15, 40].

DSA can Be a Signal for Underlying Microscopic Injury, Indicative of Subclinical Rejection (ABMR and TCMR), Which can Be Identified Through Allograft Biopsy. (1C)

Subclinical DSA as a Marker for Latent Rejection

For a valid screening strategy for disease, clinicians should also be able to identify a latent stage. In some cases, patients undergoing rejection present with clinical dysfunction of the graft as first sign. However, most have a latent phase with prevalent DSA prior to developing graft dysfunction. The first evidence of subclinical DSA as a marker for latent rejection came from preclinical studies in a non-human primate model with sequential protocol biopsies by Smith et al. [69, 70] They showed that development of dnDSA generally precedes graft dysfunction, as well as C4d-deposition or transplant glomerulopathy. Clinical studies of longitudinal protocol biopsies in stable renal transplant recipients with preformed DSA show substantial oscillations characterized by fluctuations in HLA-DSAs, C4d deposition and scores for glomerulitis and/or capillaritis in a dynamic and multidirectional fashion [71–73]. Seminal papers by Wiebe et al. [22, 34] have shown that this progressive subclinical injury can also be detected in patients with dnDSA several years after kidney transplantation. They found that of 64 patients who developed dnDSA, the majority was without graft dysfunction. Additionally, development of subclinical dnDSA was independently associated with transplant glomerulopathy (and thus chronic ABMR (cABMR)), decline in graft function, and allograft loss. Therefore, it is unlikely that chronic rejection is the result of a single spike of HLA-DSA or a single episode of ABMR. Instead, it represents a dynamic process that continues, unabated, at varying levels and eventually progresses towards chronic allograft injury, graft dysfunction and ultimately graft loss [12].

Development of latent rejection in patients with subclinical DSA has been observed in other types of organ transplants [74–76], as well as in more recent clinical studies in KTR, which show underlying rejection in roughly half of overall patients (**Table 4**). Bertrand et al. [77] recently analyzed 123 patients with subclinical dnDSA in a French multicenter cohort study and found that 41.5% of these patients had subclinical ABMR. Loupy et al. [57] showed in a large prospective cohort study of 1,001 patients with 1 year protocol biopsies that of 256 patients with subclinical DSA, 55% had ABMR. Of these cases, 78% were related to pretransplant DSA, further indicating that both pretransplant DSA and dnDSA can underlie a latent pathological process. Coemans et al. [78] recently studied longitudinal protocol and indication biopsies in a single-centre cohort of 1,000 Belgian patients. Of these, 108 had pretransplant DSA and 47 developed dnDSA. The prevalence of subclinical aABMR in protocol biopsies at 3, 12, 24 and 60 months post-transplant was 42.5%, 40.5%, 37.3% and 13.3% respectively in patients with HLA-DSA. Prevalence of transplant glomerulopathy increased over time and this was associated with previously diagnosed aABMR, further corroborating the notion that ABMR is a dynamic and continuous process [71, 72]. Schinstock et al. [30] retrospectively analyzed a single center cohort of patients with serial surveillance biopsies, but also included biopsies at graft dysfunction and upon subclinical dnDSA development. They found that of the 40 patients who were biopsied at the time of dnDSA development, 25%, 7.5% and 20% had underlying aABMR, cABMR, and TCMR respectively. Yamamoto et al. [79] reported on a Japanese cohort of 43 patients with subclinical dnDSA and found that 41.8% of patients had ABMR. Parajuli et al. [80] showed in an American retrospective single center cohort study with biopsies in case of dnDSA development or clinical indication that of 29 patients with subclinical dnDSA, 15 (51%) had underlying rejection. Of those rejections, 60% were ABMR, 20% mixed rejections, and 20% were TCMR. Waldecker et al. [81] retrospectively studied 84 German patients with indication biopsies or in case of dnDSA development from a single centre and found that out of 50 patients with subclinical dnDSA, 44% had ABMR, 15% had TCMR, 12% had mixed rejection and 15% had borderline rejection. Notably, only 14% of these patients had no histopathological signs of rejection at light microscopy. Eskandary et al. [82] retrospectively reported on the screening process for the BORTEJECT study, whereby 861 patients with stable grafts were cross-sectionally screened for presence of DSA [82]. Of 86 patients with subclinical DSA, 44 (51%) met the Banff criteria for ABMR. Lastly, Cornell et al. [83] analysed the results of a prospective trial on pretransplant desensitization with eculizumab in patients with a positive flow-crossmatch and compared the long-term outcomes to a historical matched cohort. The overall prevalence of subclinical ABMR at 3 months, 1 year and 2 years post-transplant was 41.8%, 37% and 20% respectively in a total of 78 patients.

Relationship Between *de novo* DSA and Subclinical T-cell Mediated Rejection

While most studies only reported these biopsy results in terms of either positive or negative for ABMR, the studies by Schinstock et al. [30], Parajuli et al. [80] and Waldecker et al. [81] interestingly further show that subclinical dnDSA can also be a signal for underlying TCMR. Unfortunately, no biopsies were performed in a DSA-negative control group in these studies, making it difficult to ascertain the precise odds of dnDSA to signal TCMR risk. Nonetheless, as discussed above, the association between TCMR and dnDSA development has been described previously in multiple studies. The study by Loupy et al. [57] seems to contrast this suggested association, as they showed

TABLE 4 | Summary of studies on subclinical DSA in renal transplant recipients.

Study	Type of study	Total patients (n)	Total DSA+	Biopsied patients with subclinical DSA (n)	dnDSA/ preformed DSA	Time of biopsy	Subclinical aABMR (n) (%)*	Subclinical caABMR (n) (%)*	Subclinical cABMR (n) (%)*	Subclinical TCMR (n) (%)*	Subclinical mixed rejection (n) (%)*	No rejection (n) (%)*	Outcome
Wiebe et al. [22, 34]	Retrospective Single center	508	64	45	dnDSA	6 months post-transplant At dnDSA detection Graft dysfunction	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	Time to 50% allograft loss in clinical dnDSA vs. subclinical dnDSA: 3.3 years vs. 8.8 years ($p < 0.0001$) Significantly worse allograft survival in subclinical dnDSA vs. no dnDSA + no dysfunction
Bertrand et al. [77]	Retrospective Multicenter	123	123	123	dnDSA	At dnDSA detection	32 (26%)	19 (15.5%)	Not specified	Not specified	Not specified	No ABMR: 72 (58.5%)	Significantly worse post-biopsy 8-years allograft survival and 5 years delta creatinine in subclinical aABMR and cABMR compared to dnDSA without rejection
Loupy et al. [57]	Retrospective Single center + external validation	1,001	256	256	Preformed DSA + dnDSA	1 year post-transplant		With DSA: 142 (55%)*		With DSA: 17 (6.6%)*	Not specified	With DSA: 97 (38%)*	Significantly worse post-biopsy 8-years allograft survival and delta creatinine in subclinical ABMR compared to subclinical TCMR or no rejection

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TABLE 4 | (*Continued*) Summary of studies on subclinical DSA in renal transplant recipients.

Study	Type of study	Total patients (n)	Total DSA+	Biopsied patients with subclinical DSA (n)	dnDSA/ preformed DSA	Time of biopsy	Subclinical aABMR (n) (%)*	Subclinical caABMR (n) (%)*	Subclinical cABMR (n) (%)*	Subclinical TCMR (n) (%)*	Subclinical mixed rejection (n) (%)*	No rejection (n) (%)*	Outcome
								Total: 142 (14.2%)**		Total: 132 (13.2%)**		Total: 727 (72.6%)**	No significant difference between (treated) subclinical TCMR and no rejection in either allograft survival or delta creatinine
Coemans et al. [78]	Retrospective Single center	1,000	155	At 3 months: 60 At 12 months: 37 At 24 months: 29 At 60 months: 15	Preformed DSA (108) + dnDSA (47)	3, 12, and 24 months post-transplant Additional protocol biopsy at either 3, 4 or 5 years post-transplant Indication	At 3 months: 42.5% At 1 year: 40.5% At 5 years: 13.3% At 2 years: 37.3%	Not specified	Not specified	Not specified	Not specified	No aABMR at 3 months: 57.5% At 12 months: 59.5% At 24 months: 62.7% At 5 years: 86.7%	No analysis of effect of subclinical rejection vs. no rejection on transplant outcome
Schinstock et al. [30]	Retrospective Single center	771	54	40 biopsied at detection of DSA 34 biopsied 1 year post detection of DSA Not all subclinical	dnDSA	4, 12, 24, 60 months post-transplant at dnDSA detection Graft dysfunction	At dnDSA detection: 10 (25%) 1 year post dnDSA detection 18 (53%)	Not specified	At dnDSA detection: 3 (7.5%) 1 year post dnDSA detection 13 (38.2%)	At dnDSA detection: 8 (20%) 1 year post dnDSA detection 5 (14.7%)	Not specified	Not specified	Only those with dnDSA + ABMR had evidence of graft loss at mean follow up of 3.2 ± 2.0 years 21.4% vs. 0% in dnDSA without AMR. ($p < 0.01$) No significant difference in composite endpoint of –50% eGFR or allograft loss between dnDSA without AMR vs. no dnDSA ($p = 0.26$)

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TABLE 4 | (*Continued*) Summary of studies on subclinical DSA in renal transplant recipients.

Study	Type of study	Total patients (n)	Total DSA+	Biopsied patients with subclinical DSA (n)	dnDSA/ preformed DSA	Time of biopsy	Subclinical aABMR (n) (%)*	Subclinical caABMR (n) (%)*	Subclinical cABMR (n) (%)*	Subclinical TCMR (n) (%)*	Subclinical mixed rejection (n) (%)*	No rejection (n) (%)*	Outcome
Yamamoto et al. [79]	Retrospective Single center	899	95	43	dnDSA	At dnDSA detection	18 (42%) At rebiopsy 2 years post biopsy in those without ABMR: 0 (0%)			Not specified	Not specified	No ABMR: 25 (58%) At rebiopsy 2 years post biopsy in those without ABMR: 8 (100%)	Only 1 of 11 patients at 2 years follow up without ABMR at initial biopsy had deteriorating creatinemia/ proteinuria
Parajuli et al. [80]	Retrospective Single center	45	45	29	dnDSA	At dnDSA detection "Other indications"		9 (31%)		3 (10%)	3 (10%)	14 (48%)	Significantly better 1 year post biopsy eGFR in patients with subclinical dnDSA vs. clinical dnDSA. No statistical differences in allograft loss rate but low event rate
Waldecker et al. [81]	Retrospective Single center	865	132	34	dnDSA	At dnDSA detection Graft dysfunction	11 (26%)	3 (9%)	1 (3%)	5 (15%)	4 (12%)	5 (15%)	No analysis of effect of subclinical rejection on transplant outcomes
Eskandary et al. [82]	Retrospective Single center	861	86	86	Preformed DSA + dnDSA	Cross-sectional screening		44 (51%)		Not specified	Not specified	No ABMR 42 (49%)	Only patients with subclinical ABMR had evidence of graft loss 5 vs. 0 without rejection during a median follow up of 20.5 months

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TABLE 4 | (*Continued*) Summary of studies on subclinical DSA in renal transplant recipients.

Study	Type of study	Total patients (n)	Total DSA+	Biopsied patients with subclinical DSA (n)	dnDSA/ preformed DSA	Time of biopsy	Subclinical aABMR (n) (%)*	Subclinical caABMR (n) (%)*	Subclinical cABMR (n) (%)*	Subclinical TCMR (n) (%)*	Subclinical mixed rejection (n) (%)*	No rejection (n) (%)*	Outcome
Cornell et al. [83]	Prospective trial cohort + historical retrospective matched cohort	78	78	78	Preformed DSA	3–4, 12 and 24 months post-transplant	Overall: At 3–4 months: 41.8% At 1 year: 37% At 2 years: 20%			Not specified	Not specified	Overall: No ABMR at 3–4 months: 58.2% At 1 year: 63% At 2 years: 80%	No analysis of effect of subclinical rejection vs. no rejection on transplant outcome

*ABMR, antibody-mediated rejection; aABMR, active ABMR; cABMR, Chronic ABMR; caABMR, Chronic active ABMR; DSA, Donor specific antibody; TCMR, T-cell mediated rejection. *; Proportion of total subclinical DSA patients with a biopsy. **; Proportion of total patients.*

significantly more patients without DSA developed subclinical TCMR compared to patients with DSA. However, the majority of patients with DSA in this study had preformed DSA, not dnDSA. This was also reflected in patients with subclinical TCMR, as only 5 out of 17 (29%) of these patients had dnDSA. This could imply that there is no association between preformed DSA and TCMR, while there might be one for dnDSA and TCMR. Others were also not able to relate pretransplant DSA and TCMR development [84, 85]. However, these studies did not take into account the possible presence of mixed rejection in these analyses, or pooled these type of rejections with patients with ABMR. The study by Coemans et al. [78] did adjust their analysis accordingly and found that while pretransplant DSA was not associated with isolated TCMR, it was associated with total TCMR including mixed rejections. Nevertheless, the results of these studies might be further evidence for the previously described view on rejection as a spectrum of different clinical and histological manifestations. These may occur in sequence, as TCMR can result in dnDSA development, which can lead to ABMR. In contrast, preformed DSA may not necessarily lead to development of isolated TCMR. Collectively, these results imply that a biopsy serves to diagnose latent rejection (ABMR, TCMR or mixed rejection) in around half of patients with subclinical DSA, which is in line with previous recommendations on performing an allograft biopsy these patients [15]. While the number of papers warrants strong support for this statement, the evidence is mainly observational. This is reflected in the grading.

Upon Detection of a dnDSA, the Pathogenicity and the Impact on Prognosis is Currently Best Assessed by Doing a Biopsy (1C)

Prognostic Value of a Banff Classified Rejection Diagnosis

Aside from the diagnostic purposes of an allograft biopsy in patients with subclinical DSA, it may also have prognostic value in predicting allograft loss. As stated before, not all patients who develop DSA seem to lose their graft or even show declining allograft function. Wiebe et al. [22] showed that 50% of patients still had a functioning allograft 10 years post-detection of the subclinical dnDSA. Therefore, further risk stratification regarding the pathogenicity of these subclinical dnDSA seems necessary. The Banff classified rejection diagnosis in these patients may provide further risk-stratification for detrimental transplant outcomes. Multiple studies showed that KTR with subclinical DSA and histological evidence of ABMR had significantly worse allograft survival rates and allograft functional decline than those without histopathological rejection at light microscopy [30, 57, 77, 78, 86]. Others show a similar trend, albeit not statistically significant [79, 82]. Moreover, Bertrand et al. [77] and Loupy et al. [57] showed that patients with subclinical dnDSA but without ABMR had excellent 8 years allograft survival (>90%) and stable graft function. This suggests that the rejection diagnosis could be prognostically more important for graft outcome than the dnDSA status itself in patients without graft dysfunction. This was further corroborated by Parajuli et al. [87] in a cohort of 587 patients

without rejection at an initial protocol or indication biopsy, whereafter there was no difference in 5 years allograft loss rates between DSA-positive and DSA-negative patients. Though, dnDSA positivity in patients with a negative index biopsy was associated with subsequent rejection.

Prognostic Value of Inflammation Activity

Beyond the prognostic value of the Banff classified rejection diagnosis in patients with subclinical DSA, the severity of the Banff recognized acute pathological lesions may perhaps offer further risk-stratification in patients with rejection. A prospective cohort study of 215 patients showed that while DSA were univariately associated with renal function decline, this was no longer statistically significant when analyzed with a multivariate model including MVI and tubulitis [88]. This suggests that the presence of histological markers may define a more severe phenotype in patients with DSA. Wiebe et al. [22] showed in multivariate analysis of a small subcohort of 23 subclinical patients with dnDSA that tubulitis was a strong predictor for allograft loss. Studies by De Kort et al. [89] and the iBox study by Loupy et al. [90] elegantly showed that increasing levels of MVI severity score in patients with DSA is independently associated with worse allograft survival. A more recent study on semi-supervised clustering through data-driven mathematic modeling by Vaulet et al. [91] further corroborated the prognostic value of inflammation activity as determinant of allograft loss within patients with DSA.

Prognostic Value of Chronicity Markers

Histological chronicity markers also impact allograft survival. The presence of transplant glomerulopathy is implicated as independent risk factor for allograft attrition in multivariate analysis by multiple studies [22, 78, 90, 92]. Other chronicity markers such as interstitial fibrosis or tubular atrophy also seem to independently infer risk for allograft loss in patients with DSA [90]. More recent research showed that patients with increasing chronicity scores as determined by an aggregate of several chronicity markers have significantly worse prognosis in terms of allograft survival [93]. A follow-up study by Vaulet et al. [94] validated these results, again through a semi-supervised clustering approach, and found that clusters with higher levels of chronicity were associated with increasingly higher rates of allograft loss. Importantly, they assessed the impact of time since transplant of the biopsy in this study. Even though there was an association with clustering based on chronicity, clustering solely based on time of biopsy could not discriminate in allograft loss rate. This indicated that pattern of chronicity scores was an independent risk factor for poor allograft survival, regardless of the post-transplant time of the biopsy.

Temporal Association Between Activity and Chronicity and Relation With Efficacy of Treatment

Even though activity and chronicity are viewed as separate entities for the sake of the analyses in these studies, it should not be forgotten that they are intertwined. The temporal association between aABMR and chronic lesions associated with cABMR such as transplant glomerulopathy, peritubular capillary basement membrane multilayering and transplant arteriopathy is well described in both preclinical models and clinical studies [57, 69, 70, 78, 95–97]. Previous research in patients with TCMR has shown that chronic scarring is a determinate for poor response to treatment [98, 99]. Haas et al. [100] has also previously shown that early intervention in patients with ABMR may prevent chronic lesions such as transplant glomerulopathy. Recently, Wu et al. [101] did not observe any effect of current treatment options in patients with chronic ABMR and transplant glomerulopathy further indicating that late stages are less responsive to therapy. It could thus be of interest to identify patients at an earlier stage of the ABMR disease process. If a latent phase with subclinical DSA is an earlier stage in the continuum of rejection, then a biopsy taken at this stage may theoretically show less chronicity and these patients could perhaps be more amendable to treatment. Unfortunately, very few studies investigated this. Parajuli et al. [102] found that the Banff sum chronicity and transplant glomerulopathy scores of patients with underlying ABMR at biopsy were significantly lower in subclinical ABMR compared to dysfunctioning allografts with ABMR. Additionally, Wiebe et al. [34] found that no patients with subclinical dnDSA had evidence of transplant glomerulopathy. However, more research is needed to confirm this hypothesis, as current data is insufficient to draw meaningful conclusions.

Timing of the Allograft Biopsy

While this body of evidence seems to point out the additional prognostic value and possible clinical utility of an allograft biopsy in patients with (subclinical) DSA, it does not necessarily provide direction on when to perform this biopsy within the subclinical stage. The study by Schinstock et al. [30] clearly showed that within dnDSA-positive patients with a negative index biopsy, a follow-up biopsy 1 year later yields significantly more positive cases of ABMR. This appears to contrast aforementioned studies which suggested that an initial negative biopsy infers significantly less risk in patients who have developed dnDSA. However, the findings by Schinstock et al. [30] could be explained by the fact that not all their included biopsies at either dnDSA detection or follow-up were in fact in a subclinical setting. This may affect the *a priori* probability of finding underlying rejection at follow-up. Alternatively, the worse prognosis of the minority group who do eventually develop rejection may have been masked by the majority who remained without significant graft injury in other studies. Nevertheless, Schinstock et al. [30] could indicate that performing the biopsy too early could lead to false negative findings. Whereas no histopathological rejection at light or electron microscopy might be visible in these cases, there may in fact still be rejection at the molecular level. Previous research has shown the independent prognostic value of molecular ABMR gene transcripts for allograft attrition within patients with Banff classified ABMR [103]. The INTERCOMEX study has shown the added clinical value of these molecular gene expression transcripts for identifying rejection [104]. Two studies on molecular gene transcript classifiers further show that DSA-positive patients who present with high levels of these classifiers but show no histopathologic evidence of ABMR at light

microscopy have more risk to develop histologic ABMR at subsequent biopsies compared to patients with low molecular ABMR gene transcript levels [105, 106]. Molecular analysis may thus offer additional prognostic value in case of a microscopically negative index biopsy, however these techniques are not yet available in most centers and require further validation. Some clinicians could perhaps consequently conclude that upon dnDSA detection, a certain amount of time should elapse before a biopsy is conducted, as this may decrease the chance of a negative biopsy result in those patients with molecular but not yet microscopic histological rejection. However, this may negatively impact potential efficacy of treatment in patients who would have more chronicity on later biopsies. Additionally, aforementioned studies on biopsy results upon subclinical dnDSA detection clearly show rejection in approximately 50% of cases. These would all be detected later by postponing a biopsy. An alternative strategy could entail a follow-up biopsy, if the index biopsy is negative. No study has been conducted which specifically addresses and compares the impact of these strategies on transplant outcomes. Therefore, more research on the optimal time of biopsy in patients with subclinical DSA is needed. Nevertheless, the additional prognostic value of renal biopsy information on both the Banff recognized rejection diagnosis and of the severity of the pathological lesions in patients with subclinical DSA seems clear. Therefore, despite the mostly single-center observational evidence, we strongly recommend to perform an allograft biopsy to further determine the pathogenicity and impact of developed subclinical dnDSA, if prognostication is desired.

Efforts Should be Made to Standardize Testing and Reporting of DSA, Including Information on MFI, Their Plausibility and Possible Cross-Reactive Antigens/Epitopes (1B)

A prerequisite for any screening strategy is the availability of a suitable test system which is acceptable to the population and with facilities available for diagnosis and treatment. The SAB-SPI test is currently the test system of choice to define DSA. This method is semiquantitative, highly specific, sensitive and able to detect and identify anti-HLA antibodies. However, differences within and between laboratories impair reproducibility when it comes to the definition of DSA in both clinical practice and trials. A recent systematic review showed that the reporting of DSA in clinical trials had huge variability concerning assay type, DSA verification, MFI cutoff to define DSA and the prevention of prozone [107]. The level of "not reported" was determined at +/−15% for assay type, >30% for DSA verification and MFI cut off and around 80% for prozone treatment. Not only antibody tests have to be taken into account. Senev et al. [108] showed that 23% of DSA defined on a low resolution level could not be confirmed if correlated to second field high resolution HLA-typing results. Laboratory factors, as well as donor and patient factors, are inherent limitations to the testing and reporting of DSA [2, 3]. MFI values underestimate broad reacting specificities as Bw4/Bw6 or beads saturated with antibody. MFI does not reflect titer and one should bear in mind that SAB-SPI tests are qualitative, at best semiquantitative tests [42, 109]. Potential pitfalls notwithstanding, HLA-antibody detection and antigen/epitope specificity identification have never been as good as today. HLA-antibody assessment using solid phase assays including all major HLA-loci are already recommended in the 2017 North-American Sensitization in Transplantation: Assessment of Risk workgroup (STAR) report [109]. Initiatives such as the STAR workgroup [109, 110] are essential to clarify the expectations and limitations of current clinically used DSA detection methods. Clinicians need to receive comprehensive reports in a timely manner while being informed on the limitations of individual assays and results. Additionally, HLA-immunologists need to understand the clinical course of a patient after transplantation. Whereas HLA-labs are highly involved in the definition of acceptable antigens pretransplant, they are less involved in the posttransplant follow-up of individual patients. To increase clinical utility and validity, feedback should not only go from the lab to the clinic but also *vice versa*, resulting in both standardized analytical and clinical reporting. The following information can be helpful in DSA reporting: risk category of the patient at the moment of transplantation, DSA chronology, and the indication of DSA testing. This interaction is specifically needed to address the potential pitfalls of DSA screening in the entity of DSA-negative ABMR.

International standards for HLA-labs should focus on the different aspects that can interfere with the definition of DSA as a follow-up biomarker for subclinical DSA. These can include definition of MFI (Median, Mean, trimmed mean), signal-to-background calculation or plausibility evaluation.

Although the current SAB-SPI allow identification of DSA, further research is required to standardize DSA monitoring in patients with functioning grafts. The use of SAB-SPI methods measuring C3d or C1q complement fixing of DSA can have additional value but needs further validation and cannot currently be recommended as a biomarker for subclinical DSA monitoring, as conflicting retrospective studies exist [111, 112]. Conflicting studies also exist in regards to IgG subclass differentiation [55, 113]. The role of non-HLA post-transplant does not seem to be impactful, but the number of studies is currently limited [114].

Methods to detect B cell memory [115, 116] or to detect specific antibody parameters as affinity and avidity [117] are currently not available on a large scale nor are they ready as posttransplant monitoring biomarker. Further research on these topics is required.

Whilst Post-Transplant Monitoring of Preformed DSA in Patients With Stable Graft Function Might be Helpful, Additional Clinical and Laboratory Parameters Should Also be Considered When Deciding if a Biopsy Should be Performed. (2C)

Development of dnDSA could prompt clinicians to further investigate a patient for underlying pathology. Here, we consider monitoring patients with subclinical preformed DSA. We will not argue against the validity and prognostic value of a

biopsy *per se* in these patients. However, it is more difficult to determine a prompt to biopsy in patients with preformed DSA. It could be argued that post-transplant persistence of preformed DSA could prompt a biopsy as some preformed antibodies may gradually disappear from the circulation. Previous studies indicate that persistence of preformed DSA infers a higher risk of allograft loss and rejection than DSA that have disappeared [118–123], though some contradict this conclusion [88, 124]. Additionally, studies comparing allograft loss in patients with cleared preformed DSA versus no preformed DSA give conflicting results [118, 120, 125]. Furthermore, no study has examined the predictive value of clearance of preformed DSA. Thus, it is currently uncertain whether grafts in patients with cleared preformed DSA have a survival disadvantage or suffer higher rates of rejection compared to grafts in regular non-sensitized patients. It is therefore uncertain if clearance of preformed DSA should preclude a biopsy in patients without graft dysfunction. There is currently little evidence that post-transplant change in MFI of preformed DSA in patients with stable grafts has any predictive value. Early rise in preformed DSA MFI was associated with ABMR development in older studies [126, 127]. However, more recent in-depth analysis by Philpott et al. [128] of post-transplant temporal evolution of DSA indicated that allograft survival was impacted by the speed of change in MFI, rather than eventual delta MFI during the first month. They showed that patients with modulating preformed DSA (i.e., a rise then subsequent fall of MFI) had significantly better allograft survival than patients with sustained levels of preformed DSA (i.e., rising MFI and followed by sustained or stable MFI). This would indicate that a random point measurement of DSA MFI level in the early post-transplant course would provide minimal predictive information. Preformed DSA with high delta MFI compared to pretransplant levels could still be DSA which is undergoing a modulating course, which appears to infer less risk than DSA which had a more stable course in MFI. In this study, biopsies were only performed in case of allograft dysfunction, so it is difficult to extrapolate these results to patients with stable graft function. Moreover, delta MFI should be interpreted with caution in the absence of other clinical parameters, considering that the inter-laboratory variation of MFI can be as high as 62% [129] Consensus guidelines of the STAR workgroup are in line with this notion, as they state that any increase of MFI less than 50% is likely to be meaningless in otherwise "relaxed" situations [109]. Furthermore, even if the results of Philpott et al. [128] could be extrapolated to subclinical patients, they would only support careful monitoring in the first month post-transplant, as allograft survival was dependent on the evolution of DSA in that month. Unfortunately, no studies analyzed associations between late evolution in preformed DSA MFI and transplant outcomes. This leads to the conclusion that, although patients with preformed DSA and stable grafts can have latent rejection, there is currently no evidence to support the notion that monitoring these DSA alone provides a prompt to initiate further investigation of the patient. Additional clinical and laboratory parameters should thus also be considered, before deciding upon a biopsy in patients with preformed DSA. The lack of robust evidence regarding this topic is reflected in the grading of this recommendation. Alternatively, these patients might benefit from strategies utilizing protocol biopsies [2, 130] or a combined screening strategy using additional non-invasive biomarkers of rejection. A separate workgroup within the TLJ3.0 platform will publish consensus statements on the clinical validity and utility of these biomarkers and these methods are therefore beyond the scope of this consensus report.

DSA MFI Levels or Complement Binding Ability (C1q, C4d, C3d) Should Not Influence Decision-Making Regarding Whether a Biopsy in Patients With Subclinical dnDSA Should be Performed. (2C)

Development of subclinical dnDSA may prompt further investigation of the patient, though it would be of interest to define other factors that would help stratify the risk of underlying graft pathology. This may prevent needless allograft biopsies in patients with subclinical dnDSA, considering that not all patients with dnDSA have recognizable ongoing ABMR at biopsy. Previous studies have shown that patients with ABMR more often have antibodies aimed at HLA class II, however this is also likely related to class II antibodies being the most commonly formed type. [22, 92, 131] Moreover, a recent large cohort study did not find any difference in the proportion of patients with HLA class I dnDSA who have underlying ABMR, as compared to class II dnDSA. [29] Additionally, dnDSA HLA-class specificity does not seem to be significantly associated with graft survival in multivariate analysis. [29, 131] This indicates there is not enough evidence to state that DSA HLA-class significantly attenuates the risk of a rejection diagnosis or the graft prognosis and therefore should not influence the decision to omit a biopsy in patients with subclinical dnDSA. Multiple studies have associated other DSA characteristics with worse outcomes, such as MFI level (sum of all DSA MFI or highest individual MFI) [22, 88, 132–136], certain IgG subclasses [55, 137, 138], or complement binding ability (C1q, C4d, C3d) [54]. However, most studies do not provide information on the negative predictive value of these characteristics, which would be the parameter of interest in deciding on whether to omit a biopsy. Prospective randomized studies are lacking and only a few studies investigated the predictive value of these DSA characteristics. Eskandary et al. [82] retrospectively studied 86 patients with subclinical DSA and associated highest MFI, sum of MFI and complement binding ability with underlying ABMR. However, the individual C-statistics were moderate at best for each characteristic (0.77, 0.75 and 0.65 respectively). Additionally, a combined model of maximum or sum of MFI and either C1q, C4d or C3d-positivity did not improve the predictive power of the base model of only MFI significantly. The authors found that while a higher MFI cutoff of >5000 or >10000 enjoyed a higher specificity for ABMR (0.86 and 0.99 for both MFI characteristics), the sensitivity drastically reduced from 0.82, 0,84 to 0.34, 0.43 and 0.30, 0.27 respectively. These MFI cutoffs subsequently result in low negative predictive value for ABMR in patients with subclinical dnDSA (MFI > 5000: 0.63, 0.67; MFI > 10000:

0.64, 0.65, for maximum MFI and sum of MFI, respectively). This indicates at least 30% of underlying ABMR would be missed by preclusion of a biopsy based on MFI cutoffs >5000 in subclinical patients. The fact that MFI values do not reflect the strength of the antibody titer might be an important cause of the poor correlation between MFI values and outcome [42, 109]. A recent study could not identify a relationship between MFI at first occurrence and outcome, only a profound >50% reduction of dnDSA MFI values was associated with better graft survival in a multivariate model [29]. Another study by Viglietti et al. [139] performed analyses with allograft loss as outcome in 186 patients with both subclinical and clinical DSA. They found an equally moderate C-statistic regarding maximum MFI in the total group of patients with post-transplant DSA (0.72). This was only marginally better in specifically dnDSA-positive patients (0.75). No analysis regarding specific MFI cut-offs was performed. While C1q-binding was found to significantly increase the fit of the base model, the numerical increase in C-statistic was a marginal 0.028 in dnDSA-positive patients (0.751–0.779) Interestingly, IgG3-positivity strongly increased the fit of the model with improvement of the C-statistic from 0.75 to 0.88. Yet this specific characteristic was predominately present in patients whose dnDSA were detected after development of allograft dysfunction. Only 2% of patients whose dnDSA were detected as a part of regular annual screening were IgG3-positive, yet 74% and 57% of these patients had ABMR at biopsy one and two years post-transplant respectively. These studies indicate that while some DSA characteristics such as higher MFI or IgG3 positivity might increase the likelihood of underlying pathology in dnDSA-positive patients with stable grafts, absence of these characteristics also definitely do not exclude it. Therefore, as robust supporting evidence is lacking, it seems that none of these studied DSA characteristics can be used reliably to preclude a biopsy in patients with subclinical DSA. We therefore currently do not recommend utilizing these DSA characteristics as an aid in deciding if a biopsy of patients with subclinical dnDSA should be performed.

We Recommend Optimization of Maintenance Therapy, Including Addressing Non-Adherence in Patients Who Develop Subclinical dnDSA. Additional Treatment Should Only be Considered After Performing an Allograft Biopsy. (1C)

Optimization of Maintenance Therapy

A crucial element of a screening program is whether proper treatment exists and whether there is consensus on whom to treat. Optimization of maintenance therapy, which includes promoting adherence, reducing exposure to secondary risk factors such as hypertension and maintaining appropriate calcineurin inhibitor trough levels, has been recommended in previous consensus statements for the treatment of ABMR and TCMR [15]. Moreover, the consensus on managing modifiable risk in transplantation (COMMIT) workgroup addressed non-adherence and underexposure to immunosuppression as pivotal risk factors for poor transplant outcomes [140]. The importance of adequate exposure has also previously been demonstrated in patients with DSA. Multiple studies showed that DSA-positive patients with adequate exposure have better graft survival compared to DSA-positive patients who remain non-adherent or with iatrogenic underexposure to immunosuppression [22–24]. Development of dnDSA has been heavily correlated to underexposure to immunosuppression [22–28]. This risk factor for poor transplant outcomes can be addressed and this could be done irrespective of underlying histology, because dnDSA may still signal underexposure even if there is no microscopically visible rejection. However, the recently published OuTSMART trial, which analyzed the effects of optimization of maintenance therapy based on DSA monitoring results, seems to contradict these previous retrospective studies [21]. No significant difference was found in regards to graft survival between standard of care and optimization of maintenance therapy based on DSA monitoring. This randomized controlled trial (RCT) is qualitatively better evidence than observational research. However, it should be noted that the consenting participants in OuTSMART were already highly adherent at baseline. This is reflected by the low dnDSA incidence rate of 1.6% per year and may relate to the possibility of healthy survivor bias due to cross-sectional inclusion. Even though adherence improved significantly to even greater levels, it is uncertain whether it was to be expected that this should have resulted in improved graft survival. Nonetheless, this study does appear to show that broadening the immunosuppressive regimen does not have the expected effect on graft survival. Even the sensitivity analysis, which only included patients who were optimized to a triple therapy regime upon detection of dnDSA could not demonstrate survival benefit, though the confidence interval included both estimates of highly protective as well as highly hazardous effects. This could have been related to less allograft failure in DSA-positive patients than initially expected. Interestingly, total amount of biopsy-proven rejections was significantly lower in patients in the intervention arm, indicating that increased exposure does have immunological effect. Perhaps more benefit could be demonstrated if optimization of maintenance therapy is accompanied with biopsy-guided anti-rejection treatments as subclinical rejection was likely present in only 50% of subjects. More research in terms of broadening immunosuppressive regimen as a means of optimization of maintenance therapy is thus required for this to be recommended. Nevertheless, addressing non-adherence and secondary risk factors for progression are still important aspects of treatment, which we still strongly recommend in case of development of subclinical dnDSA. The ultimate goal is to optimize graft survival which includes taking into account competing mortality risk from infections, malignancies, and other toxicities.

Maintenance Immunosuppressive Target Levels

When subclinical donor-specific antibodies emerge, it becomes crucial to detect potential non-adherence and optimize the maintenance immunosuppressive regimen, unless there are contraindications present. In case of signs of ongoing alloimmunity, the convention in many center is often to switch to triple therapy with tacrolimus, mycophenolate analogues and maintenance steroids balanced against toxic side effects. Unfortunately, there is a current lack of strong evidence for exposure targets in kidney transplant recipients

with subclinical dnDSA. To give some clinical directions, target tacrolimus exposure could be extrapolated from trough levels to prevent (additional) DSA [25, 28] rejection [141], and to improve graft survival [142–144]. Collectively, these studies suggest that maintaining the tacrolimus trough level between 5 and 8 ng/mL, which is in line with international recommendations, might prevent alloimmunity and optimize survival, albeit two studies suggested a potential lower threshold of 4 ng/mL in patients with very low intrapatient variability [141, 142]. However, whether this target range is helpful once caABMR ensues remains unknown. A study by Sablik et al. [145] did not find any survival difference between a tacrolimus trough greater or lesser than 5.9 ng/mL. Interestingly, they did find that higher intrapatient variability was significantly associated with poorer survival in patients with caABMR, suggesting that adherence and time in therapeutic range are probably more important exposure variables than attained trough levels within current clinical practice. Even less evidence is available regarding optimal mycophenolate exposure. A small single study found a trough >1.3 mg/L to prevent DSA formation [146]. It is reasonable to hypothesize that a clear exposure-relationship curve between mycophenolate and antibody formation might exist, considering the almost linear relation between MPA exposure and SARS-CoV-2 antibody formation [147]. No evidence is available for reintroduction of low-dose steroids, it is however often assumed that the anti-inflammatory effects and the diminished chance of acute rejection from maintenance steroids might have beneficial effects in the long-term but need careful balancing against side-effects [148]. Some evidence has emerged regarding the effectiveness of conversion from a CNI based immunosuppressive regime to costimulation blockade with belatacept [149]. Perhaps optimization of maintenance therapy could entail such a strategy, as it would effectively eliminate occult non-adherence due to the necessity of intravenous administration. Additionally, belatacept's immunological mode of action may be more fitted for patients who have already developed a dnDSA as it interrupts T-follicular helper cell—B-cell interaction and could thus decrease B-cell stimulation and further reduce the evolution of DSA formation [29, 150]. Some studies have shown effectiveness of belatacept on DSA levels and on the (lower) incidence of ABMR in sensitized patients [149–152]. Interestingly, DSA positivity was not associated with graft loss in a small cohort of patients converted to belatacept, though the presence of aABMR with MVI was independently associated with treatment failure [153]. It has to be noted however, that the incidence of TCMR was significantly increased, especially in patients converted within the first year post-transplant [154]. We therefore recommend more research to be conducted on the role of costimulation blockade as a means to optimize maintenance therapy in patients with subclinical DSA.

Pre-Emptive Treatment *In Lieu* of an Allograft Biopsy

In regards to further treatment of patients with subclinical dnDSA before conducting a biopsy, evidence is lacking. Only one small cohort study has been identified, in which patients with subclinical DSA were treated with bortezomib, PP, IVIG and corticosteroids without performing a biopsy to confirm rejection [155]. This study showed that patients who achieved DSA clearance had more stable 2 year allograft function compared to those with persistent DSA. However, no control group was included and thus it cannot be concluded that improvement in outcome was due to treatment. Furthermore, irrespective of efficacy, subjecting all patients with subclinical dnDSA to such a strong and broadly targeting immunosuppressive regimen might be difficult to justify, considering that roughly half of this population have no underlying observable histological injury [30, 57, 78, 80–83]. In addition, transient spontaneous negativity of dnDSA has been observed in 24% of patients with subclinical dnDSA and complete clearance of dnDSA has been observed in around 10% of patients [29]. Lastly, identification of the Banff classified type of rejection through a biopsy will ensure that patients with underlying cell-mediated rejection are not unnecessarily subjected to therapy aimed at antibodies and *vice versa*. We therefore do not recommend additional preemptive treatment of patients with subclinical dnDSA, besides optimization of maintenance therapy, without performing an additional allograft biopsy.

Treatment of Subclinical T-cell Mediated Rejection

Amongst dnDSA-positive patients with underlying rejection, those with subclinical TCMR may have the best evidence for gained benefit. Treatment of subclinical TCMR has been investigated in multiple studies (**Table 5**). A literature review by Mehta et al. [156] revealed that most available studies [157–161] at the time showed that subclinical acute TCMR (aTCMR) is associated with inferior outcomes. Choi et al. [162] observed significantly lower 10 years allograft survival in patients with untreated early subclinical TCMR vs. non-rejectors (62.3% vs. 96.2%). Consequently, ESOT advocates subclinical aTCMR to be considered as primary efficacy endpoints in clinical trials [163]. The first evidence of treatment came from a randomized trial by Rush et al. [161]. They showed that treatment of early subclinical TCMR detected in protocol biopsies leads to lower chronicity scores, less late rejections and more stable and lower creatinine levels at 2 years post-transplant than untreated patients. Another RCT by Kurtkoti et al. [160] showed similar results in regards to lower creatinine levels at 6 and 12 months. These older studies could be criticized for having been conducted before the tacrolimus era and thus being less applicable to current practice. A more recent randomized trial of early protocol biopsy and treatment of subclinical TCMR in patients with tacrolimus and mycophenolate analogues showed no benefit of treatment [164]. There was no difference in renal function at 6 months and chronic histology scores were in fact higher in the treatment arm. This study was, however, limited by the relatively low frequency of subclinical rejection at early protocol biopsy, as only 4.6% showed subclinical TCMR. Additionally, chronicity scores in the control arm appeared to improve from implantation to the 6 months biopsy in some patients with seemingly no additional intervention. This perhaps indicates other unknown factors may have influenced the results of this study and limits the potential conclusions that can be drawn from it. In terms of more recent observational research, Seifert et al. [165] analyzed protocol biopsies at 3 and/or 6 months in 120 pediatric patients. They showed that 13 treated

TABLE 5 | Summary of studies on outcome of treated and untreated subclinical.

Study	Type of study	Total patients (n)	Time of biopsy	Total subclinical TCMR (n) or (%)	Treatment of subclinical TCMR	Outcome
Nankivell et al. [157]	Retrospective Single center	961	1, 2 weeks 1, 3, 6, 12 months post-transplant Annually thereafter	6.9% of all biopsies TCMR 23.4% of all biopsies B-TCMR	Methylprednisolone in 22.9% of TCMR and 12.3% of B-TCMR	Biopsies taken >3 months post-transplant with subclinical TCMR associated with higher ci and ct scores at 1 year biopsy Persistent TCMR associated with more significant decline in eGFR at 2 years
Moreso et al. [158]	Retrospective Single center	372	Protocol biopsy during initial 6 months post-transplant "For cause"	74 subclinical TCMR 65 subclinical TCMR + CAN	None	15 years DCGS lower in patients with CAN + TCMR compared to no rejection RR 1.86 (1.11–3.12)
Scholten et al. [159]	RCT	126 1:1 TAC vs. CsA	Protocol biopsy at 6 and 12 months post-transplant At graft dysfunction	At 6 months: 7.4% TCMR and 23.4% B-TCMR At 12 months 14.3% TCMR 24.5% B-TCMR	None	Less subclinical TCMR in TAC group Subclinical TCMR not associated with creatinin clearance at 2 years
Kurtkoti et al. [160]	RCT	102 1:1 Protocol biopy vs. Only indication biopsy	Protocol biopsy at 1, 3 months post-transplant vs. Indication only	Protocol biopsy group at 1, 3 months: 17.3%, 12%	Pulse steroids	Serum creatinin significantly higher at 6 and 12 months in control group vs. protocol biopsy group At 6 months: 137 ± 35 µmol vs. 113 ± 29 µmol ($p < 0.001$) At 12 months: 134 ± 36 µmol vs. 106 ± 29 µmol ($p < 0.001$)
Rush et al. (1998) [161]	RCT	72 1:1 early biopsies vs. later biopsy	Protocol biopsy at 1, 2, 3, 6, 12 months vs. Protocol biopsy at 6, 12 months	In early biopsy group: Subclinical TCMR at 1, 2, 3, 6 months: 43%, 32%, 27%, 15% In late biopsy group: Subclinical TCMR at 6 months: 32%	Pulse steroids	Significantly higher amount of patients with ci + ct scores ≥2 in control group vs. early biopsy group 24% vs. 6% at 6 months ($p < 0.04$) Significantly higher creatinin at 2 years in control group vs. early biopsy group 183 ± 22 µmol/L vs. 133 ± 14 µmol/L ($p < 0.05$)
Choi et al. [162]	Retrospective Single center	304	Day 14 Post-transplant	40	None	10 years graft survival subclinical TCMR vs. no rejection: 62.3% vs. 96.2% ($p < 0.05$)
Rush et al. (2007) [164]	RCT	218 1:1 early (<6 months) biopsies/ treatmentvs no biopsy	Protocol biopsy at 1, 2, 3, 6 months vs. Protocol biopsy at 6months	In early biopsy group: Subclinical TCMR at 1, 2, 3, 6 months: 5.7%, 0%, 8.1%, 8.9% In late biopsy group: Subclinical TCMR at 6 months: 6.0%	Pulse steroids	Significantly higher increase in ci + ct scores ≥2 at 6 months compared to baseline in early biopsy/treatment group vs. control group (1.12 ± 1.36 and 0.57 ± 1.02, $p = 0.04$) No significant difference in creatinin clearance or proteinuria at 6 months between groups
Loupy et al. [57]	Retrospective Single center + External validation	1,001	Protocol biopsy at 1 year	132	Pulse steroids	No significant difference in 8 years allograft survival or 8 years eGFR between subclinical TCMR vs. no rejection

(Continued on following page)

TABLE 5 | (*Continued*) Summary of studies on outcome of treated and untreated subclinical.

Study	Type of study	Total patients (n)	Time of biopsy	Total subclinical TCMR (n) or (%)	Treatment of subclinical TCMR	Outcome
Seifert et al. [165]	Retrospective Single center	103	Protocol biopsy at 3, 6 months	37	Increased maintenance immunosuppression, pulse steroids or thymoglobulin at discretion of physician	Significantly higher 5 years freedom from composite endpoint of acute clinical rejection or allograft loss in no rejection vs. untreated subclinical B-TCMR ($p < 0.001$) No significant difference in 5 years composite endpoint between treated subclinical B-TCMR vs. no rejection Significantly higher 5 years composite endpoint in no rejection vs. treated subclinical TCMR
Hoffman et al. [166]	Retrospective Single center	192	Protocol biopsy at 3, 12 months	56	Pulse steroids (Banff 1A/B) or thymoglobulin (Banff ≥ 2A)	No significant difference in delta creatinin between 3 and 24 months or odds of 50% decline in eGFR between 3 months and final follow up between subclinical TCMR vs. no rejection

TCMR CAN, Chronic allograft nephropathy; ci, Interstitial fibrosis; ct, Tubular atrophy; CsA, Ciclosporin; DCGS, Death-censored graft survival; RCT, Randomized controlled trial; TAC, Tacrolimus; TCMR, T-cell mediated rejection; B-TCMR, Borderline TCMR.

patients with subclinical aTCMR still had a significantly increased risk of meeting the composite endpoint of death-censored allograft loss and acute rejection at 5 years post-transplant, compared to patients without rejection. However, choice of treatment modality of this low number of patients was at the discretion of the physician. In contrast, larger recent studies showed no significant difference in delta creatinine, odds of 50% eGFR loss, or allograft survival between subclinical TCMR patients treated standardly with pulse steroids and a control group without TCMR at protocol biopsy; [57, 166]. It should be noted that these studies were mainly performed in DSA-negative patients. Thus, less is known about treatment of DSA-positive subclinical TCMR cases, although there is a broad consensus about the detrimental long-term consequences on ongoing inflammation in renal allografts [163]. However, Cherukuri et al; [24] analyzed the effect of treatment with steroid pulses on patients with TCMR and/or DSA, although these were not specifically subclinical cases. Patients with underlying TCMR and no DSA had no significant risk of graft loss. However, TCMR with concurrent DSA was a significant risk factor for 4 years allograft attrition in multivariate analysis, even when treated. Crucially, this significant risk was attributable to non-adherence. Adherent and pulse steroid treated patients with DSA and TCMR had no increased risk of allograft loss compared to patients without DSA and rejection, whereas non-adherent, pulse steroid treated patients with DSA and TCMR had drastically lower graft survival rates. This seemingly indicates that DSA-positive patients with underlying TCMR may still be amendable to current treatment modalities, provided they are adherent. This further signals that strengthening adherence is an important treatment option and is recommended by us and others in patients with dnDSA [15, 140]. There are currently no guidelines on the treatment of subclinical TCMR [163]. A recent systematic review and meta-analysis by Ho et al. [167] showed through the included retrospective studies that most centers seem to treat subclinical TCMR (Banff 1a or higher) with pulse steroids and occasionally thymoglobulin. This is in line with two recent surveys, which show that more than 90% of North-American transplant centers have implemented pulse steroids or lymphocyte depleting antibodies as standard of care in these patients [168, 169]. Currently, ESOT is surveying this in Europe as well.

Treatment of Subclinical Antibody-Mediated Rejection

As a substantial amount (40%–50%) of patients with subclinical dnDSA will have signs of ABMR upon biopsy, it is important to review the evidence for treatment options in these patients. Recent consensus guidelines concluded that there is very little evidence for efficacy of current treatment protocols for ABMR in patients with dnDSA [15]. However, a retrospective study showed an incremental improvement in the treatment of ABMR; [170]. In addition, a small phase II prospective randomized trial with an IL-6 inhibitor has shown some promising results in chronic active ABMR (caABMR), and is currently being studied in a large multicenter phase III RCT [171, 172]. Additional evidence is emerging on the effectiveness of costimulation blockade, as discussed above, and anti-CD38 therapy in patients with aABMR and caABMR, the latter of which is currently being investigated in a phase II RCT in the form of felzartamab [149, 173]. In light of emerging data one may conclude that (early) acute ABMR with dnDSA (but without transplant glomerulopathy) could be more responsive to maintenance treatment optimization as well as PP and IVIG and eventually novel treatment regimens than patients with caABMR or

TABLE 6 | Summary of studies on outcome of subclinical ABMR with or without treatment.

Study	Type of study	Total patients (n)	Total subclinical ABMR (n) or (%)	Type 1 or type 2 ABMR	Time of biopsy	Treatment of subclinical ABMR	Outcome
Parajuli et al. [102]	Retrospective single center	220	25 (all treated)	Type 1 and 2	Detection of dnDSA Protocol biopsies in case of pretranplant DSA 50% rise in MFI Graft dysfunction	≤3 months post-transplant: Pulse steroids, IVIG, PP ≥3 months post-transplant: Pulse steroids, IVIG, situationally RTX	No significant difference in 5 years post-biopsy DCGS between treated subclinical ABMR and no rejection Significantly better 5 years post-biopsy DCGS in treated subclinical ABMR than clinical ABMR and than DSA-indication biopsies (92% vs. 54%, proportion of DSA- indication biopsies with DCGS not provided) No significant difference in post-biopsy DCGS between type 1 or type 2 subclinical ABMR.
Orandi et al. [175]	Retrospective single center	2097	77 (41 treated)	Uncertain Mostly type 1	Protocol biopsies at 1,3,6, 12 months post-transplant in HLA or ABOi incompatible transplants	PP + Situationally RTX or eculizumab	No significant difference in DCGS between treated subclinical ABMR and ABMR free matched controls. HR 1.73; 95% CI: 0.73–4.05; $p = 0.21$ Significantly worse DCGS in untreated subclinical ABMR vs. ABMR free matched controls. HR 3.34; 95% CI: 1.37–8.11; $p = 0.008$
Yamamoto et al. [79]	Retrospective single center	43	18 (all treated)	Type 2	At dnDSA detection	Plasmapheresis and RTX	Significant decrease of MFI in 6 out of 18 patients Within 10 patients with rebiopsy, 4 had improvement or no change in graft histology
Bertrand et al. [77]	Retrospective Multicenter	123	51 (19 treated)	Type 2	At dnDSA detection	A combination of IVIG/ PP/RTX	Significantly worse 8 years biopsy DCGS in subclinical ABMR patients vs. no rejection. (78% vs. 97%, $p < 0.01$) No significant difference in 8 years post-biopsy DCGS between treated and untreated subclinical ABMR
Loupy et al. [57]	Retrospective single center + External validation	1,001	142 (56 treated)	Type 1 and 2	Protocol biopsy at 1 year post-transplant	IVIG, PP, RTX	Significantly worse 8 years graft survival probability in subclinical ABMR vs. no rejection (56% vs. 90%, $p < 0.0001$ Significantly faster decline of eGFR over 8 years in subclinical ABMR vs. no rejection (p not provided) No analysis in regards to treated vs. untreated subclinical ABMR

ABMR, Antibody-mediated rejection; DCGS, Death-censored graft survival; DSA, Donor-specific antibody; dnDSA, de novo *DSA; eGFR, Estimated glomerular filtration rate; IVIG, Intravenous immunoglobulins; MFI, Mean fluorescence intensity; PP, Plasmapheresis; RTX, Rituximab.*

cABMR, albeit all the treatment options have a low amount of supporting evidence. Active research in this area is ongoing and ABMR definition is becoming more precise [174]. Thus, there could potentially be benefit in finding and treating patients with early (subclinical) forms of ABMR before they present late with irreversible chronic lesions and clinical dysfunction. Some retrospective studies seem to support this hypothesis (**Table 6**). Parajuli et al. [102] showed similarly good post-biopsy allograft survival in patients with subclinical ABMR treated with IVIG and PP, as compared to protocol biopsied dnDSA-positive patients without rejection. Additionally, patients with treated subclinical ABMR had significantly better allograft survival than DSA-negative patients with indication biopsies or patients with treated clinical ABMR. Importantly, there was no difference in outcome between subclinical ABMR based on preformed DSA (type 1) vs. dnDSA (type 2). However, it must be noted that the post-biopsy follow-up time in patients with subclinical ABMR was relatively low at 31.0 ± 15.8 months. Orandi et al. [175] showed that patients with mostly type 1 subclinical ABMR treated by PP and in some situations rituximab or eculizumab had no significantly different rate of 5 years death-censored allograft loss compared to ABMR negative matched controls, whereas untreated patients had significantly more 5 years death-censored graft attrition rates compared to their control group. In addition, Yamamoto et al [79]. described some beneficial effects of PP and rituximab in 8 out of 18 (44%) of patients with subclinical type 2 ABMR whereby DSA levels reduced significantly or histological injury stabilized upon rebiopsy. In contrast, studies by Bertrand et al. [77] and Loupy et al. [57] found that allograft survival in treated subclinical ABMR patients was still significantly worse than patients without rejection. However, only 39% of patients with subclinical ABMR in the study by Loupy et al. [57] received specific treatment for subclinical ABMR and no analysis was performed comparing the treated and untreated group. It is apparent that more robust research on the effectiveness of treatment of subclinical ABMR is warranted. Nonetheless, the overall risk-benefit balance seems to be in favor of screening of DSA, which could result in early optimization of maintenance therapy. Moreover, further biopsy-guided treatment of subclinical TCMR and subclinical ABMR may be more effective than later treatment of clinical rejections, though evidence for this notion is more limited, as reflected in the grading of this recommendation.

Cost-Effectiveness of DSA Monitoring in Patients With Stable Graft Function Will Depend on Incidence Rate of dnDSA and Importantly on Size Effect of Treatment (2D)

Assessment of the balance between medical risks and benefits of early case finding may determine that a screening program is medically justified, though this assessment does not necessarily determine whether it is cost-effective. As transplant centers have finite resources, DSA screening should be economically balanced to the cost of medical expenditure as a whole. Important aspects are the costs of the screening test and of the consequences of a missed case. The costs of a patient with graft loss due to ABMR who proceeds to renal replacement therapy far exceed the costs of those who retain their transplant by over €40.000 per year [176]. If one assumes that graft losses to ABMR account for around 1/3 of all graft losses [17] and takes into consideration the costs and benefits of potential treatment as well as morbidity and mortality rates of those treatments, then DSA screening seems justifiable on first glance. Unfortunately, evidence in the literature on this topic is very scarce. Kiberd et al. [177] performed a DSA monitoring cost-effectiveness modelling study. They found that costs per increased quality-adjusted life year (QALY) could range from $127.000 to $444.000, depending on the estimated efficacy of treatment and on the incidence rate of dnDSA. However, the model did not account for the fact that costs saved by not screening and treating early would still partly be spent later on treating patients when they do present with clinical dysfunction. This means that the presented costs per QALY are likely an overestimation, especially considering that most of the projected costs were attributed to the treatment of found cases, instead of DSA screening itself. Nonetheless, the basis for a cost-effective screening strategy is adequately illustrated through this modelling example. The only real-world data regarding cost-effectiveness comes from the previously mentioned OuTSMART study [21]. The incidence rate of dnDSA in this study population was lower than expected at 1.6% per year. This, in combination with no found benefit of optimization of maintenance therapy, resulted in a staggering incremental cost-effectiveness ratio of £1.692.222 per QALY for monitoring for DSA. As stated before, development of dnDSA pertains to multiple risk factors, and particularly to the immunological risk and epitope mismatch [4, 28, 46, 178]. The varied reported incidence rate in current literature likely attests to this, as some report a steady rate ranging from 1.5% to 5.4% per year in immunological low-risk patients [22, 179–181]. Others report increased incidence in the first year ranging from 3.2% to even 20% with a lower steady yearly rate thereafter ranging from 0.8% to 4.3% [30, 182, 183]. The lower incidence rate in OuTSMART could thus perhaps be a reflection of better organ allocation, better post-transplant overall care or it could simply reflect a different population in terms of age, healthy survivor bias from cross-sectional inclusion, ethnicity or proclivity to adhere to their medication as compared to the populations in the mentioned reports in the literature. Nonetheless, the results of this trial provide real-world validation of the modeling study by Kiberd et al. [177], as it shows that cost-effectiveness of DSA monitoring is dependent on the incidence rate of dnDSA and effect of treatment. Whether or not DSA monitoring is cost-effective, may thus in fact differ between centers, as incidence rate, local treatment protocols, and allograft biopsy strategy in case of subclinical dnDSA may differ. More trials, with standardized DSA definition and reporting, in various

populations with additional allograft biopsies in case of subclinical dnDSA are ultimately needed to fully determine the cost-effectiveness of DSA monitoring.

Monitoring for dnDSA During Functional Graft Life Is a Continuous Process and Should Not Cease Upon Detection of dnDSA (2C)

Case-finding should be a continuing process and not a "once and for all" project. As new cases of subclinical rejection accumulate over time post-transplantation, DSA screening cannot be a one-time effort [29, 34]. The intensity and the longevity of the monitoring strategy should be reflected by the *a priori* chance of development of dnDSA over time. A recent large retrospective analysis shows that of 400 patients with dnDSA, 20% were found within the first year, 60% within 5 years and 85% within 10 years post-transplant, clearly indicating that even after 10 years post-transplant, patients may still develop dnDSA [29]. Unfortunately, as shown previously, the annual dnDSA incidence rate is not fully clear. Nevertheless, all studies indicate that dnDSA are constantly evolving and that the incidence does not reduce significantly after 1 year post-transplant. This subsequently implies that any time-limited monitoring strategy, although less costly, would be medically arbitrary and would miss new subclinical cases that occurred after screening ceased. The OuTSMART trial attests to this notion, as incidence rate did not diminish after a set amount of prospective monitoring years [21]. Another point of contention is whether monitoring should be continued for persistence or development of new dnDSA once a dnDSA has been detected. A retrospective study by DeVos et al. [184] found that patients with >60% positive DSA measurements in at least 3 separate assessments are more likely to progress to allograft loss than those with <60% positive measurements. López del Moral et al. [29] showed that dnDSA which eventually disappear, either temporarily or permanently, are associated with a lower rate of allograft loss than those who persist. Additionally, they showed that development of multiple dnDSA is associated with worse allograft survival, though this association was no longer statistically significant in multivariable analysis. In contrast, Kim et al. [88] found that resolved dnDSA was not associated with less decline in renal function. These studies, while somewhat conflicting, overall seem to suggest that newly developed dnDSA which eventually disappear are less likely to be associated with subsequent allograft loss. This implies that continued monitoring after dnDSA have already developed could serve important prognostic purposes. Moreover, additional dnDSA may develop, which could be cause for an additional allograft biopsy. Current low-grade evidence thus suggests that monitoring should not be discontinued after a set amount of post-transplant years, nor upon development of dnDSA.

The Optimal dnDSA Monitoring Scheme has Not Been Established, but a Routine Approach Would Be Antibody Monitoring at Three to Six Months Post-transplant and Annually Thereafter. (2C)

Another dilemma in regards to the continuing process of case finding entails the intensity of monitoring. In an ideal world, development of dnDSA would be noted immediately. But this would require a frequency of monitoring that is unlikely to be feasible. Centers which perform routine DSA monitoring seem to do so annually with one or more additional measurements in the first year post-transplant [30, 77, 80]. A more personalized approach could be monitoring intensity based on the immunological risk, this may also be more cost-efficacious, as lower risk patients could be subjected to less frequent screening. Monitoring intensity stratification based on HLA-matching might be easy to establish. Naturally, recipients of a completely HLA-identical donor kidney have no risk of developing HLA-DSA. Completely HLA-identical transplants are, however, rare. Most DSA appear to be aimed at HLA-DQ [185], though López del Moral et al. [29] showed that the proportion of patients with a full HLA-DQ match who developed dnDSA was comparable to those with a full HLA-B or HLA-DR match. This indicates that other HLA-loci mismatches should not so easily be disregarded. More recent evidence regarding molecular eplet HLA mismatching has emerged, whereby a low DQ/DR eplet mismatch was found to carry a negligible risk for development of DQ or DR dnDSA [26, 28]. In addition, analysis of the predictive value of the PIRCHEII and HLA-matchmaker molecular eplet mismatch algorithms showed that low eplet mismatch was associated with reduced probability of dnDSA development for both class I and II HLA-loci [186]. Lastly, post-hoc analysis of the CELLIMIN trial showed that high molecular eplet mismatch load was associated with development of dnDSA for both HLA-classes [187]. These studies indicate that low levels of total eplet mismatch load could be a reason to lower DSA monitoring intensity or even omit it. Personalized DSA monitoring intensity based on molecular mismatch thus seems promising. However, further validation of this risk-stratification technique in prospective trials on DSA screening is needed and more research is thus recommended. Currently, no study has been conducted which compares outcomes of different monitoring frequency strategies. Notwithstanding, the study by Parajuli et al [102] shows that patients with subclinical dnDSA who are detected, biopsied and treated through a strategy consisting of screening after 6 months and annually thereafter have good outcome. This suggests that more intensive monitoring may be unnecessary. Additionally, a monitoring interval greater than 1 year might be ill-advised, as studies in untreated subclinical ABMR show more chronic lesions within 1 year post-diagnosis [72, 97]. This may indicate that patients detected beyond 1 year from

inception of the dnDSA may be more difficult to treat. Lastly, considering multiple studies have indicated increased incidence of development of dnDSA in the first year post-transplant, it might be advisable to perform an additional measurement within three to 6 months post-transplant [29, 30, 182, 183]. It thus appears from current low-level evidence that, until more robust immunological risk-stratification methods are validated, monitoring strategies consisting of screening within the first three to 6 months post-transplant and annually thereafter may seem pragmatic. However, more prospective research is needed to determine the optimal monitoring strategy.

SUMMARY AND NEXT STEPS

The authors suggest that, based on current available evidence and the assessment of each individual Wilson & Jungner criterium, monitoring for development of dnDSA has clinical utility to further optimize long-term graft survival. A routine approach for such a strategy could be annual monitoring with an additional assessment within the first three to 6 months post-transplant. Monitoring should not cease after a certain amount of time or after dnDSA has already developed. Subclinical dnDSA development should lead to promotion of adherence and addressment of secondary risk factors. Further treatment should only be considered after performing an allograft biopsy to diagnose underlying rejection. Evidence for further treatment guided by such biopsies in subclinical patients is limited. However, certain patients with early rejection may respond to it empirically and treatment of subclinical TCMR has become standard of care in most centers. Novel treatments may provide additional efficacy in terms of prolonging allograft survival in the near future. Ultimately, further prospective trials are necessary to fully determine the benefits of such treatment strategies and their cost-effectiveness. Monitoring preformed DSA and their evolution in the subclinical setting post-transplantation with currently available validated assays may not provide a clear enough signal for possible underlying pathology. Additional clinical and laboratory parameters should therefore be considered before deciding to perform a biopsy in these patients. However, this does not preclude DSA monitoring in these patients, as development of additional dnDSA should equally lead to further investigation and treatment of these individuals.

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

Involved in the conception or design of the work: DB, SM, KB, CL, EC, DB, CM, AD, M-PE, MN, and AV. Literature screen and review: DB, SM, KB, CL, EC, DB, CM, AD, M-PE, MN, and AV. Drafted the article: DB, SM, KB, CL, EC, DB, CM, AD, M-PE, MN, and AV. Critically revised the article: DB, SM, KB, CL, EC, DB, CM, AD, M-PE, MN, and AV. Finally approved the version to be published: DB, SM, KB, CL, EC, DB, CM, AD, M-PE, MN, and AV. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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European Society of Organ Transplantation (ESOT) Consensus Statement on Prehabilitation for Solid Organ Transplantation Candidates

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There is increasingly growing evidence and awareness that prehabilitation in waitlisted solid organ transplant candidates may benefit clinical transplant outcomes and improve the patient's overall health and quality of life. Lifestyle changes, consisting of physical training, dietary management, and psychosocial interventions, aim to optimize the patient's physical and mental health before undergoing surgery, so as to enhance their ability to overcome procedure-associated stress, reduce complications, and accelerate

Abbreviations: AGREE, Appraisal of Guidelines for Research & Evaluation; CET, Centre for Evidence in Transplantation; ESOT, European Society of Organ Transplantation; GRADE, Grading of Recommendations, Assessment, Development, and Evaluations; PICO, Population, Intervention, Comparator and Outcome; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; RCT, Randomized Controlled Trial.

post-operative recovery. Clinical data are promising but few, and evidence-based recommendations are scarce. To address the need for clinical guidelines, The European Society of Organ Transplantation (ESOT) convened a dedicated Working Group "Prehabilitation in Solid Organ Transplant Candidates," comprising experts in physical exercise, nutrition and psychosocial interventions, to review the literature on prehabilitation in this population, and develop recommendations. These were discussed and voted upon during the Consensus Conference in Prague, 13–15 November 2022. A high degree of consensus existed amongst all stakeholders including transplant recipients and their representatives. Ten recommendations were formulated that are a balanced representation of current published evidence and real-world practice. The findings and recommendations of the Working Group on Prehabilitation for solid organ transplant candidates are presented in this article.

Keywords: prehabilitation, solid organ transplant candidates, exercise, nutrition, psychosocial interventions

INTRODUCTION

Patients who need a solid organ transplant often have a compromised overall condition due to end-stage organ failure, comorbidities, deconditioning, and treatment-related adverse effects such as dialysis in end-stage kidney disease (ESKD), left ventricular assist device (LVAD) in heart failure, and oxygen therapy in end-stage pulmonary disease (ESPD) [1–3]. Although considered a frail patient population with malnutrition, low physical fitness, fatigue, and often secondary psychological challenges, it is imperative for such patients to attain, and maintain, their optimal physical and mental wellbeing, as this will help them tolerate the waiting time and the stress of transplant surgery and expedite recovery after the transplant. The time spent on the transplant waitlist provides a window of opportunity to work towards enhancing the overall condition of such patients.

Prehabilitation refers to the optimization of patient's overall physical and psychological condition before undergoing surgery, in order to enhance his/hers ability to overcome the stress associated with the procedure, to reduce the risk of complications and to accelerate post-operative recovery, with the ultimate goal to improve survival and quality of life [4]. The approach focuses on achieving lifestyle changes and should consist of physical training, dietary management, and psychological interventions [4]. By providing a multimodal program, the complex interaction between the physical and psychological health of a patient is addressed, which is important to maximize the outcomes of the interventions [5].

Prehabilitation has shown promising results in non-transplant patients undergoing major abdominal or orthopedic procedures [6–10], with reduced overall post-operative complications and morbidity, improved aerobic capacity, and improved functional recovery and shorter length of stay. The conclusions from two systematic reviews supported the feasibility and safety of such interventions in waitlisted solid organ transplant candidates [11, 12]. In addition, observed beneficial effects included improvements in cardiorespiratory function, exercise capacity, muscular strength, mental/physical composite scores and health-related quality of life [11, 12]. There is a growing awareness and evidence that prehabilitation may not only benefit clinical transplant outcomes, but may also improve the transplant candidate's overall health and quality of life, through adoption of a sustainable, healthy lifestyle. Despite this growing awareness and promising data, evidence-based recommendations for physical exercise, nutritional, or psychological prehabilitation interventions in candidates for solid organ transplants are not available. With regard to exercise interventions, recommendations on the role of exercise in solid organ transplantation were made by Janaudis-Ferreira et al in a position statement paper in 2019 [13].

The limited clinical guidance on how to implement prehabilitation for solid organ transplant candidates was presented as one of the priority themes at the first European Society of Organ Transplantation (ESOT) consensus conference in November 2022. Under the oversight of the ESOT guideline taskforce, and in keeping with the procedures recently established by the ESOT Consensus Platform for Organ Transplantation, leading experts presented in-depth literature evidence and proposed recommendations, which were publicly discussed and assessed by an independent jury, and consensus was formed [14]. Participants in the consensus process included not only transplant, prehabilitation and medical specialists, but also allied health professionals, patients and patient representatives.

This document presents the 2022 ESOT consensus findings and recommendations on implementing prehabilitation in the care for solid organ transplant candidates. These guidelines and recommendations undergo continual review and will be updated to reflect new evidence as it becomes available.

METHODS

The consensus development process was governed by the dedicated ESOT Guidelines Taskforce and co-organized by the ESOT sections European Liver and Intestine Transplant Association, European Kidney Transplant Association,

European Pancreas and Islet Transplant Association, European Cardio Thoracic Transplant Association, European Transplant Allied Healthcare Professionals, the ESOT Education Committee and Young Professionals in Transplantation.

The consensus development process followed the methodology stipulated by the ESOT Consensus Platform as recently published in detail [14]. In brief, the subsequent steps were as follows:

i) Prehabilitation for solid organ transplant candidates was selected as a priority topic for the first ESOT Consensus Conference, as published [14].
ii) A specific steering committee was selected, consisting of experts in the topic field, members from the Centre for Evidence in Transplantation, a Young Professional in Transplantation representative, and a guideline taskforce member to liaise with ESOT.
iii) The steering committee identified key relevant questions related to prehabilitation of solid organ transplant candidates (heart, lung, liver, kidney) using to the Population, Intervention, Comparator and Outcome (PICO) methodology [15] (**Table 1**).
iv) The staff of the Centre for Evidence in Transplantation performed systematic literature reviews that were informed by the PICO questions and thus related to exercise, nutritional and psychological interventions in solid organ transplant candidates. The search strategy is presented in **Supplementary Table S1**. The PRISMA diagrams from the evidence review are shown in **Figures 1–3**. As the number of publications was expected to be limited, selection was not limited to randomized clinical trials but also included studies that used a pre/post or case-control design, prospective and retrospective studies (cohorts or registry), feasibility studies and pilot studies. Reviews and meta-analyses were included for hand searching of bibliographies for additional literature. Studies were included only if a minimum of 80% of study participants were formally waitlisted for a solid organ transplant. Case reports on fewer than 10 patients, conference abstracts, and letters to the editor were excluded, as was non-English literature. The literature evidence relating to the PICO questions was summarized, as shown in **Tables 2–4** and **Supplementary File S1**.
v) The steering committee integrated the literature evidence and formulated recommendations (**Supplementary File S1**). When proposing recommendations for each question, the quality of evidence was considered as evaluated by the GRADE approach [16]. This included risk of bias (**Figures 4–6**), which was assessed by two independent reviewers, and an additional third one if disagreement occurred. The strength of the individual recommendations was rated as strong or weak.
vi) Jury members, who were not part of the steering committee were selected and vetted by the guideline taskforce and were comprised of allied health professionals, patients (representatives), transplant physicians, and transplant surgeons.
vii) Consensus was generated using discussion within the entire working group and modified Delphi methodology including consensus polling, followed by jury voting of the recommendations during a session at the ESOT Consensus conference in Prague [17].
viii) A committee of validating experts validated the recommendations using the AGREE II guidelines [18].

RESULTS

A total of 4 PICO questions were identified, along with key criteria for analysis, as presented in **Table 1**. The systematic

TABLE 1 | PICO questions and criteria for analysis.

1	In adult candidates for lung, liver, kidney and heart transplantation: What is the evidence for the effectiveness of pre-transplant exercise training, nutritional support and psychosocial interventions, as measured by the criteria prehabilitation efficacy outcomes, clinical outcomes and patient-reported outcomes Criteria for analysis: Effectiveness of the prehabilitation program: Maximal exercise capacity, Functional exercise capacity, Muscle strength, Nutritional status, Body composition, BMI, Cardio metabolic risk profile, Distress (anxiety/depression), Fatigue, Frailty Clinical outcomes: Mortality (pre-/post-transplant); Hospital (re-)admissions (pre-/post-transplant); Length of hospital stay (pre-/post-transplant); Complications after transplant surgery; Graft survival; Rejection episodes Patient reported outcomes: Health related Quality of Life (HRQoL), Activities of daily living
2	In adult candidates for lung, liver, kidney, and heart transplantation: What is the evidence for the type of pre-transplant exercise, nutritional support and psychosocial interventions?
3	In adult candidates for lung, liver, kidney, and heart transplant candidates: Which relevant outcomes need to be measured to evaluate the effect of the pre-transplant exercise and physical therapy, nutritional support and psychosocial interventions?
4	In adult candidates for lung, liver, kidney and heart transplantation: What is the evidence for the feasibility of prehabilitation, as measured by the criteria enrolment, retention, acceptability, fidelity, safety? Criteria for analysis: Enrolment: the number of screened patients who met the eligibility criteria (n/%), the number of eligible patients who were recruited for the study (n/%) Retention: the number of participants that were retained in the intervention study, drop-out rate (n/%), reasons for drop-out Acceptability: the perception among professionals and participants that the intervention is agreeable, appropriate, or satisfactory Fidelity: the degree to which the intervention was implemented as it was intended, as measured by adherence to the program protocol by the interventionist and participants, Safety: occurrence of adverse events

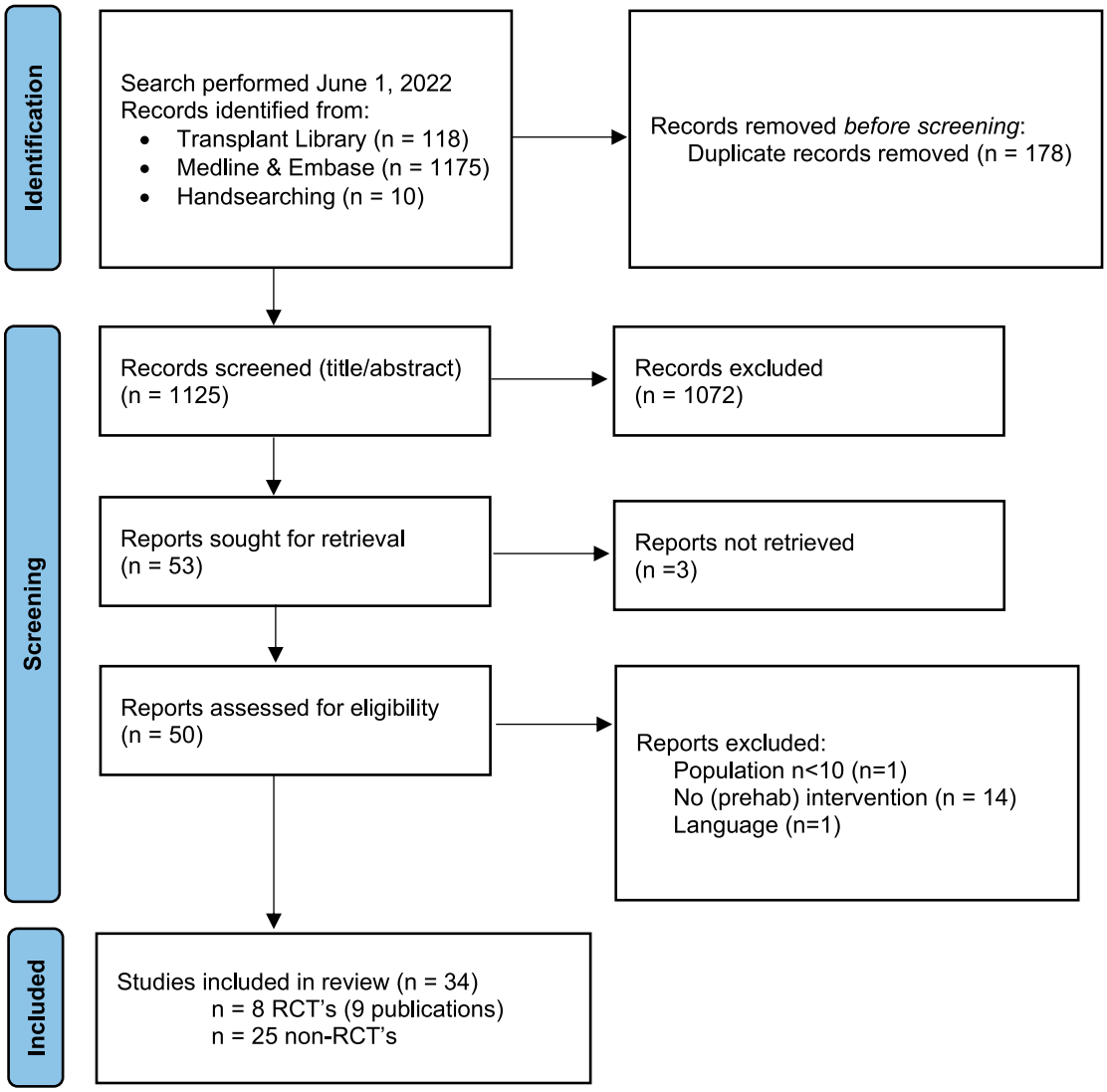

FIGURE 1 | Flow chart of study selection process exercise interventions with reasons for exclusion. Note: n = number of studies. Figure adapted from: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffman TC, Mulrow CD, et al. The PRIMSA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372:n71, doi:10.1136/bmj.n71. For more information visit http://www.prisma-statement.org/.

review of literature yielded 34 studies on exercise, 7 on nutritional, and 10 on psychological interventions (**Figures 1–3**). Summaries of the literature evidence were generated and are presented in **Tables 2–4** and **Supplementary File S1**. A total of 26 recommendations were formulated (**Supplementary File S2**). At the Consensus Conference, the literature summaries and recommendations were presented, discussed, and amended according to the ESOT consensus-finding process. In response to the considerations voiced during the discussion, and in an attempt to avoid overlap, the number and nature of the recommendations was revised to 10 well-defined recommendations, i.e., 4 general and 6 specific ones. In a first voting round, 100% agreement was achieved on 7 out of 10 recommendations, whereas 3 recommendations reached 86% agreement (1.2, 2.4, 2.5) due to being considered as too exclusive of certain patient groups. Consensus was reached to amend these recommendations to be more inclusive, and in a second voting round, 100% agreement was achieved on all 10 recommendations.

RECOMMENDATIONS

PICO Question 1

In adult candidates for lung, liver, kidney, & heart transplantation: what is the evidence for the effectiveness of pre-transplant exercise training, nutritional support and psychosocial interventions as measured by prehabilitation efficacy outcomes, clinical outcomes and patient-reported outcomes.

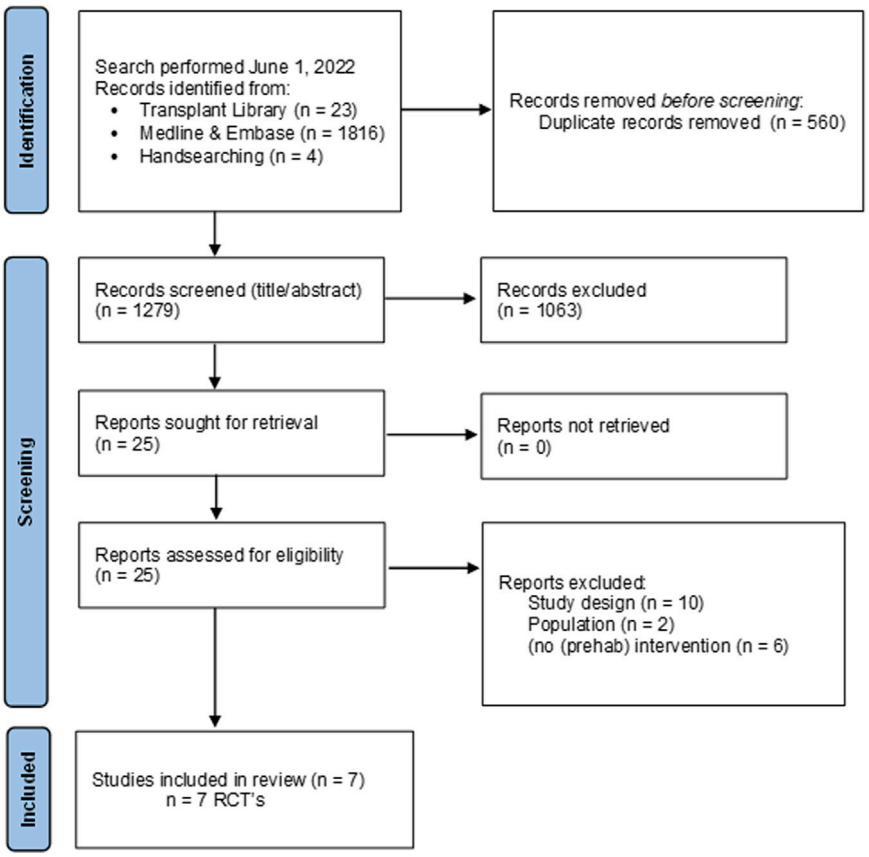

FIGURE 2 | Flow chart of study selection process nutritional interventions with reasons for exclusion.

To date, multimodal prehabilitation programs that offer a combination of exercise, nutritional, and psychosocial interventions, have not been studied in solid organ transplant candidates. Rather, literature is limited to studies investigating a single type of intervention. Based on the committee's literature review and analysis of the predefined criteria prehabilitation effectiveness, clinical and patient-reported outcomes, one general recommendation and two specific recommendations were made.

Recommendation 1.1

Studies are needed that evaluate multi-modal prehabilitation interventions in candidates for all types of solid organ transplantation and that focus on core outcomes and implementation. Such studies should be of high quality, and preferably–but not exclusively–adequately powered RCTs.

Quality of Evidence: not applicable.

Strength of Recommendation: Strong.

Rationale: Although supportive, the current evidence (**Table 2–4**) based on the effectiveness of pre-transplant exercise, nutritional, and psychosocial interventions is weak because of the limited number of randomized studies; 8 for exercise interventions [19–25], 7 for nutritional interventions [26–32], and 6 for psychosocial interventions [33–38]. In addition, 25 non-randomized studies regarding exercise [39–64], and 4 non-randomized studies on psychosocial interventions [65–68] were retrieved by literature review (**Supplementary File S1**). The small sample size per study and the limited size and heterogeneity of the total populations studied, the variability in interventions and outcomes measures, the generally low-to-moderate quality of the methodology, and–as a result–the inconsistency of findings across studies (**Tables 2–4**), warrants high-quality studies on multimodal prehabilitation before solid organ transplantation.

Recommendation 1.2

It is suggested that exercise-based interventions are included in the prehabilitation care of solid organ transplant candidates, with the objective to improve cardiorespiratory fitness and/or inspiratory muscle strength.

Quality of Evidence: Low.

Strength of Recommendation: Weak.

Rationale: Although the number and size of RCTs is limited, studies have shown that exercise training was associated with clinically meaningful improvement in cardiorespiratory fitness in heart transplant candidates [19, 21, 22, 24] and a clinically meaningful gain in inspiratory muscle strength in heart and in liver transplant candidates [19, 23, 24].

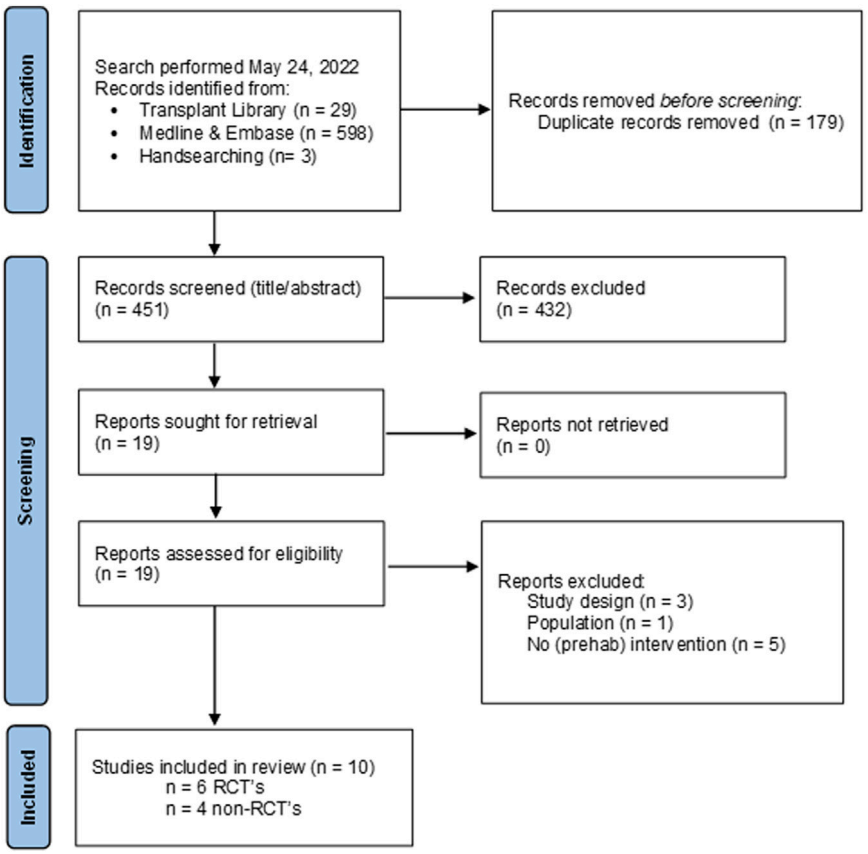

FIGURE 3 | Flow chart of study selection process psychosocial interventions with reasons for exclusion.

Recommendation 1.3

It is suggested that probiotic therapy be used in candidates for liver transplantation to reduce their susceptibility to post-transplant infections.

Quality of Evidence: Very low.

Strength of Recommendation: Weak.

Rationale: Two studies were identified in which pre-transplant probiotic and symbiotic therapy were associated with reduced post-transplant infection rates in recipients of a liver transplant [26, 28]. However, both studies had small sample sizes ($n = 44/n = 50$) and used different products.

PICO Question 2

In adult candidates for lung, liver, kidney, & heart transplantation: which type(s) of exercise, nutritional support and psychosocial interventions are recommended in the pre-transplant phase?

As there are no established prehabilitation programs for solid organ transplant candidates, evidence review was focused on studies that addressed interventions that could be of value in a multimodal prehabilitation program. One general recommendation and four specific recommendations were established.

Recommendation 2.1

Studies are needed to identify the optimal component(s) and the mode of delivery of pre-transplant multimodal prehabilitation programs in solid organ transplant candidates. Such studies should be of high quality and be preferably -but not exclusively-adequately powered RCTs.

Quality of Evidence: not applicable.

Strength of Recommendation: Strong.

Rationale: Because of the heterogeneity in the study populations and in the nature and delivery mode of the interventions described in the current literature (**Tables 2–4**; **Supplementary File S1**), it remains unclear which organ transplant candidates would benefit most from which intervention program. Most exercise intervention studies used aerobic training [19–22, 24], peripheral muscle training [41], inspiratory muscle strength training [25, 51], or a combination of these training modalities. Nutrition intervention studies mostly used nutritional support to optimize energy intake and/or obtain weight loss [29–32, 69, 70]. Whilst psychosocial interventions predominantly included cognitive behavioral therapy [33, 34, 36, 37, 65, 67], psycho-educational interventions [35, 68] and stress management and relaxation techniques [38, 67] or a combination of these interventions. Studies are needed that will help determine the modalities of the intervention, and for each modality (exercise, nutrition or psychosocial), the intervention characteristics (frequency, intensity and timing), the delivery mode (type of interventionist, level of supervision, home-based versus in- or outpatient) for each type of donor organ recipient.

TABLE 2 | Summary of RCTs- Exercise interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – Feasibility outcomes
1	Laoutaris, 2011 (Greece)	HTx candidates with LVAT or BiVAT n = 21	All participants were advised to walk every day for 30–45 min.	VO_2peak	↑ within I; I ≈ C	Enrolment: 21/23 (91%) of eligible patients
		I n = 14 100% male Age 37 (±18)	I: 10 week, homebased aerobic exercise (45 min, 3–5x/week, intensity y12–14/20 RPE) and hospital-based IMT (until exhaustion, 2–3x/week, intensity 60% MIP)	6MWT	↑ within I; I ≈ C	Attrition: 15/21 (71%) completed (I 10/14 (71%); C 5/7 (71%)), all drop out due to Tx
		C n = 7 80% male Age 42 (±15)	C: Usual care	QOL (MLwHFQ)	↑ within I; I ≈ C	Fidelity (participants): NR
			Assessments: - Pre-intervention - Post-intervention	PIMax and sustained PImax	↑ within I; I ≈ C	Fidelity (interventionist): NR
				Lung volumes (inspiratory capacity)	↑ within I; I ≈ C	Acceptability: patients enjoyed training and seemed more enthusiastic compared with patients in the control group
				Dyspnea after 6MWT	I ≈ C	Safety: no adverse events occurred during the training period
2	Gloeckl, 2012 (Germany)	LuTx candidates following COPD stage IV diagnosis n = 71	All participants received strength training (four to six exercises, 3 sets of 30 repetitions, at maximal tolerated load), breathing therapy, education, and psychological support.	6MWT	≈ increase in I andC	Enrolment: 71/97 of eligible patients
		I n = 36 49% male Age 52 (±6)	I: 3-weeks, hospital-based high-intensity interval training, 10–36 min per session, 5–6x/week, 1–2 sessions/day, intensity repeated bouts of 30 s at 100% Wmax alternated by 30 s rest	QOL (SF-36 PCS and MCS)	PCS: ≈ within I; ↑ within C; I ≈ C MCS: ↑ within I; ≈ within C; I ≈ C	Attrition: 60/71 completed (I 30/36 (83%); C 30/35 (86%)). Dropout due to: I acute exacerbation (n = 4), non-compliance with study protocol (n = 1), other (n = 1); C acute exacerbation (n = 3), Tx (n = 1), other (n = 1)
		C n = 35 44% male Age 55 (±7)	C: 3-weeks, hospital-based, moderate-intensity aerobic exercise, 10–30 min/session, 5–6x/week, 1-2 sessions/day, intensity 60% Wmax	W_{max}	≈ increase in I and C	Fidelity (participants): no difference in number of exercise sessions or total work performed per group. I: 14.9 (±1.9); C: 14.7 (±1.5)
			Assessments: - Pre-intervention - Post-intervention	During exercise - SpO_2 - $TCPCO_2$ - Dyspnea - leg fatigue	 I ≈ C I ≈ C ↓ in I I ≈ C	Fidelity (interventionist) NR
				Unintended breaks (number and time) during exercise	↓ number and ↓ duration in I	Acceptability: NR
				PaO_2 and $PaCO_2$	I ≈ C	Safety: no serious adverse events occurred
				lung function (DLCO, FEV1, FEV1/IVC)	I ≈ C	

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TABLE 2 | (*Continued*) Summary of RCTs- Exercise interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – Feasibility outcomes
3	Hayes, 2012 (Australia)	HTx candidates with LVAT n = 14	All participants followed a progressive walking program: They were advised to walk a minimum of 5 days per week at 13 RPE and increased their walk progressively up to 60 min.	VO_2peak	↑ in; I ≈ C	Enrolment: 14/18 (78%) of eligible patients
		I n = 7 86% male Age 48.7 (±14.5)	I: 8-week, gym-based aerobic and strength training (60 min/session, 3x/week, intensity cycling at 50% VO_2reserve; treadmill at 60% of the speed averaged during the 6MWT; strength: three upper limb and three lower limb exercises using weight machines and free weights, 2 sets of 10 reps).	6MWT	↑ in I and C; I ≈ C	Attrition: 14/14 (100%) completed
		C n = 7 86% male Age 45.9 (±14.6)	C: Usual care, which included a walking program.	QOL (SF36)	↑ within I; I ≈ C	Fidelity (participants): I: participation in 21.3 ± 1.5 of possible 24 sessions. Reasons for missed sessions: conflicting medical appointment (79%) and conflicting family demands (21%). C: 100% compliance to the walking program
			Assessments: - Pre-intervention - Post-intervention	W_{max}	↑ in I and C	Fidelity (interventionist): NR Acceptability: NR Safety: no adverse events occurred
4	Adamopoulos, 2013 (Greece)	HTx candidates with LVAT or BiVAT n = 22	All participants were advised to walk every day for 30–45 min.	Thyroid hormone signalling (TRα1, p/t-AKT and p/t-JNK)	↑ within I; I ↑ C	Enrolment: 22/26 (85%) of eligible patients
		I n = 11 91% male Age 39.7 (±4.3)	I: 12 weeks, aerobic training (home-based, 45 min/session, 4x/week, intensity 12-14/20 RPE) and IMT (hospital-based, until exhaustion, 3x/week, intensity 60% PImax	VO_2peak	↑ within I; I ↑ C	Attrition: 22/22(100%) completed
		C n = 11 82% male Age 40.9 (±4.9)	C: Usual care	NT-proBNP (marker of heart failure)	↓ within I; I ↓ C	Fidelity (participants): NR
			Assessments: - Pre-intervention - Post-intervention			Fidelity (interventionist): NR Acceptability: NR Safety: NR

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TABLE 2 | (*Continued*) Summary of RCTs- Exercise interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – Feasibility outcomes
5	Limongi, 2014 and 2016 (Brazil)	LiTx candidates n = 49		MIP	↑ in I and C	Enrolment: 49/49 (100%) of eligible patients
		I n = 22 79% male Age 55.8 (±5.4)	I: 3-months, home-based, daily exercises illustrated in a manual (3 × 15 repetitions of diaphragmatic breathing exercises, diaphragmatic isometric exercise, Threshold IMT®, lifting upper limbs with a bat and strengthening the abdomen). Duration training sessions varied by patient. Intensity reported only for diaphragmatic breathing (1 kg on the belly). Supervision once a month at distance.	MEP	↑ in I and C	Attrition: 37/49 (76% completed; I 14/22 (64%); C 23/27 (85%)). Dropouts due to I: LiTx (n = 2), death (n = 3), declined to perform exercise (n = 3), C LiTx (n = 1), death (n = 3)
		C n = 27 78% male Age 55.4 (±9.9)	C: Usual care	Spirometry (FVC, FEV1)	no changes	Fidelity (participants): NR
			Assessments: - Pre-intervention - Post-intervention	QOL (SF-36)	↑ in I and C on general health and mental health subscale; ↑ within I on functional capacity, not in C, but without between group differences	Fidelity (interventionist): NR
				Surface EMG of diaphragm	I ↓ C	Acceptability: NR
				Surface EMG of rectus abdominis	no changes	Safety: NR
				Ascites presence	I ≈ C	
6	Forestieri, 2016 (Brazil)	HTx candidates n = 24		6MWT	↑ within I; I ↑ C	Enrolment: 24/27 (89%) of eligible patients
		I n = 12 71% male Age 48.3 (±10.2)	I: ~22 days, hospital-based, intermittent aerobic (stationary cycle ergometer exercise: 5 periods of 3 min cycling and 1 min res, 20 min/session, 2x/day, intensity 3–4/10 RPE	MIP	↑ within I; I ↑ C	Attrition: 18/24 (75%) completed (I 7/12 (58%); C 11/12 (92%)). Dropouts due to: I: incapacity to complete the stationary cycle ergometer exercise (n = 5); C: acute severe arrhythmias (n = 1)
		C n = 12 82% male Age 48.0 (±11.2)	C: ~19 days, hospital-based, breathing exercises and global active exercises of the upper and lower limbs in the upright seated position (2x/day, intensity: 3–4/10 RPE)	FVC	NR	Fidelity (participants): 42% lost for follow-up
			Assessments: - Pre-intervention - Post-intervention	FEV1	NR	Fidelity (interventionist): NR
				NT-proBNP	NR	Acceptability: 42% were incapable to complete the intervention program Safety: NR

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TABLE 2 | (*Continued*) Summary of RCTs- Exercise interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – Feasibility outcomes
7	Pehlivan, 2018 (Turkey)	LuTx candidates n = 34	All participants participated in a home-based pulmonary rehabilitation program: breathing exercises (local expansion exercises, diaphragmatic breathing, and pursed lip breathing), free walking, and upper and lower body strengthening with resistance bands. Participants completed a weekly chart that was reviewed by the physiotherapist.	MIP	↑ within I; I ↑ C	Enrolment: 34/38 (89%) of eligible patients
		I n = 17 59% male Age 36.1 (±15.9)	I: 3-months, 5x/week (supervised 2x/week; home-based 3x/week) standard pulmonary rehabilitation (aerobic exercises: treadmill, cycle and arm ergometer, 15 min per exercise modality/ session, intensity: 50%–70% of HRmax and resistance exercises: dumbbell and free weight bags, 8–12 reps, one to two sets/ session, intensity 20%–40% 1-RM) + IMT with Powerbreathe device (15 min/ session, 2x/day, 5 days/week, intensity initial 30% of MIP, progressed to 60% MIP)	MEP	↑ in I and C	Attrition: 34/34 (100%) completed
		C n = 17 65% male Age 39.0 (±12.4)	C: Usual care, including standard pulmonary rehabilitation (see above)	6MWT	↑ in I and C, but greater in I than C	Fidelity (participants): NR
			Assessments: - Pre-intervention - Post-intervention	mMRC dyspnea scale	↓ in I and C	Fidelity (interventionist): NR
				FVC FEV1 DLCO DLCO/VA	no changes no changes no changes I ↑ C	Acceptability: NR Safety: NR
8	Manzetti, 1994 (United States)	LuTx candidates n = 21 22% male Age 40 (±10)		W_{max}	no changes	Enrolment: 36/91 (40%) eligible for participation, 15/36 (42%) of eligible patients declined participation due to financial reasons (n = 10) or transport issues or inability to perform activities of daily living independently (n = 5)
		I: n = 5 % Male NR Age NR	I: 6-week, health education program + supervised aerobic training (treadmill, bicycle ergometer, 30 min/session, 2x/ week, around aerobic threshold or 80% maximal ventilation) + strength training of upper extremity (low intensity)	6MWT	↑ in I and C, I ≈ C	Attrition: 9/21 (43%) completed; drop-outs due to Tx (n = 9) or hospitalization (n = 3). Number of drop-outs per group NR
					↑ or ≈ I and C, I ≈ C	Fidelity (participants): NR

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TABLE 2 | (*Continued*) Summary of RCTs- Exercise interventions.

First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – Feasibility outcomes
	C: n = 4 % male NR Age NR	C: 6-week, health education program Assessments: - Pre-intervention - Post-intervention	QoL (QWB, QLI, SFSD)		Fidelity (interventionist): NR Acceptability: NR Safety: NR

I, Intervention group; C, comparator group; Tx, transplantation; NR, not reported; 1RM, one-repetition maximum; 6MWD, six-minute walking distance; 6MWT, six-minute walking test; BiVAT, biventricular assist device; COPD, chronic obstructive pulmonary disease; DLCO/VA, alveolar volume ratio of carbon-monoxide diffusion capacity; DLCO, diffusion capacity of the lung for carbon monoxide; EMG, electromyography; FEV1, forced expiratory volume in 1 second; HRmax, maximal heart rate; HTx, heart transplantation; IMT, inspiratory muscle training; IVC, inspiratory vital capacity; LiTx, liver transplantation; LuTx, lung transplantation; LVAT, left ventricular assist device; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; MLwHFQ, Minnesota Living with Heart Failure Questionnaire; mMRC dyspnea scale, modified Medical Research Council dyspnea scale; NA, Not applicable; NR, not reported; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; P_aCO_2, partial pressure arterial carbon dioxide; P_aO_2, partial pressure arterial oxygen; PImax, maximal inspiratory pressure; QOL, quality of life; RPE, rate of perceived exertion; SpO_2, saturation of peripheral oxygen; T_CPCO_2, transcutaneously measured pressure of arterial carbon dioxide; VO_2peak, peak or maximal oxygen consumption; W_{max}, peak work rate at the end of a cardiopulmonary exercise test; QWB, Quality of Well-being scale; QLI, Quality of Life Index; SFSD, Symptom Frequency/Symptom distress scale.

Recommendation 2.2

Solid organ transplant candidates who are underweight may be offered nutritional interventions with the aim to achieve optimal target weight before the transplant.

Quality of Evidence: Very low.

Strength of Recommendation: Weak.

Rationale: Evidence from two intervention studies in lung transplant candidates [29, 30] have indicated that increased caloric intake before transplantation may allow solid organ transplant candidates, especially those who are underweight, to reach a pre-transplant target weight. However, these studies had a small sample size and were conducted in different settings (hospital vs. outpatient clinic).

Recommendation 2.3

Solid organ transplant candidates who are overweight may be offered nutritional interventions with the aim to achieve optimal target weight before the transplant.

Quality of Evidence: Very low.

Strength of Recommendation: Weak.

Rationale: One study (n = 43) [32] showed that a weight-loss program, consisting of bibliotherapy and voice call counselling by a dietician, was successful in reducing body weight in adult candidates for heart transplantation.

Recommendation 2.4

It is suggested that cognitive behavioral therapy and psychoeducational interventions are considered for solid organ transplant candidates who have symptoms of anxiety and/or depression.

Quality of Evidence: Very low.

Strength of Recommendation: Weak.

Rationale: Six studies utilized elements of cognitive behavioral therapy (CBT) and psychoeducational interventions [33, 34, 36, 37, 65, 67] of which five reported a significant decrease in symptoms of anxiety and depression or mood [33, 34, 36, 37, 65] in lung, liver, and kidney transplant candidates. However, studies differed regarding duration (8–12 weeks), modality (group vs. individual; remote vs. in person), and most studies had small sample sizes (n = 29 to n = 71) (**Table 4**). Only the study of Blumenthal et al (2006)) [36] had an adequate sample size (n = 328).

Recommendation 2.5

It is suggested to consider stress-reducing interventions such as mindfulness-based stress reduction or relaxation techniques in candidates for solid organ transplantation to reduce anxiety or stress levels.

Quality of Evidence: Very low.

Strength of Recommendation: Weak.

Rationale: In two studies among kidney and kidney-pancreas transplant candidates, stress-reducing interventions were associated with alleviated symptoms of anxiety [65] or depression [38, 65] directly after the intervention. However, this effect was not maintained long-term. In addition, sample sizes were small (n = 41/n = 63) and the intervention differed regarding content and interventionist.

PICO Question 3

In adult candidates for lung, liver, kidney, & heart transplantation: what are the outcomes relevant to exercise and physical activity, nutritional support and psychosocial interventions that should be measured pre-transplant?

In order to reliably assess the effects of prehabilitation interventions, it is imperative to standardize outcome

TABLE 3 | Summary of RCTs- Nutritional interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
1	Grat, 2017 (Poland)	Liver Tx candidates N = 55		90-day mortality rate	I ≈ C	Enrolment: 209/491 (43%) eligible for participation; 55/209 (26%) of eligible patients participated. Refusal to participate probably due to administrational factors
		I n = 26 81% male Age 52 (47–58)	I: once daily intake of a 4-strain probiotic preparation before breakfast (ProBacti 4 Enteric®: 3 × 10^9 colony-forming units of *Lactococcus lactis* PB411 (50.0%), *Lactobacillus casei* PB121 (25.0%), *Lactobacillus acidophilus* PB111 (12.5%), and *Bifidobacterium bifidum* PB211 (12.5%) from enrolment until transplantation. Duration of intervention was <2–>10 weeks depending upon timing Tx	30-day and 90-day infection rate	I ↓ C	Attrition: 50/55 ((91%) completed (I 24/26 (92%); C 26/29 (90%)). Dropouts (n = 5) all discontinued treatment Post-Tx outcomes available of I: 21/26 (81%) and C: 23/29 (79%)
		C n = 29 74% male Age 50 (35–61)	C: placebo	5-days post-Tx: - AST - ALT - Bilirubin concentration - INR	 I ↑ C I ↑ C I ↓ C I ≈ C	Fidelity (participants): I 2/26 (8%) and C: 3/29 (10%) discontinued treatment
			Assessments: - Baseline - Pre-Tx: follow-up with intervals of 10 weeks - Post-Tx: 90 days follow-up	Pre-transplant: - Waitlist mortality - Hospitalizations - Infections - Complications	 None I ≈ C I ≈ C I ≈ C	Fidelity (interventionist): NR
				Post-transplant - Primary non-function - Early allograft dysfunction - Complications	 I ≈ C I ≈ C I ≈ C	Acceptability: NR
				MELD-score changes CTP changes	I ≈ C I ≈ C	Safety: NR
2	Plank, 2015 (New Zealand)	Liver Tx candidates N = 101		Body composition - Body weight (kg) - Total body protein - Total body fat	 I ≈ C I ≈ C I ≈ C	Enrolment: NR
		I n = 52 Male 63% Age 53 (25–68)	I: daily intake of immuno-nutrition, two 74 g sachets per day until the day of transplant, consisting of 7.5 g arginine, 2 g omega-3 fatty acids + 0,8g Ribonucleic acid. for 56–65 days (median)	Muscle function - Hand grip strength - Respiratory muscle strength	 I ≈ C I ≈ C	Attrition: 101/120 (84%) completed (I 52/60 (87%); C 49/60 (82%)). Dropouts: I: delisting (n = 8), C: death (n = 4), delisting (n = 7)
		C n = 49 Male 73% Age 50 (22–59)	C: daily intake with a similar amount of an isocaloric, but not isonitrogenous, control product	Plasma phosphatidyl-choline fatty acids	I ↑ C at pre-Tx and day 10 measurements	Fidelity (participants): NR
			Assessments: - Baseline - Prior to Tx - 10, 30, 90, 180, 360 days after Tx	Fatigue (NR)	I ≈ C	Fidelity (interventionist): NR
				Graft rejection Length of stay at ICU	I ≈ C I ≈ C	Acceptability: NR Safety: intolerance to immune-nutrition in four participants
				Length of stay at hospital	I ≈ C	

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TABLE 3 | (*Continued*) Summary of RCTs- Nutritional interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
3	Eguchi, 2011 (Japan)	Living donor Liver TX candidates N = 50		Infectious complications	I ↓ C	Enrolment: NR
		I n = 25 52% male Age 56 (33–66)	I group 1: 2 days preoperative and group 2: 2 weeks post-operative synbiotic therapy (*Bifidobacteriu breve*, *Lactobacillus casei* and *Galactooligosa charides*)	Mortality	I ≈ C	Attrition: NR
		C n = 25 64% male Age 57 (25–68)	C: placebo	Length of stay at ICU	I ≈ C	Fidelity (participants): NR
			Assessments: Not specified	Length of stay at hospital	I ≈ C	Fidelity (interventionist): NR Acceptability: NR Safety: all participants tolerated synbiotic therapy
4	Park, 2003 (United States)	Heart Tx candidates with BMI > 25 kg/m^2 N = 43	All participants had one consultation session by a graduate student in clinical psychology under the supervision of the study's registered dietitian, who provided the recommendations such as energy balance	Body weight change	I ↑ C	Enrolment: 43/54 (80%) of referred patients
		I n = 21 81% male Age 47.8 (±8.5)	I : 3-months weight-loss program comprised of bibliotherapy (written, 20-page manual containing brief lessons about cognitive and behavioral weight loss strategies), and telephone-based counseling (1x/week, 15–20 min) delivered by a therapist who has a bachelor's or master's degree in psychology.			Attrition: 36/43 (84%) completed (I 17/21 (81%); C 19/22 (86%)
		C n = 22 68% male Age 48.1 (±9.4)	C: 3-months weight-loss program comprised of bibliotherapy without counseling			Fidelity (participants): I returned more food diaries than C, but not significant; I returned more postcards than C, but not significant Fidelity (interventionist): NR
			Assessments: - Pre-intervention - Post-intervention			Acceptability: NR Safety: NR
5	Forli, 2001(a) (Norway)	Lung Tx candidates N = 65		Change in body weight.	I ↑ C1 and C2	Enrolment: 6/71(8%) of eligible patients excluded for various reasons: refused intervention (n = 1), dietary wishes (n = 1), absent during night/weekend (n = 1), short hospital stay (n = 1), death (n = 1).
		I n = 18 44% male Age 49 (44–53)	I: intensified nutritional support comprised of energy-rich diet and supplements, provided by a dietician during hospital stay	BMI (kg/m^2)	C1 ↓ I and C2	Attrition: 49/65 (75%) completed. Drop-outs due to: not willing to record data (n = 1), missing data (n = 3), oedema (n = 2), death (n = 1)
		C1 n = 19 53% male Age 48 (44–52)	C1: normal hospital diet	Total energy intake/kg	C2 ↓ I and C1	Fidelity (participants): NR
		C2 n = 28 43% male Age 51 (48–55)	C2: normal weight lung Tx candidates	Total energy intake/REE predicted	I ↑ C1 and C2, C1 ↑ C2	Fidelity (interventionist): NR
			Assessments: - During hospitalization for lung Tx screening, exact moments NR			Acceptability: NR Safety: NR

(Continued on following page)

TABLE 3 | (*Continued*) Summary of RCTs- Nutritional interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
6	Forli, 2001(b) (Norway)	Lung Tx candidates with underweight N = 71		Body composition - Change in body weight - Change in Fat mass - Change in Fat free mass	 ↑ in I (+2.9 kg) and C1 (+2.3 kg) group, not in C2 group I ↑ C1 ≈ C2 C1 ↑I ≈ C2	Enrolment: NR
		I n = 21 48% male Age 47 (28–59)	I: intensified sessions dietary counselling with suggestions for individual meal plans facilitating weight gain, booklet with dietary information and recipes, supplements, and support by telephone by the dietitian each month after hospital discharge. Mean intervention time was 22 weeks.	Blood samples - Albumin concentration - Phosphate concentration	 I ≈ C1 ≈ C2 I ≈ C1 ≈ C2	Attrition: 54/71 (76%) completed (1 18/21 (86%); C1 13/21 (62%); C2 23/29 (79%)). Dropouts: I and C1 death (n = 8), Tx (n = 3), infection (n = 14); C2 death (n = 2), Tx (n = 4), infection (n = 7)
		C1 n = 21 48% male Age 46 (25–60)	C1: one session of individual dietary counselling with the dietitian. No follow-ups. The mean intervention time was 20 weeks.	Lung function test: - PaO_2 - $PaCO_2$ - FVC - FEV1 - TLCO	 I ↓ C1 ≈ C2 I ≈ C1 ≈ C2 I ≈ C1 ≈ C2 I ≈ C1 ≈ C2 I ≈ C1 ≈ C2	Fidelity (participants): NR
		C2 n = 29 41% male Age 52 (26–60)	C2: normal weight Lung Tx candidates Assessments: - Pre-intervention - 4–5 months after discharge	Exercise testing: - handgrip strength - 6MWT	 I ≈ C1 ≈ C2 I ≈ C1 ≈ C2	Fidelity (interventionist): NR Acceptability: NR Safety: NR
7	Le Cornu, 2000 (United Kingdom)	Liver Tx candidates N = 82	No information who provided the advice in both groups nor the number of sessions	Biochemical parameters - Bilirubin - Creatinine - Urea - Alkaline phosphatase - Aspartate transaminase - INR	 ≈ within ≈ within I and C ↑ within I; ≈ within C ≈ within I; ↓ within C ≈ within I and C ≈ within I; ↑ within C	Enrolment: 116/328 (35%) patients were eligible, 82/116 (71%) of eligible patients consented
		I n = 42 69% male Age 52 (27–67)	I: Standard dietary advice to increase energy intake on top of the dietary recommendations they already had to follow for underlying medical conditions and daily enteral supplementation (750 calories out of 20 g protein and 33.5 g fat).	Anthropometric measurements: - Mid-arm circumference - Triceps skinfold thickness	 I ≈ C I ≈ C	Attrition: 80-28 (98%) completed (I 41/42 (98%); C 39/40 (98%)). Dropouts due to I: lost to follow-up n = 1); C: delisted (n = 1)
		C n = 40 79% male Age 50 (24–68)	C: Standard dietary advice to increase energy intake on top of the dietary recommendations they already had to follow for underlying medical conditions.	Handgrip strength	I ≈ C	Fidelity (participants): NR
			Assessments: - Screening - Monthly follow-up until Tx or death	Energy Intake	I ≈ C	Fidelity (interventionist): NR
				Survival (pre-transplant) Days on ventilatory support Length of ICU stay Length of Hospital stay	I ≈ C I ≈ C I ≈ C I ≈ C	Acceptability: NR Safety: NR

I, Intervention group; C, comparator group; Tx, transplantation; NR, not reported; AST, asparate; ALT, alanine aminotransferase; INR, Internationalized Normalized Ratio; MELD, Model for End-stage Liver Disease; CTP, Chil-Turcotte-Pugh; ICU, Intensive Care Unit; BMI, Body Mass Index; PaO_2, Arterial O_2; $PaCO_2$, Arterial CO_2; FVC, Forced Vital Capacity; FEV1, Forced Expiratory Volume/1s; TLCO, Lung transfer factor carbon monoxide; 6MWT, six Minutes Walking Test.

TABLE 4 | Summary of RCTs - Psychosocial interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
1	Napolitano, 2002 (United States)	Lung Tx candidates N = 71		Health-related quality of life (SF36, PQLS)	SF36: I ↑ C on overall quality of life, mental health, role limitations due to emotional functioning, and vitality score PQLS: I ↑ C on overall score and subscales psychological functioning and physical functioning	Enrolment: 81/91 (89%) of eligible patients
		I n = 36 31% male Age 44.2 (±12.7)	I: 8-weeks, weekly, telephone-based psychological treatment comprised of supportive counselling and CBT, delivered by clinical psychology graduate	Anxiety (GHQ) Depression (GHQ)	I ↓ C on total score and subscales scores (anxiety, depression, social dysfunction, and somatic symptoms)	Attrition: 71/81 (88%) completed baseline (n = 2 delisted, n = 1 Tx, n = 6 withdrew consent, n = 1 died). 66/71 (93%) completed follow-up (I 34/36 (94%), missing data due to Tx (n = 2); C 32/35 (91%), missing data due to loss to follow-up (n = 3))
		C n = 35 31% male Age 46.6 (±12.4)	C: care as usual	Social support (PSSTx)	I ↑ C	Fidelity (participants): all participants received all sessions
			Assessments: - Pre-intervention - Post-intervention	Distress (PSTx)	I ≈ C	Fidelity (interventionist): NR Acceptability: NR Safety: NR
2	Rodrigue, 2005 (United States)	Lung Tx candidates N = 35		Quality of Life (QOLI)	I ↑ C at 1 and 3-month follow-up	Enrolment: 35/58 (60%) of eligible patients
		I n = 17 35% male Age 48.8 (±10.0)	I: 8–12-weeks, weekly, telephone-based CBT delivered by clinical psychology graduate students and interns	Mood (POMS)	I ↓ C at 3 month follow-up	Attrition: 35/35 (100%) at baseline, 31/35 (89%) completed all assessments
		C n = 18 33% male Age 49.0 (±11.3)	C: supportive treatment, delivery NR	Social intimacy (MSIS)	I ↑ C at 1 month follow-up	Fidelity (participants): I: 88% full treatment, C: 89% full treatment
			Assessments: - Pre-intervention - 1 month post-intervention - 3 month post-intervention	FEV1	I ≈ C	Fidelity (interventionist): NR
				Physical functioning (6MWT)	I ≈ C	Acceptability: I: high levels of comfort, rapport, helpfulness and convenience, low levels of distraction. 87% would participate again, 32% preference telephone counselling Safety: NR

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TABLE 4 | (*Continued*) Summary of RCTs - Psychosocial interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
3	Sharif, 2005 (Iran)	Liver Tx candidates N = 110		Health-related Quality of life (CLDQoL)	I ↑ scores on domains fatigue, emotional function, and total QOL score at 1-month follow-up. I ↑ on all domains of QoL at 3-month follow-up	Enrolment: NR
		I n = 55 76% male Age NR	I: 4-weeks, three individual sessions and one group session, 90 min/week, psycho-educational treatment, mode of delivery NR		Comparison with control group not reported	Attrition: NR
		C n = 55 75% male Age NR	C: educational booklet			Fidelity (participants): NR
			Assessments: - Pre-intervention - Post-intervention			Fidelity (interventionist): NR
						Acceptability: NR Safety: NR
4	Blumenthal, 2006 (United States)	Lung Tx candidates N = 328		Health-related Quality of life (PQLS, SF36, GHQ)	PQLS: I ≈ C SF36: I ↑ C on PSC and subscales mental health and vitality; I ≈ C on subscales general health, physical functioning, pain, physical role GHQ: I ↑ C	Enrolment: 389/625 (62%) of eligible patients. Drop out after randomization I: 34/200 (17%); C 27/189 (14%) due to death, Tx or delisting
		I n = 166 45% male Age 50 (±11)	I: 12-weeks, telephone-based, 30 min/week, supportive counseling and training in cognitive-behavioral coping skills coping skills delivered by trained social worker or psychologists	Anxiety (STAI)	I ↓ C	Attrition: I: 126/166 (76%) and C: 147/162 (91%) completed all assessments
		C n = 162 43% male Age 50 (±12)	C: care as usual	Depression (BDI)	I ↓ C	Fidelity (participants): 10.6 out of 12 sessions, 77% completed all sessions
			Assessments: - Pre-intervention - Post-intervention	Perceived stress (PSS)	I ≈ C	Fidelity (interventionist): 97.6% adhered to the protocol
				Life-orientation (LOT-R)	I ↑ C	Acceptability: NR
				Social Support (PSSC)	I ≈ C	Safety: no adverse events
				Shortness of breath (SDS-BQ)	I ≈ C	
				Survival pre-transplant	I ≈ C	

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TABLE 4 | (*Continued*) Summary of RCTs - Psychosocial interventions.

	First author, year (country of origin)	Sample characteristics Tx-type, total N, n per group, % male, age (y) (mean (sd) or median (range))	Intervention(s) and measurement points	Effectiveness Outcomes	Results – Effectiveness outcomes ↑ = significant increase ↓ = significant decrease ≈ no difference	Results – feasibility outcomes
5	Rodrigue, 2011 (United States)	Kidney Tx candidates N = 62		Health-related Quality of life (QoLI, SF-36)	QoLI: I1 ↑ (clinical relevant) C, I2 ≈ C SF36: I1 ↑ (clinical relevant) C, I2 ≈ C	Enrolment: 65/110 (59%) of eligible patients (n = 18 excluded, n = 27 refused)
		I1 n = 22 64% male Age 53.2 (±11.1)	I1: 8-weeks,in person, 50 min/ week, QoL-therapy, delivered by trained social workers or psychologists	Mood (POMS)	I1 ↑ (clinical relevant) C, I2 ≈ C	Attrition: 62/65 (95%) completed baseline, 51/62 (82%) completed all assessments
		I2 n = 20 40% male Age 48.6 (±11.9)	I2: 8-weeks, in person, 50 min/ week, supportive care, delivered by trained social workers or psychologists	Distress (HSCL)	I1+ I2	Fidelity (participants): I1 17/22 (77%) and I2 17/20 (85%) received full treatment
		C n = 20 45% male Age 52.7 (±12.7)	C: care as usual	Social Intimacy (MSIS)	I1 ↑ (clinical relevant) C, I2 ≈ C	Fidelity (interventionist): NR
			Assessments: - Pre-intervention - 1-week post-intervention - 12-week post-intervention	No mental unhealthy days	I1 ≈ I2 ≈ C	Acceptability: high level of comfort, rapport, supportiveness and overall helpfulness Safety: NR
6	Gross, 2017 (United States)	Kidney and kidney pancreas Tx candidates N = 63		Health-related quality of life (SF36)	I ↑ C on MCS at 6-month follow-up I ↑ within group PCS score at 2-month follow-up	Enrolment: 63/388 (16%) of eligible patients
		I n = 32 43% male Age 50 (±12)	I: 8-weeks, group-based, combined in-person and telephone-based, mindfulness stress reduction training, delivered by a certified mindfulness-based stress reduction teacher	Anxiety (STAI)	I ≈ C	Attrition: 51/63 (81%) completed assessment at 2 months after baseline (I 27/32 (84%); C 24/31 (77%)). 42/63 (67%) completed all assessments (I 22/32 (69%); C 20/31(65%))
		C n = 31 43% male Age 50 (±12)	C: 8-week, group based, combined in-person and telephone-based, weekly, structured support group, delivered by a group facilitator	Depression (CES-D)	I ↑ C at 2-month follow-up I ≈ C at 6-month follow-up	Fidelity (participants): attendance seven out of eight sessions; in both groups; n = 4 never attended
			Assessments: - Baseline - 2 months after baseline - 6 months after baseline	Sleep quality (PSQI)	I ≈ C	Fidelity (interventionist): no treatment contamination found
				Pain (SF12 pain item)	I ≈ C	Acceptability: 90% reported continuing meditation practices, 67%–80% indicated that MBSR was helpful
				Fatigue (PROMIS-Fatigue SF)	I ≈ C	Safety: No intervention-related adverse events occurred

Tx, transplant; I, intervention group; C, comparator group; NR, Not reported; CBT, cognitive behavioural therapy; SF-36, short form 36 questionnaire; GHQ, general health questionnaire; PQLS, pulmonary-specific quality-of-life-scale; PSSTx, perceived social support related to transplantation; PSTx, perceived stress related to transplantation QOLI, quality of life inventory; POMS, Profile of Mood States Short-Form; MSIS, 17-item Miller Social Intimacy Scale; FEV1, Forced expiratory volume; 6MWT, six minute walk test; CLDQoL, Chronic Liver Disease Quality of Life; PQLS, pulmonary specific Quality of Life Scale; STAI, State-Trait Anxiety Inventory- State form; BDI, Beck Depression Inventory; PSS, perceived stress scale; LOT-R, life orientation test- revised; PSSC, Perceived social support scale; SDS-BQ, University of California San Diego Shortness of breath Questionnaire; COPE Inventory; POMS, Profile of Mood States-Short Form; HSCL, Hopkins Symptom Checklist-25; No of unhealthy mental health days in the past month, subjective reporting of number of days experiencing stress; depression or anxiety in the past month; CES-D, The Centre for Epidemiologic Studies Depression Scale; PSQI, The Pittsburg Sleep Quality Index; SF-12, Short-Form 12 questionnaire; MCS, Mental Composite Score of the SF-12; PCS, Physical Composite Score of the SF-12; PROMIS-Fatigue, PROMIS-Fatigue Short Form v1.

FIGURE 4 | Risk of Bias assessment RCTs exercise intervention studies.

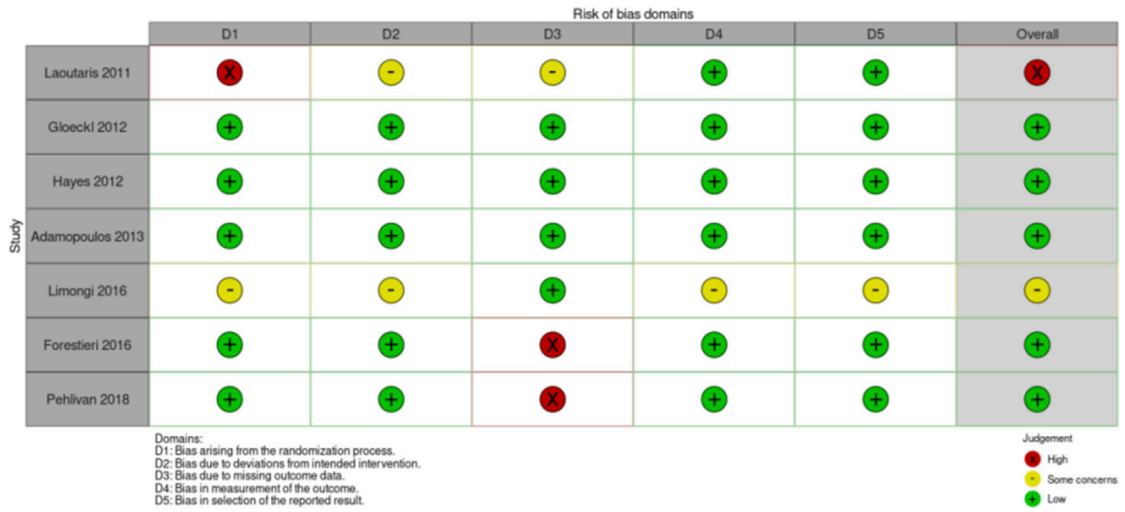

FIGURE 5 | Risk of Bias assessment RCTs nutritional intervention studies.

measures, their definitions, and the tools to measure them. Literature was reviewed with respect to the outcomes evaluated as well as the tools to measure them. One general recommendation was formulated.

Recommendation 3.1

It is strongly recommended that a *core outcome measurement set* is defined for future multimodal prehabilitation studies in solid organ transplant candidates.

Quality of Evidence: not applicable.

Strength of Recommendation: Strong.

Rationale: The studies retrieved during this review varied widely with respect to the clinical and patient-reported outcomes that were utilized, and the methods to assess them (**Tables 2–4**). Most exercise intervention studies included cardiorespiratory fitness including peak or maximal oxygen consumption (VO_2peak) and/or six-minute walking distance (6MWD)], Health-related Quality of Life (HRQoL), dyspnea, or maximal inspiratory pressures outcome measures. Nutritional intervention studies mostly monitored weight changes, infection rates, body composition and survival as either primary or secondary outcomes. The outcomes in studies that used psychosocial interventions included mostly HRQoL as well as parameters of mood, social intimacy and coping, while the use of clinical outcomes was rare. All stakeholders including solid organ transplant candidates and recipients, transplant

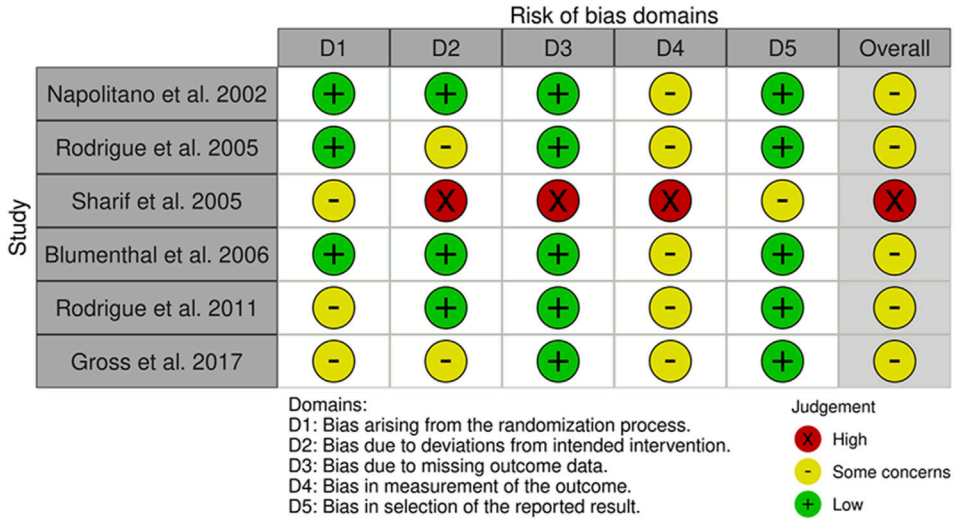

FIGURE 6 | Risk of Bias assessment RCTs psychosocial intervention studies.

professionals, and researchers in the field of transplantation strongly supported that a *core outcome set* be defined to facilitate comparative studies and give impetus to the field. A *core outcomes set* refers to a minimum set of outcome measures that are critical to patients, caregivers, and health professionals for decision making [71]. Selected outcomes that have so far not been considered but do carry clinical relevance during the pre-transplant waiting time are health-related physical fitness parameters such as muscular fitness, motor fitness, body composition and (cardio)metabolic health, as well as patient-reported outcomes such as fatigue, medication adherence and lifestyle, and clinical outcomes such as duration of intensive care stay, hospitalization, (re-)admissions, complications, graft function and survival, and waitlist and post-transplant mortality.

PICO Question 4

In adult candidates for lung, liver, kidney, & heart transplantation: what is the evidence for the feasibility (enrolment, retention, acceptability, fidelity, safety) of prehabilitation?

Implementation of prehabilitation in clinical practice of solid organ transplantation should be supported by evidence of feasibility. Two systematic reviews have previously concluded that exercise prehabilitation is feasible and safe for solid organ transplant candidates [11, 12]. The review by Wallen et al was performed with focus on the feasibility outcomes enrolment, retention, acceptability, fidelity and safety [11]. One general recommendation was made.

Recommendation 4.1

It is strongly recommended that future studies on multimodal prehabilitation in solid organ transplant candidates include the specific assessment of feasibility.

Quality of Evidence: Moderate.

Strength of Recommendation: Strong.

Rationale: One study was identified that was specifically designed to assess the feasibility of delivering a psychosocial prehabilitation in solid organ transplant candidates. This study showed that a stress management and relaxation training program in liver transplant candidates was efficiently deliverable and considered acceptable and tolerable by the patients [67]. However, the enrolment rate was low, (29%) and the attrition rate was moderate (68%). Amongst the remainder of the literature, most studies reported on some aspect(s) of feasibility as a secondary outcome, mainly regarding enrolment and attrition (**Tables 2–4**). The feasibility measures fidelity of participants and/or interventionist and safety were less reported (**Tables 2–4**). For the exercise intervention studies, the enrolment rate was approximately 86%, while the average attrition rate ranged between 71% and 100% [19–25]. However, drop-outs were often due to transplant surgery. In the studies on nutritional interventions, feasibility measures were poorly reported. If reported, the enrolment rate was found to be low to moderate [26, 31, 32]. Attrition rates ranged between 62% and 98% [26, 27, 29–32]. In the psychosocial intervention studies, enrolment rates ranged between 24% and 59%, attrition rates between 69% and 88%, and acceptability of the intervention was high [33, 34, 36–38]. Only two studies reported the occurrence of adverse events [27, 65], but no serious adverse events occurred.

Overall, the consensus was that these studies do support the notion that it is feasible, acceptable and safe for adults to participate in exercise, nutritional, and psychosocial interventions during the waiting-list period (**Tables 2–4**). Although enrolment in studies differed significantly across studies, the overall willingness to participate in studies was found to be good and the attrition rates are adequate, and few adverse events are reported. Fidelity of participants as well as the

interventionist and acceptability of the intervention are less reported. Nonetheless, implementation of prehabilitation in a clinical practice has not been established so far. Future dedicated studies should focus on the feasibility of implementation in clinical practice by assessing factors related to potential implementation strategy effects (e.g., adoption, fidelity, reach, sustainability) and factors to inform the design or development of the implementation strategy (e.g., acceptability, adaptability, feasibility, compatibility, complexity, self-efficacy, context, costs) [72].

DISCUSSION AND FUTURE PERSPECTIVES

The newly established ESOT consensus platform has proven successful in supporting the development of evidence-based consensus recommendations for prehabilitation in candidates for solid organ transplantation. Ten recommendations were formulated for which full consensus was reached within two voting rounds. This indicated that a high degree of consensus existed amongst all stakeholders from the prehabilitation, rehabilitation and transplantation fields, including transplant recipients and their representatives, and that the recommendations are a balanced representation of current published evidence and expert opinion.

Published evidence on prehabilitation before solid organ transplantation was found to be limited and consisted of studies addressing unimodal prehabilitation interventions with heterogeneous design, methodology and relatively small sample sizes. Nevertheless, by consensus and expert opinion, the available evidence on effects of prehabilitation on physical functioning, nutritional status, and psychosocial wellbeing and the evidence on safety of prehabilitation interventions was felt sufficiently strong to recommend that multimodal, patient-tailored prehabilitation should be offered as standard of care to patients awaiting solid organ transplantation. Specific recommendations included exercise-based intervention as well as psychological and stress management support for all solid organ transplant candidates, nutritional intervention for those who are over- or underweight, and probiotic supplementation for candidates for liver transplantation.

Because of the shortage in clinical evidence, however, particularly strong recommendations were formulated regarding the urgent need for high quality, but not exclusively, randomized controlled trials and implementation research studies that address the feasibility and effectiveness of pre-transplant multimodal prehabilitation. Two RCTs on multimodal prehabilitation interventions in kidney transplant candidates are currently underway: the FRAIL-MAR-study (NCT04701398) [73] and the PreCareTx-study (NCT05489432).

In addition, it was strongly recommended that priority should be given to the definition and consistent use of a Core Outcome Set to be measured by all future trials modalities, timing, duration and delivery modes of an optimal prehabilitation program.

From the in-person public discussions during the Consensus ESOT Conference, additional constructive perspectives emerged. It was advocated that clinical guidelines should be broadly applicable to transplant candidates irrespective of organ type, while leaving room for organ-specific recommendations, such as probiotics for liver transplant candidates. It was noted that intoxication-related interventions are not included in the recommendations, as intoxication (i.e., tobacco smoking or alcohol abuse) is typically addressed prior to patients joining the waitlist. The suggestion was made to formulate recommendations regarding pre-transplant peer support, however, such was considered premature as no evidence base could be found in the literature review. Lastly, the consideration was made that the designing of future studies or the future revisiting of the new guidelines may benefit of being informed by the prehabilitation literature in the broader field of surgery. However, unlike elective surgery, the waiting time is often unpredictably long while physical and mental condition may deteriorate due to the underlying disease. Therefore, prehabilitation should be offered throughout the waiting period from the moment of listing until transplantation.

These new evidence-based recommendations on prehabilitation serve to support best clinical practice in solid organ transplantation and help identify priorities for future research, thus optimizing patient health and post-transplant clinical outcome. The final recommendations will be included in the ESOT guidelines for transplant management, and under the auspices of the ESOT consensus development platform, will undergo continuous review and updating as new evidence becomes available.

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

Involved in the conception or design of the work: CA, SS, EC, YO, JK, TJ-F, SM, EK, MS, PF, DM, and SG. Literature screen and review: CA, SS, EC, YO, JK, TJ-F, SM, EK, MS, CM, FD, DM, and SG. Drafted the article: CA, SS, EC, YO, CM, DM, and SG. Critically revised the article: JK, TJ-F, SM, EK, MS, FD, PF, JG, AM, PG, MP, VL-L, CW, DK, DM, and SG. Finally approved the version to be published: CA, SS, EC, YO, JK, TJ-F, SM, EK, MS, CM, FD, PF, JG, AM, PG, MP, VL-L, CW, DK, DM, and SG. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

DM is a senior researcher of The Research Foundation- Flanders.

The remaining authors declare that the research was conducted in the absence of any commercial or financial

relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11564/full#supplementary-material

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European Society for Organ Transplantation (ESOT) Consensus Statement on the Role of Pancreas Machine Perfusion to Increase the Donor Pool for Beta Cell Replacement Therapy

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The advent of Machine Perfusion (MP) as a superior form of preservation and assessment for cold storage of both high-risk kidney's and the liver presents opportunities in the field of beta-cell replacement. It is yet unknown whether such techniques, when applied to the

Abbreviations: cDCD, Controlled Donation after Circulatory Death; CET, Centre for Evidence in Transplantation; ECTTA, European Cardio Thoracic Transplant Association; EKITA, European Kidney Transplant Association; ELITA, European Liver and Intestine Transplant Association; EPITA, European Pancreas and Islet Transplant Association; ESOT, European Society for Organ Transplantation; ETAHP, European Transplant Allied Healthcare Professionals; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; MP, Machine Perfusion; PICO, Population, Intervention, Comparator and Outcome; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SCS, Static cold storage; TJL 3.0, Transplantation Learning Journey 3.0; YPT, Young Professionals in Transplantation.

pancreas, can increase the pool of suitable donor organs as well as ameliorating the effects of ischemia incurred during the retrieval process. Recent experimental models of pancreatic MP appear promising. Applications of MP to the pancreas, needs refinement regarding perfusion protocols and organ viability assessment criteria. To address the "Role of pancreas machine perfusion to increase the donor pool for beta cell replacement," the European Society for Organ Transplantation (ESOT) assembled a dedicated working group comprising of experts to review literature pertaining to the role of MP as a method of improving donor pancreas quality as well as quantity available for transplant, and to develop guidelines founded on evidence-based reviews in experimental and clinical settings. These were subsequently refined during the Consensus Conference when this took place in Prague.

Keywords: islet transplantation, ischemia-reperfusion injury, machine perfusion, persufflation, whole pancreas transplantation

INTRODUCTION

Description of Health Problem

An estimated 537 million adults aged 20–79 years worldwide (61 million in Europe) have diabetes, approximately 10% of which have type 1 diabetes. By 2030, 643 million (67 million in Europe), and by 2045, 783 million (69 million in Europe) adults aged 20–79 years are projected to be living with diabetes. Thus, while the world's population is estimated to grow 20% over this period, the number of those with diabetes is estimated to increase by 46% [1].

Diabetes is a major driver for mortality worldwide and is the leading cause of kidney failure, peripheral vascular disease, and adult-onset blindness. Excluding the mortality risks associated with the COVID-19 pandemic, it is estimated that 6.7 million adults between the age of 20–79 died as a result of diabetes related complications during 2021. When inadequately managed, diabetes significantly elevates the risk of a host of micro- and macro-vascular complications. Because of this, optimizing glycemic control is critical in order to delay and potentially prevent the onset of chronic diabetic complications [2, 3]. Despite the tremendous expenditure in human, material, and financial resources, only about 50% of patients achieve optimal treatment. In selected cases, beta cell replacement, by pancreas or islet transplantation, can provide durable glycemic control and improve survival, therefore, all efforts must be made to offer patients this type of treatment. Such patients include those with type 1 diabetes mellitus who already have end stage renal disease or who experience recurrent severe hypoglycemia or hyperglycemia despite optimal medical management through exogenous insulin administration [4]. Moreover, the proportion of patients with type-2 diabetes undergoing simultaneous pancreas and kidney transplant continues to increase, reaching 23% in 2020. Less often, pancreas transplants are undertaken for other forms of diabetes mellitus, including, cystic fibrosis-related diabetes mellitus and post pancreatectomy diabetes mellitus [5].

The authors acknowledge that during the past decade, the annual number of pancreas transplants performed in some European Countries as well as the United States has steadily declined [6, 7]. This trend is related to a number of factors but predominantly is due to the susceptibility of the pancreas to ischemia-reperfusion injury. As all categories of beta cell replacement are life-enhancing and life-extending procedures, an initiative is needed to "re-invigorate" the rates of pancreas donation while maintaining organ quality. The impact MP has shown promise in other areas of organ transplantation inspiring research in the field of beta-cell replacement. It is hoped further studies relating to MP will improve pancreas utilization for both whole pancreas and islet transplantation.

Description of Target Population

Preservation of the pancreas is critical to maintain function of the organ and tissue during storage and has been the focus of research for decades [8, 9]. The gold-standard method for human beta cell preservation is hypothermic preservation by static cold storage (SCS), but may be insufficient when processing the marginal pancreases, thus opening up the possibility of improvement by new technologies [10, 11]. Recent innovations have focused on expanding the pancreas donor pool, including organs from older donors, those with higher BMI and those recovered from controlled donation after circulatory death (cDCD) [12–14]. In this sense, machine perfusion, in its' hypothermic, normothermic or persufflation modalities, could be the key to improving the quality of the donor pancreas as well as the pool of organs available for pancreas and islet transplantation [15, 16].

In contrast to other solid organs [17], the role of *in-situ* Normothermic Regional Perfusion after cDCD is emerging whilst *ex situ* machine perfusion for pancreas and islet transplant is in its infancy. The intrinsic characteristics of the pancreas, with its low blood flow and complex vascular anatomy, makes it highly susceptible to ischemic injury during preservation, resulting in detrimental effects on the organ's microcirculation [18]. This makes the design of the conditions, and the perfusion parameters of machines, more complex than for other solid organs and thus calls for further expert evaluation. Machine perfusion allows "real-time" investigations of the organ including perfusate analysis which opens the potential for

objective assessment criteria for transplantation, a situation which has yet to be established. This would prove to be invaluable for the assessment and utilization of "marginal organs" [19]. There are further gains to be translated clinically with the strategy of persufflation demonstrated to extend the duration of preservation and improve subsequent isolated viable islet yields [20].

Besides these initial encouraging data, preservation technologies still await a breakthrough. The relevant literature cites studies encompassing small numbers with varied protocols and outcome measures [21]. Because of this, it is imperative to develop optimal assessment parameters to evaluate organ quality and viability. Recent experimental animal and human models of *ex-situ* pancreas MP appear promising [22–24]. However, application of MP to the pancreas requires standardization that considers the unique characteristics of the pancreas and includes the highest quality evidence to inform MP protocols.

In the current era of MP technology, a consensus report is needed to define the role of pancreas machine perfusion. This consensus document, acknowledges the notable progress made in the research field of pancreas and islet MP and attempts to begin the bridge to clinical realization. This should, in turn, change the work dynamics for the transplant community, facilitating decision making based on objective morphological and functional criteria. MP could provide the paradigm shift providing opportunities for assessment, drug therapies, cellular therapies and facilitate further research and innovation.

Aim of the Guideline

To address the role of pancreas machine perfusion in increasing the donor pool for beta cell replacement, the European Society for Organ Transplantation (ESOT) assembled a consensus conference within the Transplantation Learning Journey 3.0 (TLJ3.0) framework. The Working Group comprised a global panel of experts in islet and pancreas transplantation: biomedical science researchers, biologists, transplant surgeons, urologists, endocrinologists, and pathologists. Guidelines on key aspects of pancreatic MP experimental models were developed examining their potential benefits, technical aspects, and their clinical implications. In addition, a group of senior jurors from the field was present during all proceedings. Summaries of the evidence were presented to the entire group of expert panelists and jurors. The consensus findings and recommendations of the ESOT Consensus guideline on the "Role of pancreas machine perfusion to increase the donor pool for beta cell replacement," are presented in this document for healthcare providers involved in this field. This guideline will be updated over time to reflect new evidence as it becomes available.

METHODS

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives. The detailed description of methodology used was reported previously [25].

Briefly, key issues related to the topic, namely: "Role of pancreas machine perfusion to increase the donor pool for beta cell replacement" were identified by the working group, and specific clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome) [25]. All PICO questions are listed in **Table 1**. Following the definition of the PICOs, literature searches (not preregistered) were developed by expert staff (with extensive systematic review experience) from the Centre for Evidence in Transplantation (CET) and were subsequently integrated, when needed, by the steering committee experts (**Supplementary Appendices S1, S2**).

The Transplant Library was searched on 30 October 2022. The Transplant Library includes all randomized controlled trials and systematic reviews in the field of solid organ transplantation published as full text or in abstract form, sourced mainly from MEDLINE/PubMed and hand-searches of congress proceedings. The search strategy used is as follows: (Pancreas transplantation or pancreas or islets of Langerhans transplantation or islet) and (perfusion or organ preservation or persufflation or perfusion or preservation or two layer method or two-layer method or TLM). Searches were expanded to include non-randomized studies. MEDLINE and EMBASE were searched on 30 October 2022 using the search strategy below: (Pancreas transplantation or pancreas transplant or islets of Langerhans transplantation or islet or organ transplant or simultaneous pancreas kidney or simultaneous pancreas-kidney or SPK) and (persufflation or two layer method or two-layer method or TLM or cardiopulmonary bypass or heart-lung bypass or extracorporeal circulation or extracorporeal membrane oxygenation or ECMO or regional perfusion or machine perfusion or perfusion or *ex-situ* perfusion or oxygenation or hypothermic perfusion or normothermic perfusion). Citations in articles were then reviewed and analyzed to extract unidentified articles.

A PRISMA flowchart describing the number of studies identified by the literature search and number of studies selected for inclusion (**Supplementary Appendix S3**) in the consensus statement appears in **Figure 1**.

A summary of the evidence addressing each key question by the included studies was prepared in an evidence table and sent to all members of the workgroup and the jury (**Supplementary Appendix S4**). The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C. Very low quality of evidence was not considered. For evaluation of the quality of evidence according to GRADE [26] the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance, and publication bias. Strength of recommendation was rated as 1 (strong) or 2 (weak). The Delphi method was applied with a view to reaching a group opinion/consensus during the conference. For each PICO question, recommendation, quality of the evidence and strength of the recommendation were voted on by an independent jury (4 members). Each recommendation was retained if more than 3 jury members agreed with it.

TABLE 1 | PICO question on the topic "Role of pancreas machine perfusion to increase the donor pool for beta cell replacement".

***Ex-situ* hypothermic machine perfusion in whole pancreas transplantation**	
PICO 1	For whole pancreas transplantation, should *ex-situ* hypothermic machine perfusion be performed at a pressure less than 30 mmHg?
PICO 2	For whole pancreas transplantation, should *ex-situ* hypothermic machine perfusion be beneficial if the duration is more than 1 h and less than 6 h?
PICO 3	For whole pancreas transplantation, should *ex-situ* hypothermic machine perfusate temperature be maintained at a range between 4°C and 12°C?
PICO 4	For whole pancreas transplantation, should *ex-situ* hypothermic machine perfusion be performed with Belzer-MPS or IGL-1?
PICO 5	For whole pancreas transplantation, could *ex-situ* hypothermic machine perfusion be performed by continuous or pulsatile perfusion?
PICO 6	Should *ex-situ* hypothermic machine perfusion for whole pancreas transplantation be performed simultaneously through the superior mesenteric artery and the splenic artery?
PICO 7	For whole pancreas transplantation, should *ex-situ* hypothermic machine perfusion be performed after a completed back table preparation to reduce organ leakage?
PICO 8	Does the decrease in resistance indexes during *ex-situ* hypothermic machine perfusion correlate with better preservation of the whole pancreas?
***Ex-situ* normothermic perfusion in whole pancreas transplantation**	
PICO 1	Could *ex-situ* normothermic machine perfusion be a method for evaluating whole pancreas after cold preservation for whole pancreas transplantation?
PICO 2	For whole pancreas transplantation, should *ex-situ* normothermic machine perfusion be performed at temperatures ranging from 34°C to 37°C, with a perfusate solution containing an oxygen carrier?
PICO 3	For whole pancreas transplantation, should *ex-situ* normothermic machine perfusion be performed at a maintenance pressure range from 25 to 50 mmHg?
PICO 4	For whole pancreas transplantation, does *ex-situ* normothermic machine perfusion require a balance of pressure and flow to ensure minimal damage to the endothelium?
PICO 5	In *ex-situ* normothermic machine perfusion for whole pancreas transplantation, does the addition of an oncotic factor to the perfusate ensure there is an oncotic pressure to minimize edema formation?
PICO 6	For whole pancreas transplantation, should *ex-situ* normothermic machine perfusion be beneficial if the duration is more than 1 h and less than 6 h?
PICO 7	For whole pancreas transplantation, could *ex-situ* normothermic machine perfusion be performed by continuous or pulsatile perfusion?
PICO 8	In case of prolonged perfusion, does *ex-situ* normothermic machine perfusion require the management of exocrine secretions to potentially prevent the development of tissue injury?
PICO 9	During *ex-situ* normothermic machine perfusion for pancreas transplantation, could the endocrine function of the pancreas graft be assessed by hormone secretion tests?
PICO 10	During *ex-situ* normothermic machine perfusion for pancreas transplantation, could preservation of pancreatic exocrine function be assessed by amylase and lipase levels in the perfusate?
PICO 11	Should *ex-situ* normothermic machine perfusion for pancreas transplantation be performed simultaneously through the superior mesenteric artery and the splenic artery?
***In-situ* normothermic regional perfusion in whole pancreas transplantation**	
PICO 1	Is *in-situ* normothermic regional perfusion a reliable and reproducible method for donation after cDCD in the scenario of whole pancreas transplantation?
PICO 2	For whole pancreas transplantation, is *in-situ* normothermic regional perfusion in the setting of cDCD compatible with the procurement of liver and kidneys?
PICO 3	For whole pancreas transplantation, is *in-situ* normothermic regional perfusion in the setting of cDCD compatible with the procurement of heart and lungs?
PICO 4	Should post-mortem *in-situ* normothermic regional perfusion in the setting of cDCD be run for a duration of 1–4 h in the context of whole pancreas transplantation?
PICO 5	Should valid parameters (machine perfusion-monitoring flow and temperature, analytical/biochemical parameters, and functional warm ischemia time) be defined to assess the quality of the pancreatic graft before deciding the suitability/validity of the organ for whole pancreas transplant?
PICO 6	Could *in-situ* normothermic regional perfusion in donation in the setting of cDCD improve graft and patient outcomes compared with *in-situ* cooling and rapid procurement in whole pancreas transplantation?
PICO 7	Does *in-situ* normothermic regional perfusion in the setting of cDCD have the potential to expand the donor pool for whole pancreas transplantation?
***Ex-situ* hypothermic machine perfusion in islets transplantation**	
PICO 1	Should *ex-situ* hypothermic perfusion of the pancreas for islet isolation be performed in the same manner as for vascularized pancreas transplantation with regards to: temperature, pressure, perfusate composition, oxygenation, duration, and timing?
PICO 2	In islet transplantation, could *ex-situ* hypothermic perfusion be used to increase cellular energy reserves, especially in controlled donation after circulatory death procedures?
PICO 3	Could *ex-situ* hypothermic perfusion be used to avoid night-time islet isolations?

(Continued on following page)

TABLE 1 | (*Continued*) PICO question on the topic "Role of pancreas machine perfusion to increase the donor pool for beta cell replacement".

***Ex-situ* normothermic perfusion in islets transplantation**	
PICO 1	Could *ex-situ* normothermic machine perfusion be a reliable method for evaluating whole pancreases after cold preservation in islet transplantation?
PICO 2	In islet transplantation, should *ex-situ* machine perfusion be performed at physiologic temperature, with perfusate solution containing an oxygen carrier to sustain metabolic activities of the cells?
PICO 3	In islet transplantation, should *ex-situ* normothermic machine perfusion be performed at a maintenance pressure range from 25 to 50 mmHg?
PICO 4	In islet transplantation, does *ex-situ* normothermic machine perfusion require a balance of pressure and flow to ensure minimal damage to the endothelium?
PICO 5	In *ex situ* normothermic machine perfusion for islet transplantation, does the addition of an oncotic factor to the perfusate ensure there is an oncotic pressure to minimize edema formation?
PICO 6	In islet transplantation, should *ex-situ* normothermic machine perfusion be beneficial if the duration is more than 1 h and less than 6 h?
PICO 7	In islet transplantation, could *ex-situ* normothermic machine perfusion be performed continuous or pulsatile perfusion?
PICO 8	In the case of prolonged perfusion, does *ex-situ* normothermic machine perfusion require the management of exocrine secretions to prevent the development of tissue injury?
PICO 9	During *ex-situ* normothermic machine perfusion for islet transplantation, could the endocrine function of the pancreas graft be assessed by hormone secretion tests?
PICO 10	During *ex-situ* normothermic machine perfusion for islet transplantation, could preservation of pancreatic exocrine function be assessed by amylase and lipase levels in the perfusate?
PICO 11	Should *ex-situ* normothermic machine perfusion for islet transplantation be performed simultaneously through the superior mesenteric artery and the splenic artery?
***In-situ* normothermic regional perfusion in islets transplantation**	
PICO 1	Is *in-situ* normothermic regional perfusion in the setting of cDCD a reliable and reproducible method for donation after controlled circulatory death in the scenario of islet transplantation?
PICO 2	For islet transplantation, is *in-situ* normothermic regional perfusion in the setting of cDCD compatible with the procurement of other abdominal organs (kidneys, liver)?
PICO 3	For islet transplantation, is *in-situ* normothermic regional perfusion in the setting of cDCD compatible with the procurement of thoracic organs (heart, lungs)?
PICO 4	Should post-mortem *in-situ* normothermic regional perfusion in the setting of cDCD be run for a duration 1–4 h in the context of islet transplantation?
PICO 5	Should valid parameters (machine perfusion-monitoring flow and temperature, analytical/biochemical parameters, and functional warm ischemia time) be defined to assess the quality of the pancreatic graft before deciding the suitability/validity of the organ for islet transplant?
PICO 6	Could *in-situ* normothermic regional perfusion in donation after controlled circulatory death improve isolation outcomes (yield, function, and viability) and post transplantation outcomes compared to *in-situ* cooling and rapid procurement in islet transplantation?
PICO 7	Does the *in-situ* normothermic regional perfusion in the setting of cDCD have the potential to expand the donor pool for islet transplantation?
Persufflation in islets transplantation	
PICO 1	In islet transplantation, should persufflation be performed using a humidified gaseous flow of 40% oxygen and 60% nitrogen?
PICO 2	Should persufflation be performed at a temperature of 4°C–8°C in an organ preservation solution?
PICO 3	Should persufflation be performed using a gaseous flow rate of 20–25 mL/hr?
PICO 4	Should persufflation be performed by canulation of the superior mesenteric artery and the splenic artery and optionally the pancreaticoduodenal artery?
PICO 5	Should arterial leakages be closed until the gaseous outflow is mainly venous when starting persufflation?
PICO 6	Can persufflation be used to prevent further cold ischemic damage for up to 24 h?
PICO 7	Can persufflation be performed during organ transport or as an end-ischemic strategy?
PICO 8	Can persufflation attenuate pro-inflammatory signaling in isolated islets?

cDCD, controlled Donation after Circulatory Death.

Complete information including: the list of consensus conference workgroup domains (and topics noted below); the process regarding consensus conference participant selection; development and refinement of consensus statements, and modified Delphi methodology including consensus polling, were previously reported in the in-person conference held in Prague, Czech Republic, 13–15 November 2022 [25].

RESULTS

Ex-Situ Hypothermic Machine Perfusion in Whole Pancreas Transplantation

No clinical studies are presently reported regarding the implementation of *ex-situ* hypothermic machine perfusion in whole pancreas transplantation. All studies are pre-

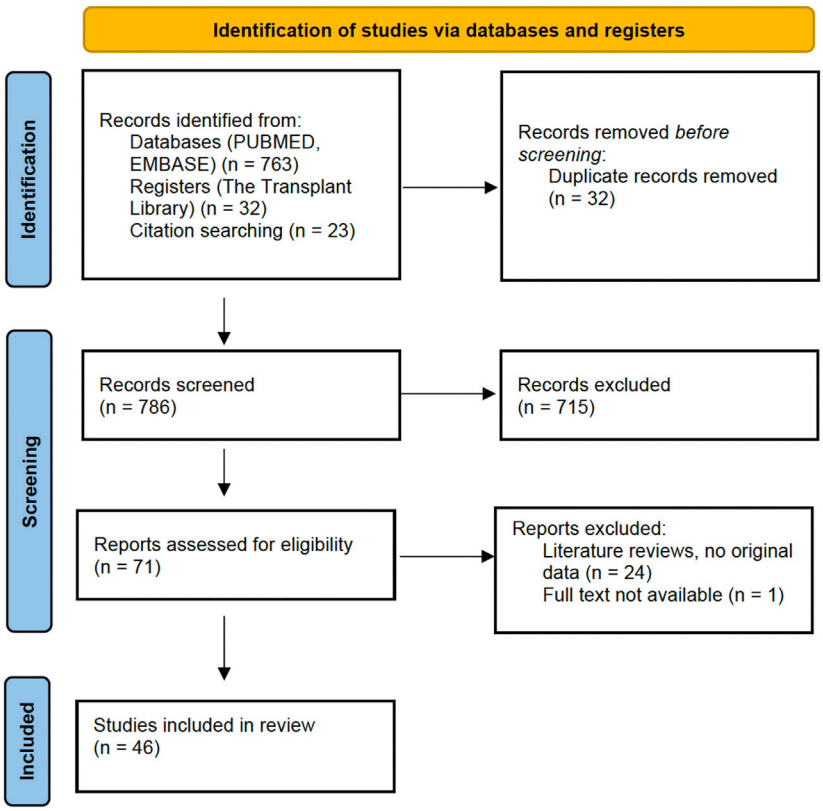

FIGURE 1 | PRISMA Flowchart: Literature search.

clinical studies in animal or human models. No human whole pancreas transplants have been performed after preservation with hypothermic machine perfusion. Therefore, the quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 6 recommendations and 2 for 2 recommendations.

Recommendation 1.1: For whole pancreas transplantation, *ex-situ* hypothermic machine perfusion should be performed up to a pressure of 30 mmHg.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 1.2: For whole pancreas transplantation, *ex-situ* hypothermic machine perfusion should be performed for a duration greater than 1 h but less than 6 h.

Quality of Evidence: C.

Strength of Recommendation: 2.

Recommendation 1.3: For whole pancreas transplantation, non-oxygenated hypothermic perfusate temperature should be maintained at a temperature range between 4°C and 12°C.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 1.4: *Ex-situ* hypothermic machine perfusion should be performed with a colloid based solution, clinically licensed for machine use (for abdominal organs).

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 1.5: For whole pancreas transplantation, *ex-situ* hypothermic machine perfusion can be performed by either continuous or pulsatile perfusion.

Quality of Evidence: C.

Strength of Recommendation: 2.

Recommendation 1.6: *Ex-situ* hypothermic machine perfusion for whole pancreas transplantation must be performed simultaneously through the superior mesenteric artery and the splenic artery.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 1.7: For whole pancreas transplantation a complete back table preparation must be performed prior to *ex-situ* hypothermic machine perfusion to reduce leakage of the perfusate.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 1.8: During *ex-situ* hypothermic machine perfusion, a decrease in resistance index may be correlated with better preservation of the whole pancreas.

Quality of Evidence: C.

Strength of Recommendation: 1.

Ex-Situ Normothermic Perfusion in Whole Pancreas Transplantation

No clinical studies are available regarding the implementation of *ex-situ* normothermic perfusion in whole pancreas transplantation.

All studies are pre-clinical studies in either animal or human models. No human whole pancreas transplants have been performed after preservation with *ex-situ* normothermic perfusion. Therefore, the quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 9 recommendations and 2 for 2 recommendations.

Recommendation 2.1: *Ex-situ* normothermic machine perfusion can be a method for evaluating the whole pancreas after cold preservation.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.2: For whole pancreas transplantation, *ex-situ* normothermic machine perfusion with a perfusate solution containing an oxygen carrier should be performed within a temperature range of 34°C–37°C.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.3: For whole pancreas transplantation, *ex-situ* normothermic machine perfusion should be performed at a maintenance pressure range from 25 to 50 mmHg.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.4: For whole pancreas transplantation, *ex-situ* normothermic machine perfusion requires a balance of pressure and flow to preserve the endothelium.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.5: In *ex-situ* normothermic machine perfusion for whole pancreas transplantation, addition of oncotic agents to the perfusate could help to minimize graft edema.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.6: For whole pancreas transplantation, *ex-situ* normothermic machine perfusion should be performed for a duration longer than 1 h.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.7: For whole pancreas transplantation, *ex-situ* normothermic machine perfusion can be performed by either continuous or pulsatile perfusion.

Quality of Evidence: C.

Strength of Recommendation: 2.

Recommendation 2.8: *Ex-situ* normothermic machine perfusion for whole pancreas transplantation requires diversion of exocrine secretions to prevent tissue injury.

Quality of Evidence: C.

Strength of Recommendation: 2.

Recommendation 2.9: During *ex-situ* normothermic machine perfusion for whole pancreas transplantation, the endocrine function of the pancreas graft can be assessed by hormone secretion tests.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.10: During *ex-situ* normothermic machine perfusion for whole pancreas transplantation, amylase, and lipase perfusate levels are not reliable exocrine markers for tissue viability or injury.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 2.11: *Ex-situ* normothermic machine perfusion for whole pancreas transplantation must be performed simultaneously through the superior mesenteric artery and the splenic artery.

Quality of Evidence: C.

Strength of Recommendation: 1.

In-Situ Normothermic Regional Perfusion in Whole Pancreas Transplantation

Regarding the implementation of *in-situ* normothermic regional perfusion in whole pancreas transplantation, a total of nine studies reported outcomes after cDCD pancreas transplantation have been published so far. These are cohort ($n = 2$) or case studies ($n = 7$). A total of 59 human whole pancreas transplants have been reported in the literature. The quality of evidence was Grade A for one recommendation and C for 7 recommendations. The strength of recommendation was 1 for 5 recommendations and 2 for 2 recommendations.

Recommendation 3.1: *In-situ* normothermic regional perfusion is a reliable and reproducible method for donation after controlled circulatory death in the scenario of whole pancreas transplantation.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 3.2: For whole pancreas transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD is compatible with the procurement of liver and kidneys.

Quality of Evidence: A.

Strength of Recommendation: 1.

Recommendation 3.3: For whole pancreas transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD is compatible with the procurement of heart and lungs.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 3.4: In the context of whole pancreas transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD should be maintained between 1 and 4 h.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 3.5: In the context of whole pancreas transplantation after *in-situ* normothermic regional perfusion of cDCD, valid assessment parameters of graft quality still need to be defined.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 3.6: For whole pancreas transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD might improve graft and patient outcomes when compared to *in-situ* cooling and rapid procurement.

Quality of Evidence: C.

Strength of Recommendation: 2.

Recommendation 3.7: *In-situ* normothermic regional perfusion in the setting of cDCD has the potential to expand the donor pool for whole pancreas transplantation.

Quality of Evidence: C.
Strength of Recommendation: 2.

Ex-Situ Hypothermic Machine Perfusion in Islets Transplantation

Regarding the implementation of *ex-situ* hypothermic machine perfusion in islet transplantation, no clinical studies are available. All studies are pre-clinical in either animal or human models. No human islet transplants have been performed after preservation with hypothermic machine perfusion. Therefore, the quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 3 recommendations.

Recommendation 4.1: *Ex-situ* hypothermic perfusion of the pancreas for islet transplantation should be performed in the same manner as for whole pancreas transplantation with the addition of oxygenation.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 4.2: In the context of pancreas for islet transplantation, oxygenated *ex-situ* hypothermic perfusion could be used to increase cellular ATP levels, especially during recovery from cDCD.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 4.3: In the context of pancreas for islet transplantation, oxygenated *ex-situ* hypothermic machine perfusion has the potential to prolong cold preservation times, which may be helpful for logistical considerations in islet isolation and transplantation.

Quality of Evidence: C.
Strength of Recommendation: 1.

Ex-Situ Normothermic Perfusion in Islets Transplantation

Regarding the implementation of *ex-situ* normothermic perfusion in islet transplantation, given the absence of references describing the use of *ex-situ* normothermic perfusion in islet transplantation, the same PICO questions have been raised as described for whole pancreas transplantation. In this sense, we consider that recommendations can be extrapolated but with a low level of evidence. Therefore, the quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 9 recommendations and 2 for 2 recommendations.

Recommendation 5.1: *Ex-situ* normothermic machine perfusion has the potential for evaluating the donor pancreas after cold preservation for islet transplantation.

Quality of Evidence: C.
Strength of Recommendation: 2.

Recommendation 5.2: For islet transplantation, *ex situ* normothermic machine perfusion with a perfusate solution containing an oxygen carrier should be performed within a temperature range of 34°C–37°C.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.3: If *ex situ* normothermic machine perfusion of the whole pancreas is to be performed prior to islet transplantation, it should be carried out at a maintenance pressure ranging between 25–50 mmHg.

Quality of Evidence: C.
Strength of Recommendation: 2.

Recommendation 5.4: During *ex-situ* normothermic machine perfusion of the pancreas prior to islet transplantation, consideration for pressure and flow is necessary to minimize injury to the endothelium.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.5: During *ex-situ* normothermic machine perfusion of the pancreas for islet transplantation, the addition of oncotic agents to the perfusate could help to minimize graft edema.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.6: *Ex-situ* normothermic machine perfusion of the pancreas for islet transplantation should be performed for a duration longer than 1 h.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.7: *Ex-situ* normothermic machine perfusion of the pancreas prior to islet transplantation can be performed by either continuous or pulsatile perfusion.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.8: *Ex-situ* normothermic machine perfusion of the pancreas prior to islet transplantation requires diversion of exocrine secretions to prevent tissue injury.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.9: During *ex-situ* normothermic machine perfusion of the pancreas for islet transplantation, the endocrine function of the pancreas can be assessed by hormone secretion tests.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.10: During *ex-situ* normothermic machine perfusion of the pancreas for islet transplantation, amylase and lipase perfusate levels are not reliable exocrine markers for tissue viability or injury.

Quality of Evidence: C.
Strength of Recommendation: 1.

Recommendation 5.11: *Ex-situ* normothermic machine perfusion of the pancreas for islet transplantation must be performed simultaneously through the superior mesenteric artery and the splenic artery.

Quality of Evidence: C.
Strength of Recommendation: 1.

In-Situ Normothermic Regional Perfusion in Islets Transplantation

Regarding the implementation of *in-situ* normothermic regional perfusion in islet transplantation, a total of 2 studies reporting outcome after cDCD pancreas transplants have been published so far. A total of 5 clinical islet transplants have also been reported in the

literature. The quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 7 recommendations.

Recommendation 6.1: *In-situ* normothermic regional perfusion is a reliable and reproducible method for recovery of the pancreas in a cDCD when utilized in the scenario of islet transplantation.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.2: In the context of pancreas for islet transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD is compatible with the procurement of liver and kidneys.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.3: In the context of pancreas for islet transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD is compatible with the procurement of heart and lungs.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.4: In the context of pancreas for islet transplantation, *in-situ* normothermic regional perfusion in the setting of cDCD should be maintained between 1 and 4 h.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.5: Valid assessment parameters of pancreas graft quality still need to be defined in the context of pancreas for islet transplantation after *in-situ* normothermic regional perfusion for cDCD.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.6: *In-situ* normothermic regional perfusion in donation after cDCD may improve islet isolation and transplant outcomes compared to *in-situ* cooling and rapid procurement.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 6.7: *In-situ* normothermic regional perfusion in the setting of cDCD has the potential to expand the donor pool for islet transplantation.

Quality of Evidence: C.

Strength of Recommendation: 1.

Persufflation in Islet Transplantation

No clinical studies are available regarding the implementation of persufflation in islet transplantation. All studies are pre-clinical in either animal or human models. No human islet transplants have been reported after preservation with persufflation. Therefore, the quality of evidence was Grade C for all recommendations. The strength of recommendation was 1 for 6 recommendations and 2 for 1 recommendation. The jury could not deliberate on one query (PICO 8) due to lack of evidence.

Recommendation 7.1: In the context of pancreas for islet transplantation, persufflation should be performed using a humidified gaseous flow of 40% oxygen.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.2: In the context of pancreas for islet transplantation, persufflation should be performed at a temperature of 4°C–8°C in an organ preservation solution.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.3: In the context of pancreas for islet transplantation, persufflation should be performed using a gaseous flow rate of 20–25 mL/hr.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.4: In the context of pancreas for islet transplantation, persufflation can be performed by cannulation of both the superior mesenteric artery and the splenic artery and optionally, the gastroduodenal artery.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.5: In the context of pancreas for islet transplantation, a back table preparation must be performed prior to persufflation to stop arterial gaseous leaks.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.6: In the context of pancreas for islet transplantation, persufflation has the potential to prolong cold preservation up to 24 h.

Quality of Evidence: C.

Strength of Recommendation: 1.

Recommendation 7.7: In the context of pancreas for islet transplantation, persufflation can be performed during organ transport or as an end-ischemic strategy.

Quality of Evidence: C.

Strength of Recommendation: 2.

SUMMARY AND NEXT STEPS

Improved preservation of the pancreas with a view to undertaking either whole pancreas or islet transplantation is a particularly interesting and developing field. In recent years there has been a significant increase in the number of pre-clinical and clinical studies reporting the use of hypothermic and normothermic machine perfusion as well as normothermic regional perfusion. However, there are very few studies relating to pancreas and islet transplantation. With the likely development of this field, it is important to have consensus to allow systematic reporting and comparison.

The clinical implementation of normothermic regional perfusion is the only modality that has been successfully reported for both whole pancreas and islet transplantation. None of the other preservation modalities have been implemented in pancreas and islet transplantation and can therefore not be considered at present as a preservation modality that can be used in humans. Normothermic regional perfusion is already considered the standard of care in a minority of European countries but it is hoped that further funding in other countries will support its wider application. These data highlight the major difficulty in obtaining high quality data on organ preservation for either

whole pancreas transplantation or islet transplantation, when compared to other organ transplants (kidney-liver) where it has been more widely reported. These ESOT TLJ3.0 guidelines are the first to specifically address perfusion preservation modalities in both pancreas and islet transplantation. Despite the lack of high-quality data, these guidelines define the current technical modalities of perfusion in both hypothermic and normothermic conditions. They also aim to define the main outcomes expected in various perfusion modalities. The development of a consensus on perfusion modalities through preclinical studies allows us to consider high quality clinical studies to accurately assess the role of perfusion in whole pancreas and islet transplantation. In the authors' opinion, this consensus provides the technical basis to guide future clinical studies with a view to conducting the first human clinical feasibility trials.

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

Involved in the conception or design of the work: JF-F, BM, FM, JD, CD, ME, PJ, HL, GO, VP, RP, TR, WS, FV, TB, DJ-TT, NK, AW, AO, SW, and JB. Literature screen and review: JF-F, BM, FM, JD, CD, ME, PJ, HL, GO, VP, RP, TR, WS, FV, TB, DJ-TT, NK, AW, AO, SW, and JB. Drafted the article: JF-F, BM, FM, JD, CD, ME, PJ, HL, GO, VP, RP, TR, WS, FV, TB, DJ-TT, NK, AW, AO, SW, and JB. Critically revised the article: JF-F, BM, FM, JD, CD, ME, PJ, HL, GO, VP, RP, TR, WS, FV, TB, DJ-TT, NK, AW, AO, SW, and JB. Finally approved the version to be published: JF-F, BM, FM, JD, CD, ME, PJ, HL, GO, VP, RP, TR, WS, FV, TB, DJ-TT, NK, AW, AO, SW, and JB. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11374/full#supplementary-material

SUPPLEMENTARY APPENDIX S1 | Evidence report - Pancreas Machine Perfusion.

SUPPLEMENTARY APPENDIX S2 | Congress abstracts Pancreas Machine Perfusion.

SUPPLEMENTARY APPENDIX S3 | Studies included in review.

SUPPLEMENTARY APPENDIX S4 | Preliminary Statements Draft - Role of Pancreas Machine Perfusion to Increase the Donor Pool for Beta Cell Replacement.

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European Society for Organ Transplantation (ESOT)-TLJ 3.0 Consensus on Histopathological Analysis of Pre-Implantation Donor Kidney Biopsy: Redefining the Role in the Process of Graft Assessment

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The ESOT TLJ 3.0. consensus conference brought together leading experts in transplantation to develop evidence-based guidance on the standardization and clinical utility of pre-implantation kidney biopsy in the assessment of grafts from Expanded Criteria Donors (ECD). Seven themes were selected and underwent in-depth analysis after formulation of PICO (patient/population, intervention, comparison, outcomes) questions. After literature search, the statements for each key question were produced, rated according the GRADE approach [Quality of evidence: High (A), Moderate (B), Low (C); Strength of Recommendation: Strong (1), Weak (2)]. The statements were subsequently presented in-person at the Prague kick-off meeting, discussed and voted. After two rounds of discussion and voting, all 7 statements reached an overall agreement of 100% on the following issues: needle core/wedge/punch technique representatively [B,1], frozen/paraffin embedded section reliability [B,2], experienced/non-experienced on-call renal pathologist reproducibility/accuracy of the

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Abbreviations: ESKD, end-stage kidney disease; ECD, expanded criteria donors; KDPI, kidney donor profile index; DCD, donor after circulatory death; PICO, patient/population, intervention, comparison, outcomes; CET, center of evidence in transplantation; WB, wedge biopsy; NB, needle core biopsy; DGF, delayed graft function; PB, punch biopsy; IFTA, interstitial fibrosis and tubular atrophy; ICC, intra-class correlation coefficient; GS, glomerulosclerosis; cv, vascular chronicity; TA, tubular atrophy; HES, haematoxylin-eosin saffron; IF, interstitial fibrosis; FFPE, formalin-fixed paraffin embedded; WSIs, whole-slide images; RGF, reduced graft function; ROC, receiver operating characteristic; AUC, area under the ROC curve; RENFAST, Rapid evaluatioN of fibrosis and vesselS thickness; RENTAG, robust evaluatioN of tubular atrophy and glomerulosclerosis; OPTN, organ procurement and transplant network; MA, moderate arteriolosclerosis; UNOS, united network for organ sharing.

histological report [A,1], glomerulosclerosis/other parameters reproducibility [C,2], digital pathology/light microscopy in the measurement of histological variables [A,1], special stainings/Haematoxylin and Eosin alone comparison [A,1], glomerulosclerosis reliability *versus* other histological parameters to predict the graft survival, graft function, primary non-function [B,1]. This methodology has allowed to reach a full consensus among European experts on important technical topics regarding pre-implantation biopsy in the ECD graft assessment.

Keywords: kidney transplantation, expanded criteria donors, histopathology, pre-implantation kidney biopsy, consensus paper

INTRODUCTION

Kidney transplantation is the first-line treatment for end-stage kidney disease (ESKD), but organ availability does not meet the needs of the large number of potential recipients. For this reason, during the last years, the use of expanded criteria donors (ECD), aged more than 60 years or aged 50–59 years with at least two criteria among hypertension, serum creatinine more than 1.5 mg/dL or death from cerebrovascular accident, has steadily increased [1–3].

Considering the marginal nature of these organs, pre-implantation kidney biopsies have been used to provide a window on the state of the renal graft and it is considered in some settings a valuable decision-making tool as it helps to identify chronic or acute organ damage in order to estimate renal function after transplantation [4–6]. However, in spite of the well-reported clinical utility of this procedure, its use in the daily clinical practice is still debated and poorly standardized.

The role of pre-implantation biopsy in the decision to utilize kidney grafts from ECDs has been somehow controversial: on the one hand, an accurate histological assessment would provide additional information regarding the actual state of a sub-optimal organ, on the other hand the correlation between histological lesions in different compartments (glomerular, tubular, interstitial, vascular) and graft outcome after renal transplantation is not fully understood [5, 7]. Moreover, some histological features may lead transplant centers to discard organs otherwise acceptable based on the Kidney Donor Profile Index (KDPI) or on clinical/functional data [8, 9]. The absence of a clear threshold, as defined by alterations in each compartment of the renal architecture, that accurately predicts an acceptable outcome if the transplant proceeds, makes it challenging to define acceptance criteria based on histological evaluation. In addition, the assessment of pre-implantation kidney biopsies is not standardized in terms of technical procedures and pathologists' evaluation.

The ultimate goal of the present work is to collect evidence and set up guidelines on the role of pre-implantation biopsy aiming to improve the outcomes and minimize the organ discard: the specific object of this preliminary activity was to reach a consensus about relevant operational procedures as the sampling, processing, staining and reading of the specimens. Currently, no such consensus around pre-implantation biopsy-related technical issues exists, nor does it relate to the impact of histopathological alterations in the different kidney compartments on graft function and survival.

The main reason of this lack of consensus is the difficulty in standardizing the procedure because of different scoring systems, the type of biopsy (wedge vs. needle core), and the differences in reported outcomes. In addition, the pathologists' expertise has to be taken into account, as it is known to influence the correlation with the outcome [9, 10]. As reported by Azancot et al. [9], donor histology and graft outcome were correlated when the biopsy was evaluated by renal pathologists, but not when they were evaluated by on-call pathologists.

The evaluation of pre-implantation renal biopsies requires specific ultra-specialist training, but in many cases, it is entrusted to an on-call pathologist who often has little knowledge in nephropathology and does not have the opportunity to deal with more expert colleagues [11].

In this context, the possibility of digitizing the slides is essential, allowing for remote evaluation/second opinion [12]. Additionally, the development of digital pathology and modern computerized image analysis tools could also assist the pathologist in slide reading and diagnostic definition [11–13].

All these tools could reduce inter-observer variability, as there is still little agreement among general pathologists, who tend to give higher scores, especially for glomerulosclerosis and arterial thickness, which are the most important parameters for evaluating chronic renal damage [5].

Finally, the employment of pre-implantation kidney biopsy for the evaluation of donor after circulatory death (DCD) is essential [14], but the impact of the specific histological lesions in the Bayesian context of the clinical scenario should be better evaluated.

The deep analysis of the current literature evidence and a peer discussion of all aforementioned issues could help reach a general consensus with a practical clinical impact in kidney transplantation.

For this purpose, in order to develop evidence-based guidance on the standardization and clinical utility of pre-implantation kidney biopsy for the assessment of grafts from ECD, a global panel of four histopathologists, four nephrologists and two transplant surgeons underwent in-depth analysis after the formulation of PICO (patient/population, intervention, comparison, outcomes) questions to develop guidelines on key aspects of the role of pre-implantation histopathology in the process of graft assessment.

TABLE 1 | List of all PICOs and recommendations.

PICO	Recommendation	Quality of evidence	Strength of recommendation
1. For evaluating chronic lesions in ECD kidneys (P), is the needle core biopsy (I) comparable/inferior/superior to wedge biopsy (C) or punch biopsies in terms of representatively of the entire renal parenchyma (O)?	For the evaluation of chronic lesions in ECD kidneys, needle core and wedge biopsy are both suitable, even though differences may be found in terms of glomerular and vascular assessment. Punch biopsies have potentially similar suitability, although more evidence is required	Moderate (B)	Strong for (1)
2. For the evaluation of chronic lesions in ECD kidneys (P), is the frozen section (I) comparable/inferior/superior to paraffin embedded section (C) in terms of reliability of the reading from pathologists?	For the evaluation of chronic lesions in ECD kidneys the frozen section is inferior to paraffin embedded section in terms of reliability of the reading from pathologists. Frozen sections should not be considered as a first option; however, it could be suitable for use in selected cases such as clinical urgency or other specific contexts	Moderate (B)	Weak against (2)
3. For score assessment of pre-implantation kidney biopsy in the evaluation of ECD (P) is the experienced renal pathologist (I) comparable/inferior/superior to on-call pathologist (C) in terms of reproducibility and accuracy of the histological report (O)?	For score assessment of pre-implantation kidney biopsy in the evaluation of ECD the experienced renal pathologist is superior to non-experienced pathologist in terms of reproducibility and accuracy for the prediction of total parenchyma status	High (A)	Strong for (1)
4. In the quantification of chronic damage in ECD kidneys (P), is glomerulosclerosis (I) more reproducible (O) in comparison with other parameters (interstitial fibrosis, tubular atrophy, wall/lumen ratio, arteriolar hyalinosis) (C)?	In the quantification of the chronic damage in ECD kidneys, glomerulosclerosis is more reproducible in comparison with other parameters (interstitial fibrosis, tubular atrophy, wall/lumen ratio, arteriolar hyalinosis)	Low (C)	Weak for (2)
5. In the quantification of the chronic damage in ECD kidneys (P) is measurement of histological variables with digital pathology (I) comparable/inferior/superior (O) when compared with light microscopy (C)?	In the quantification of the chronic damage in ECD kidneys measurement of histological variables with digital pathology is potentially comparable with light microscopy	High (A)	Strong for (1)
6. In the quantification of the chronic damage in ECD kidneys (P) is measurement of histological variables with the aid of special stainings (Periodic-Acid Schiff, Silver, Picro Sirius Red, Trichrome stainings) (I) comparable/inferior/superior (O) if compared with Haematoxylin and Eosin alone (C)?	In the quantification of chronic damage in ECD kidneys, the use of additional histochemical stainings (including, but not limited to PAS, Silver, Trichrome and/or Picro Sirius Red) is superior to the use of H&E alone in any diagnostic kidney pathology context but can likely not be performed under time constraints in the context of (on-call) organ utilization decision making	Low (C)	Strong for (1) (expert-opinion)
7. In the quantification of the chronic damage in ECD kidneys (P), is glomerulosclerosis percentage (I) more representative than other parameters (interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and cv score) (C) to predict the graft survival, graft function, primary non-function (O)?	Even though no studies are available for head-to-head comparison between GS and the other parameters, the degree of GS in procurement kidney biopsies from ECDs is associated with graft survival	Moderate (B)	Strong for (1)

After a literature search by the Center of Evidence in Transplantation (CET), the relative statements for each key question were produced, rated according to the quality of evidence using the GRADE approach. The statements were subsequently presented in-person at the kick-off meeting in Prague, discussed and voted [15].

METHODS

The consensus development process was organized by a dedicated Guidelines Taskforce within ESOT and its sections ELITA, EKITA, EPITA, ECTTA, ETHAP, Education Committee, YPT, Transplant International editorial board members and patient representatives. A detailed description of the methodology used was reported previously [15].

Briefly, key issues related to transplantation topic were identified by each working group and specific clinical questions were formulated according to the PICO methodology (PICO, Population, Intervention, Comparator and Outcome) [16]. All PICO questions are listed in **Table 1**.

Following the definition of the PICOs, literature searches were developed by an expert staff from the CET who have expertise in conducting systematic reviews and subsequently integrated, when needed, by the steering committee experts.

The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. For evaluating the quality of evidence according to GRADE [15] the following features were considered: study design, the risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance and publication bias. The strength of recommendation was rated 1 (strong) or 2 (weak).

Complete information, including the list of consensus conference workgroup domains (and topics noted below), and

the process regarding consensus conference participant selection, development and refinement of consensus statements are previously reported beforehand the in-person conference held in Praque, Czech Republic, 13–15 November, 2022 [15].

RESULTS

After all the methodological steps and two rounds of discussion and voting, 7 statements reached an overall agreement of 100%.

PICO 1

For evaluating chronic lesions in ECD kidneys (P), is the needle core biopsy (I) comparable/inferior/superior to wedge biopsy (C) or punch biopsies in terms of representatively of the entire renal parenchyma (O)?

Analysis of the Evidence for PICO 1

To evaluate chronic lesions in ECD, several techniques are employed, but, to date, no consensus concerning the best procedure for this invasive diagnostic process is available.

A large number of studies, most of them including both ECDs and standard criteria donors, have compared wedge biopsy (WB) *versus* needle core biopsy (NB), demonstrating slight differences. In particular, WB, being more superficial, may provide more glomeruli compared with NB. This may over-estimate the degree of glomerulosclerosis [17–19] and underestimate the extent of the arterial intimal thickening [20]. Different studies also analyzed the correlation between WB or NB and histology of the nephrectomy in the same kidney (Muruve et al., n = 9; Mazzuco et al.; n = 154) [17, 21] or in the biopsies performed in the early post-transplant period (Bago et al., n = 271; Husain et al.; n = 392) [19, 22], leading to similar conclusions. Also, two other studies had similar results comparing directly WB *versus* NB in the evaluation of the same organ (Yushkov et al. [23]; Haas et al. [20]). In 226 donors, Yushkov et al. [23] found that optimized needle biopsies were significantly more sensitive in identifying allograft tubulointerstitial scarring as well as intimal fibrous narrowing than WB. However, the technique of NB implied 2 cores of 14-gauge needles. Haas et al. found more severe arteriosclerosis in NB, partly due to the higher number of arcuate arteries in NB compared to WB, but this study was performed in healthy living donors.

Subsequently, Yong et al. [18], demonstrated that WB could be superior to NB in predicting delayed graft function (DGF). However, in this study, the two techniques were not compared in the same patient cohorts and all comorbidities associated with DGF were not considered in the statistical analysis.

Only one study (Bago Horwath et al. [22]), compared punch biopsy (PB) with WB in both pre-implantation and post-transplant biopsies performed for cause within 2 months demonstrated that PB was superior to the other techniques for the diagnosis of Interstitial Fibrosis and Tubular Atrophy (IFTA) and chronic vascular changes.

Recommendation 1.1

For the evaluation of chronic lesions in ECD kidneys, needle core and wedge biopsy are both suitable, even though differences may be found in terms of glomerular and vascular assessment. Punch biopsies have potentially similar suitability, although more evidence is required.

Quality of Evidence: Moderate (B).

Strength of Recommendation: Strong for (1).

PICO 2

For the evaluation of chronic lesions in ECD kidneys (P), is the frozen section (I) comparable/inferior/superior to paraffin embedded section (C) in terms of reliability of the reading from pathologists?

Analysis of the Evidence for PICO 2

In a large clinical study, including kidneys in which more than one biopsy was performed [24], authors observed that different procurement biopsies of the same kidney were poorly reproducible (64% of cases, k = 0.14). The correlation between procurement and reperfusion biopsies was also poor, including percentage of glomerulosclerosis, which had 63% agreement (k = 0.15), interstitial fibrosis/tubular atrophy and vascular chronicity, with agreement rates of 82% (k = 0.13) and 80% (k = 0.15), respectively.

A smaller study published by Sagasta et al. [25] found that agreement between observers (on call pathologist *versus* trained pathologist) using the same frozen sections was weaker than the correlation between frozen and paraffin-embedded sections.

Concordance was lower also in the retrospective review of frozen sections (Kendall's Tau b for Remuzzi score: 0.03), and better in the original report (Kendall's Tau b for Remuzzi score: 0.67). This comparison revealed that the trained pathologist assigned higher scores when using frozen *versus* paraffin-embedded sections and hypothetically reducing organ acceptance.

Another study [26] showed that frozen and paraffin-embedded sections showed comparable histological changes. Although frozen sections underestimated glomerulosclerosis and arteriolosclerosis and overestimated acute tubular necrosis and interstitial fibrosis those differences were not statistically significant.

Teixera et al. [27] used an aggregate score (MAPI) to assess agreement between frozen sections and paraffin-embedded biopsies, showing improved Kappa coefficient when the total score was used in comparison with the individual parameters. In details, the retrospective review of pathological reports of frozen sections (on-call pathologist) and their corresponding permanent sections (trained pathologist), showed Kappa values ranging from 0.29 to 0.51 for the individual MAPI parameters 0.59 when using the total MAPI score.

Recommendation 2.1

For the evaluation of chronic lesions in ECD kidneys the frozen section is inferior to paraffin embedded section in terms of reliability of the reading from pathologists. Frozen sections should not be considered as a first option; however, it could

be suitable for use in selected cases such as clinical urgency or other specific contexts.

Quality of Evidence: Moderate (B).

Strength of Recommendation: Weak Against (2).

Comment to Recommendation 2.1

In this recommendation, the terms "clinical urgency" was referred to the need to accelerate the transplant procedure due to many factors including very long cold ischemia-time or other logistic necessities.

PICO 3

For score assessment of pre-implantation kidney biopsy in the evaluation of ECD (P) is the experienced renal pathologist (I) comparable/inferior/superior to on-call pathologist (C) in terms of reproducibility and accuracy of the histological report (O)?

Analysis of the Evidence for PICO 3

In a study that included 92 biopsies, 78 kidneys from transplanted and 14 from non-transplanted patients, correlation between the on-call pathologists and the trained pathologist was weak in all the parameters on frozen sections [25]. Trained pathologists assigned higher Remuzzi scores to pre-implantation biopsies from expanded criteria donors than on-call pathologists.

A larger study by Azancot A et al. [9] demonstrated poor to fair agreement for scores generated by on-call and experienced renal pathologists for all histological variables other than glomerulosclerosis, which, conversely, was highly reproducible. In this study, on-call pathologists tended to have higher aggregate scores with a tendency to overcall chronic damage, possibly leading to higher organ discard. It should be highlighted that whilst there was no association between the readings from the on-call pathologist and outcome, evaluation of biopsies by a renal pathologist was significantly and independently associated with estimated 12-month glomerular filtration rate and composite graft outcome.

Subsequently, Girolami et al [5] analyzed the Remuzzi score of 46 discarded kidneys reviewed by three general and two experienced renal pathologists (the original report was blinded) and the intra-class correlation coefficient (ICC) demonstrated that trained pathologists achieved higher values of ICC, reaching excellent or good agreement in most of the parameters, while general pathologists' values were mainly fair or good.

Notably, the Banff Histopathological Consensus Criteria for Pre-implantation Biopsies endorse a training of general pathologists assigned to donor biopsy evaluation [28].

Recommendation 3.1

For score assessment of pre-implantation kidney biopsy in the evaluation of ECD the experienced renal pathologist is superior to non-experienced pathologist in terms of reproducibility and accuracy for the prediction of total parenchyma status.

Quality of Evidence: High (A).

Strength of Recommendation: Strong for (1).

Comment to Recommendation 3.1

Based on the literature reports and after our collegial discussion, we recommend, wherever possible, to involve a specialist pathologist for pre-implantation kidney biopsy assessment to minimize the risk of erroneous discard of organs due to the lack of expertise.

PICO 4

In the quantification of chronic damage in ECD kidneys (P), is glomerulosclerosis (I) more reproducible (O) in comparison with other parameters (interstitial fibrosis, tubular atrophy, wall/lumen ratio, arteriolar hyalinosis) (C)?

Analysis of the Evidence for PICO 4

In a study of 44 donor biopsies (50% needle, 50% wedge), glomerulosclerosis (GS), vascular chronicity (cv), tubular atrophy (TA) and interstitial fibrosis (IF) were scored by 3 independent pathologists. The ICCs were 0.87 for GS (the highest), 0.51 for cv, 0.71 for TA and 0.35 for IF. ICC was similar for wedge and needle biopsies [29].

In a more recent study [5], 46 discarded kidneys were identified with their 75 corresponding biopsies (83% wedge and 17% needle). The biopsies were reviewed by three general and two specialist pathologists. Specialist pathologists achieved higher values of ICC with excellent-to-good agreement, while general pathologists' agreement was fair-to-good. Interestingly, the ICC was highest for GS and was comparable between the general and specialists, whereas ICC for IFTA and vascular changes was poor-to-fair for on-call pathologists and good-to-excellent for experienced renal pathologists. However, the percentage of GS was significantly higher in the biopsies than in discarded organs, demonstrating a "true" sampling error of GS as the majority of biopsies were wedge biopsies.

Using artificial intelligence, a deep neural network segmented normal and sclerotic glomeruli in 98 hematoxylin, eosin and saffron (HES) frozen and 51 formalin-fixed paraffin embedded (FFPE) whole-slide images (WSIs) from 83 donor kidney biopsies, to quantify global glomerulosclerosis. Annotation by three expert pathologists served as the ground truth. A total of 1,544 globally sclerosed and 6,914 non-globally sclerosed individuals were labeled in 149 images. The study demonstrated higher performance of the artificial intelligence model than pathologists. Model accuracy further increased by pooling multiple sections, resulting in a decreased likelihood of erroneous organ discard. However, this study did not compare the reproducibility of GS with other chronic parameters in the biopsy [30].

Two studies from the same center at Columbia University focused on the reproducibility of chronic scores in sequential biopsies from the same donor. Husain et al. [31], included 1,010 cases among which 606 had more than one procurement biopsies. Information about GS, IF, TA, cv was retrieved from the reports. A score from 0 to 3 was assigned for each parameter. Agreement between sequential biopsies reports for kidney that underwent multiple procurement biopsies was evaluated. There was poor overall agreement for the 3 histologic compartments, and agreement was highest for vascular disease and lowest for GS.

More recently, they compared protocol kidney biopsies performed at day 7 and 14 in 69 patients and obtained the reported GS, IFTA, cv and arteriolar hyalinosis scores. Agreement between day 7 and day 14 was best for cv (concordance 78%, k = 0.60). For GS, only a moderate correlation between both time points was found (r^2 = 0.25) [32].

Recommendation 4.1

In the quantification of the chronic damage in ECD kidneys, glomerulosclerosis is more reproducible in comparison with other parameters (interstitial fibrosis, tubular atrophy, wall/lumen ratio, arteriolar hyalinosis).

Quality of Evidence: Low (C).

Strength of Recommendation: Weak for (2).

PICO 5

In the quantification of the chronic damage in ECD kidneys (P) is measurement of histological variables with digital pathology (I) comparable/inferior/superior (O) when compared with light microscopy (C)?

Analysis of the Evidence for PICO 5

The study of Altini et al. [33] detected and classified glomeruli (n: 2,500) in kidney biopsies of 26 subjects using a model based on Convolutional Neural Networks. Global accuracy was higher than 0.98 with precision in classifying healthy and sclerosed glomeruli ranging 0.834–0.935 and 0.806–0.976.

The paper by Bevilacqua et al. [34] tested a Computer-Aided Diagnosis system for segmentation and discrimination of blood vessels *versus* tubules from 10 biopsies in the kidney tissue through the elaboration of histological images: regions of interest identified were in 221:71 vessels and 150 tubules. Results demonstrated that the supervised artificial Neural Network approach was consistent and reveals good performance, after a training phase based on vessels and tubules samples. Accuracy was higher than 0.93, with precision higher than 0.88 in the validation set and higher than 0.91 in the test set.

Luo et al. [35] used donor kidney biopsy WSIs as a source of features in addition to clinical characteristics for graft function prediction, building neural network models to predict stable eGFR and reduced graft function (RGF) in deceased-donor kidney transplant recipients who underwent pre-transplantation biopsy. They tested six prediction models on 219 WSIs. Overall, donor kidney biopsy WSIs were a useful predictor for graft function recovery, showing distinct improvements in the prediction performance of the deep learning algorithm plus the clinical characteristics model. Compared with the clinical data model, the area under the receiver operating characteristic (ROC) curve (AUC) of the clinical data plus the image model for eGFR classification increased from 0.69 to 0.83. Additionally, the predictive performance for RGF increased from 0.66 to 0.80.

In a proof-of-concept study, So et al. [36] reported noteworthy differences in Multiphoton Microscopy derived collagen parameters between donor kidneys with varying KDPI scores. They evaluated the amount (CART) and quality (CRI) of collagen deposition in 20 preimplantation biopsies. Although CART values were identical across all samples, biopsies classified with >85% KDPI demonstrated a significantly higher CART (51.94 vs. 45.61; p = .011) than biopsies with 20%–85% KDPI percentages. Conversely, they had lower CRI compared to biopsies with 20%–85% KDPI scores (4.15 vs. 4.53; p = .025).

Cascarano et al. [37] collected 26 digital slides taken from the kidneys of 19 donors with Periodic Acid-Schiff staining with the aim to develop a neural network able to detect and classify glomeruli. The workflow allowed the classification of sclerotic and non-sclerotic glomeruli with good performances: 0.99 accuracy, 1.00 precision.

Marsh et al. [38] developed a deep learning model for glomerulosclerosis on a population of mixed wedge and core kidney biopsy cases: 98 frozen and 51 permanent sections. Glomerular counts were compared against annotation ground truth, with accuracy assessed by Pearson correlation coefficient. The model correlated very well with pathologists' annotations, with a correlation coefficient higher than 0.900.

Salvi et al. [39] developed two models: RENFAST (Rapid EvaluatioN of Fibrosis And vesselS Thickness) for vessels and interstitial fibrosis detection and RENTAG (Robust EvaluatioN of Tubular Atrophy and Glomerulosclerosis) for glomeruli and tubules detection and classification. The RENFAST algorithm is developed and tested on 350 periodic acid–Schiff images for blood vessel segmentation and on 300 Masson's trichrome stained images for detecting renal fibrosis. In the test set, the algorithm exhibited excellent segmentation performance in both blood vessels (accuracy: 0.8936) and fibrosis (accuracy: 0.9227). The algorithm takes an average computational time 2.91 s against 20 min for pathologist assessment. RENTAG was developed using 61 WSIs for glomerulosclerosis assessment while 22 WSIs were employed for tubular atrophy quantification. The algorithm showed Dice scores of 0.95 and 0.91 for glomeruli and tubules with 100% sensitivity and PPV and little time of computation required.

Eccher et al. [40] evaluated 62 consecutives, previously reported pre-implantation kidney biopsies scanned with the ScanScope Digital Slide Scanner. The slides were assessed for percentage glomerulosclerosis, tubular atrophy, interstitial fibrosis and vascular narrowing using the Remuzzi criteria by two pathologists, one using glass slides and the other using the WSIs viewed on a widescreen computer monitor. After a 2-week washout period, all the slides were re-assessed by the same pathologists using the opposite mode of reporting to that used in the first evaluation. Very high glass-digital intra-observer concordance was achieved for the overall score and for individual grades by both pathologists (κ range, 0.841–0.973).

Recommendation 5.1

In the quantification of the chronic damage in ECD kidneys measurement of histological variables with digital pathology is potentially comparable with light microscopy.

Quality of Evidence: High (A).

Strength of Recommendation: Strong for (1).

Comment to Recommendation 5.1

Artificial intelligence could potentially help pathologists in their assessment of histological variables in kidney, also reducing

interobserver variability. The future potential in terms of 1) infrastructure and organization of care and 2) algorithmic assessment of digital pathology and artificial intelligence needs further evidence.

PICO 6

In the quantification of the chronic damage in ECD kidneys (P) is measurement of histological variables with the aid of special stainings (Periodic-Acid Schiff, Silver, Picro Sirius Red, Trichrome stainings) (I) comparable/inferior/superior (O) if compared with Haematoxylin and Eosin alone (C)?

Analysis of the Evidence for PICO 6

The literature search did not identify articles that fit the search criteria related to the PICO question. Generally, the Scientific Committee strongly beliefs that for any renal pathology setting, only performing an H&E staining is in principle inferior to a dedicated panel of special histochemical staining that also includes Periodic-acid Schiff, Silver, Trichrome and/or Picro Sirius Red stainings.

However, in the setting of (on-call) organ usage decision making specifically, where the optimal decision-making competes with time constraints, processing of special histochemical stains (either performed on frozen sections or fast formalin-fixation protocols) will likely result in an unwanted delay of the organ transplant procedure with a consequent increase of ischemia time for several hours.

Recommendation 6.1

In the quantification of chronic damage in ECD kidneys, the use of additional histochemical stainings (including, but not limited to Periodic-Acid Schiff, Silver, Trichrome and/or Picro Sirius Red) is superior to the use of H&E alone in any diagnostic kidney pathology context but can likely not be performed under time constraints in the context of (on-call) organ utilization decision making.

Quality of Evidence: Low (C).

Strength of Recommendation: Strong for (1) (expert-opinion).

Comment to Recommendation 6.1

The absence of extensive literature on this topic may not allow for a high quality of evidence, but after discussion, the panel concluded that the strength of this recommendation (expert opinion) was high.

PICO 7

In the quantification of the chronic damage in ECD kidneys (P), is glomerulosclerosis percentage (I) more representative than other parameters (interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and cv score) (C) to predict the graft survival, graft function, primary non-function (O)?

Analysis of the Evidence for PICO 7

In a recent study [41], Stewart et al analyzed a large dataset of 3,851 ECDs recovered in the United States from 2008 to 2012 and reported a significant effect of glomerulosclerosis (GS>10%) on kidney graft survival, even after adjustment for potentially confounding donor and recipient variables. Conversely, the effects of interstitial fibrosis and vascular changes on the outcome were attenuated after adjustment. The BARETO (Biopsy, Anatomy, and Resistance Effects of Transplant Outcomes) study found a clinically and statistically significant effect of GS on 10-year graft survival among ECD kidney transplants. Kidneys having GS>10% were found to have 18% higher risk of graft failure compared with kidneys with GS 0%–5%.

The effect waned beyond 10%, suggesting little or no incremental risk associated with a GS of 20% compared with a GS of 10%. Regarding vascular changes, their data suggest a possible meaningfully large effect of mild-moderate (>25%) or worse vascular changes on long-term graft survival. Interstitial fibrosis seemed to have minimal, if any, prognostic value. These results agreed with those previously published by Anglicheau et al. [42] demonstrating that GS was an independent histological predictor of low eGFR at 1 year and death-censored graft survival. Also, in this case, the cut-off of GS more that 10% was the most significant.

Cheungpasitporn et al. [43] analyzed kidney graft outcomes related to the degree of GS in numerous datasets (>22,000 kidneys) ECDs with a KDPI score >85% from 2005 to 2014. They found that GS >10% is independently related to increased risk of graft loss. Kidneys with >10% GS were associated with 27% higher risk of graft failure compared to kidneys with 0%–10%. Of note, there was no difference in graft survival between 11% and 20% and >20% GS.

These results were in contrast with those previously published by Bodzin et al. [44] using the Organ Procurement and Transplant Network (OPTN) data. Multivariate analysis demonstrated that kidneys from ECDs with 0%–5% GS had no significant differences in graft function compared with those having more than 10% GS.

Additionally, Kayler et al. [45], analyzing a large dataset of kidney transplant recipients (*n*: 597) showed that only the presence of moderate arteriosclerosis and/or moderate arteriolosclerosis (MA), defined as > or = 25% luminal narrowing, was a significant predictor of graft outcome in recipients of ECD kidneys as defined by United Network for Organ Sharing (UNOS) criteria (univariate $p = 0.02$).

Increasing degree of GS in ECD organs was not associated with earlier graft failure in the multivariate analysis ($p = 0.30$).

GS>20% and interstitial fibrosis>25% had a low frequency in the material reviewed, likely reflecting organ use practices and a demonstrable effect on graft outcome could not be demonstrated.

Finally, Sung et al. [46], in another large multivariate analysis performed using the Scientific Registry of Transplant Recipients (SRTR)/Organ Procurement and Transplantation Network (OPTN) data, found that in ECD kidneys, GS was not reliably associated with DGF or graft failure.

Recommendation 7.1

Even though no studies are available for head-to-head comparison between GS and the other parameters, the degree

of GS in procurement kidney biopsies from ECDs is associated with graft survival.

Quality of Evidence: Moderate (B).

Strength of Recommendation: Strong for (1).

Comment to Recommendation 7.1

Based on the aforementioned literature evidence, we cannot draw definitive conclusions regarding the clinical impact of GS in comparison with other chronicity items (IF, TA and cv) to predict graft function and survival. In particular, available studies have only partially considered the quality of the histological interpretation (often performed by non-experienced pathologists), the quality/quantity of the kidney tissue sampling, and the correct data adjustments for demographic and clinical features (e.g., donor/recipient age, recipient's' dialysis vintage, HLA matching, comorbidities, immunosuppressive therapy, rate of rejections, infections). No studies have then considered primary non function as the target clinical outcome. Further studies must address these research gaps.

SUMMARY AND NEXT STEPS

This methodology has allowed us to reach a full consensus on important technical topics regarding pre-implantation biopsy in the process of ECD graft assessment and, at the moment, it represents the first attempt in Europe to standardize procedures in this field, including: needle core/wedge/punch technique representatively, frozen/paraffin embedded section reliability, experienced/non-experienced on-call renal pathologist reproducibility and accuracy of the histological report, glomerulosclerosis/other parameters (interstitial fibrosis, tubular atrophy, wall/lumen ratio, arteriolar hyalinosis) reproducibility, digital pathology/light microscopy in the measurement of histological variables, special stainings (Periodic-Acid Schiff, Silver, Picro Sirius Red, Trichrome)/Haematoxylin and Eosin alone comparison in the measurement of histological variables, glomerulosclerosis percentage/interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and intima fibrosis score reliability to predict transplant outcome. Due to the low number of papers published in this field, a main limitation of this consensus is the inclusion of data available from some studies comprising both ECDs and SCDs. However, when possible, we have drawn our conclusions deeply analyzing the specific results referred to ECDs.

We expect that this can have an important clinical impact and represents the basis for the European guideline. In the future, we expect to go into more details on several technical issues and better analyze the relationship of this procedure with the daily clinical practice and hard transplant outcomes, and to review and discuss the role of preimplantation biopsy in ECD kidney acceptance and, ultimately, allocation.

AUTHOR CONTRIBUTIONS

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

WORKGROUP COMPOSITION

The ESOT workgroup on Pre-implantation donor kidney biopsy comprised of all main authors, as well as the following panel of independent experts: Klemens Budde, Anthony Dorling, Giuseppe Ietto, Amanda Klein, Georgios Liapis, Maarten Naesens, Mikhail Nozdrin, Stella Stabouli, Stathis Tsiakas, Fabio Vistoli, Angeliki Vittoraki and Oleksii Voroniak.

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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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