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ABOUT COVER

Editor-in-Chief of *World Journal of Transplantation*, Dr. Vassilios Papalois is Professor of Transplantation Surgery and Consultant Transplant and General Surgeon at the Hammersmith Hospital, Imperial College, London, United Kingdom. His clinical work focuses on high-risk and extended criteria kidney and pancreas transplantation. He has been active in surgical research for more than 30 years and has published more than 200 papers in peer reviewed journals (receiving more than 200 citations per year internationally), 20 book chapters, and 7 books. His research focuses on pre-transplant assessment and reconditioning of marginal kidney and pancreas grafts, use of stem cells for treatment of chronically scarred allografts, clinical ethics, and health policy. He has been awarded a Silver National Award for Clinical Excellence by the United Kingdom Department of the Health, and a Senior Clinical Investigator Award by the European Society of Organ Transplantation. (L-Editor: Filipodia)

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OPINION REVIEW

Practical recommendations for kidney transplantation in the COVID-19 pandemic

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Abstract

Kidney transplantation at the time of a global viral pandemic has become challenging in many aspects. Firstly, we must reassess deceased donor safety (for the recipient) especially in communities with a relatively high incidence of coronavirus disease 19 (COVID-19). With respect to elective live donors, if one decides to do them at all, similar considerations must be made that may impose undue hardship on the donor. Recipient selection is also problematic since there is clear evidence of a much higher morbidity and mortality from COVID-19 for patients older than 60 and those with comorbidities such as hypertension, diabetes, obesity and lung disease. Unfortunately, many, if not most of dialysis patients fit that mold. We may and indeed must reassess our allocation policies, but this must be done based on data rather than conjecture. Follow-up routines must be re-engineered to minimize patient travel and exposure. Reliance on technology and telemedicine is paramount. Making this technology available to patients is extremely important. Modifying or changing immunosuppression protocols is controversial and not based on clinical studies. Nevertheless, we should reassess the need for induction therapy across the board for ordinary patients and the more liberal use of mammalian target of rapamycin inhibitors in transplant patients with proven infection.

Key Words: COVID-19; Kidney transplantation; Organ donation; Coronavirus; SARS-CoV2

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Core Tip: Transplantation in areas with a high rate of the coronavirus disease 19 (COVID-19) infection may be risky for recipients, as there may be a risk of COVID-19 transmission from infected donors. All preventive measures should be taken while treating kidney transplant patients.

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INTRODUCTION

Kidney transplantation (KT) is the treatment of choice for end-stage kidney disease[1]. The progress in immunosuppression along with the advances in surgical techniques has led to an improvement in transplantation outcomes. However, the increased risk of infection in immunocompromised patients can negatively affect the results of transplantation. The appearance of the new coronavirus disease 2019 (COVID-19), which is highly infectious and carries a high mortality risk, presents significant challenges to transplantation in general, to KT in particular and to living donor KT specifically.

COVID-19 is caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV2)[2]. COVID-19 was confirmed following several severe cases of pneumonia in the city of Wuhan in China in December 2019[3], and shortly thereafter, this disease spread worldwide[4] affecting more than 1.5 million people with more than 110 thousand deaths[5]. In Israel, the first diagnosed case of COVID-19 was announced on February 21, 2020. Since then, more than 11000 cases have been confirmed of which 110 died[6]. As reported elsewhere, COVID-19-related mortality is far more prevalent in older patients and those with comorbidities[7].

During the SARS epidemic in 2003 and the Middle East respiratory syndrome (MERS) in 2018, there was no increased mortality among immunocompromised patients[8,9]. Conversely, in the current COVID-19 pandemic, several reports have demonstrated the severity of this disease among immunocompromised transplanted patients[10,11].

Under the current circumstances, there are clear obstacles and challenges that almost all transplant centers in the world encounter due to the lack of evidence-based medicine regarding kidney transplant management in this setting. In this report, we highlight our local measures and guidelines that were adopted by the KT unit at Hadassah – Hebrew University Medical Center in Jerusalem, Israel.

DECEASED DONORS

We expected the number of organs from deceased donors to decrease during the pandemic, as a result of the extreme load on the intensive care units causing care to be diverted from brain dead potential donors. Also, at times of societal stress, the tendency to donate organs goes down and lastly due to the social distancing there are far less road accidents and brain injuries. Surprisingly, our center was only minimally affected regarding deceased donors during this period.

The risk of transmission of COVID-19 by a deceased donor is not yet known, but we believe that there is a possibility of viral transmission, since it was reported that there is a 15% chance of isolating the virus from blood[12]. Moreover, some pathological changes were reported in organs other than the lungs in COVID-19 patients[13].

In order to minimize the previously mentioned potential hazards, whether they are from the donor, the recipient or the team, we adopted the recommendations of the National Transplantation Steering Committee for consideration of a potential deceased kidney donor. These criteria include: (1) The donor must have a negative nasopharyngeal swab for COVID-19; (2) The donor should have no history of traveling abroad in the last 14 days and no exposure to a proven COVID-19 patient; (3) Every potential donor with diagnosed pneumonia should test negative for COVID-19, if no test can be performed the donor is rejected; (4) A donor that was treated by a medical

team that took care of proven COVID-19 patients should be rejected; and (5) In the case of a donor with cardiac death (DCD), if there is insufficient time to gather all this information, the donor should be rejected. By accumulating knowledge on COVID-19 disease, we believe the following additional factors should be considered: (1) The presence of upper respiratory symptoms or fever; (2) Lymphopenia; (3) Chest computed tomography (CT) scan with findings that can be attributed to COVID-19 infection; and (4) High suspicion of COVID-19 infection, based on epidemiologic and clinical signs, even if COVID-19 polymerase chain reaction (PCR) is negative. We also apply the same criteria for liver donors.

The importance of performing a chest CT scan and considering lymphopenia for every potential donor stems from the report published by Guan *et al*[14] who demonstrated that in a large cohort of 1099 COVID-19 patients, 96% of the patients had specific abnormal findings in the lungs, and 82.1% had lymphopenia.

Regarding the 5th recommendation of the steering committee for a DCD donor, we recommend that the technique of machine perfusion should be utilized. This can provide a relatively safe environment for the kidneys and even enhance their performance while allowing additional time for missing data to be acquired. The application of these strict criteria on potential deceased kidney donors should decrease the risk of infection for both the transplant team and future recipients.

LIVING DONORS

Transplantation from living donors brings additional considerations. These are elective, pre-scheduled carefully planned transplantations[15]. Thus, stringent safety criteria must be implemented in order to protect the donor, the recipient and the team.

We believe that donors must undergo a period of 14 d isolation prior to transplantation. This may prove to be an undue and indeed unbearable burden for some donors and is to be explained at length during medical and psychosocial evaluation. Of note, PCR tests still show significant percentages of false negative results, and antibody detection assays are not yet commonly available.

The recent outbreak resulted in the Ministry of Health and transplantation centers temporarily withholding all living-related transplantation activities. This will eventually lead to an increased number of patients on dialysis treatment, with its prognostic and financial implications.

KIDNEY TRANSPLANT RECIPIENTS

In Israel, there are more than 857 patients on the waiting list. All of which are treated by dialysis, nevertheless, this number does not include patients who may need preemptive kidney transplant. In 2019, a total of 411 KT were performed in Israel, 248 from living donors and 163 from deceased donors[16].

In order to minimize the damage from the decreased number of donations, every effort should be made to stratify the patients who may be able to benefit from a kidney transplant in this pandemic era.

In Israel, we have implemented an old for old allocation policy for many years with great success. However, in these times, when it is clear that COVID-19 infection severity and mortality increase with age and comorbidities[7,17] we may need to reconsider this policy. Our present approach is that older recipients (> 65 years) should be informed of their inherent greater risk and if they decline the offer it should be rerouted to a younger patient. Although there is presently no data to make any projection or firm recommendation, we believe that due to the pandemic a reassessment of allocation policies in order to maximize safety and reduce mortality, morbidity and graft loss may be required.

Finally, according to the recommendations that were published on March 20, 2020 by the European Dialysis Working Group of ERA-EDTA, dialysis patients should be instructed to stay away from crowds whenever possible, to use individual means of transportation, to use protective measures in order to conserve their hygiene , and even to avoid personal contact with family members[18]. We suggest that these recommendations should be applied to kidney transplant recipients during and after hospitalization.

POST-OPERATIVE FOLLOW-UP

The clinical course following KT is fraught with complications in the best of cases. In order to minimize this, patients are advised to adhere to a strict follow-up routine. COVID-19 may expose these patients to added hazards when traveling and visiting medical clinics. As a result, we suggest tailoring an individual follow-up strategy that balances the risks with the needed intensity of visits for each patient. The plethora of technology devices and applications allowing effective telemedicine should be used as much as possible. However, patients who lack smart phones or computers with internet access may present a problem. In Israel this is almost universally due to religious prohibition and can be dealt with in an ad hoc manner. In places where economic considerations prevent patients from accessing technology, reach-out should be made to insurers, providers and charitable institutions to step into the gap. Telemedicine will assume an important future role in the care of these patients. Particular emphasis should be placed on strict adherence to the government's instructions regarding social isolation, hygiene habits and awareness of the signs and symptoms related to COVID-19. This means that patients arriving at clinics must have N95 masks and wear gloves. This personal protective equipment should be prescribed and delivered to transplant patients.

IMMUNOSUPPRESSION

Intuitively, one would tend to decrease immunosuppression in the face of a viral pandemic. We do not have any information as to whether that will benefit patients and the consequences are almost surely increased rates of rejection, increased immunosuppression, infection and graft loss. Thymoglobulin, a T cell depleting agent, is routinely used as an induction treatment. It has been linked to an increased rate of viral infections such as CMV, HSV and BK and to viral-related complications *e.g.*, post-transplant lymphoproliferative disorder[19]. Thus, it makes sense to speculate that it will increase the rate and the severity of COVID-19 infections. Its advantage is that it decreases the rate of rejection and allows the use of lower CNI levels. If the recipient is of higher immunological risk, the importance of thymoglobulin induction rises. Therefore, should we avoid thymoglobulin and move to non-depleting regimens, *e.g.*, Basiliximab (CD25R antagonist) or avoid induction at all? This will increase the risk of acute rejection, and if rejection occurs this could result in a whole anti-rejection treatment protocol accumulating to a much larger dose of immunosuppression. The issue of induction therapy for all needs to be examined and perhaps there is logic in using induction for higher immunological risk recipients. Nevertheless, at this time, due to lack of evidence-based reports, we believe institutions should continue their induction practices as before.

Corticosteroids have a major role in all anti-rejection protocols. In our institution, high dose methylprednisolone is given with induction with rapid tapering off down to 40 mg/day on day 6. Routinely, we do not use steroid-avoidance or steroid withdrawal protocols. Should one move to steroid-avoidance protocols now? No decrease in CMV infection rate was found when steroid avoidance or withdrawal protocols were compared to steroid maintenance protocols[20], and data regarding BK nephritis rates are conflicting. When investigating the previous, SARS-COVID experience, the Chinese reported advantageous outcomes when combining high dose steroids with hydroxychloroquine[21] and recently, a favorable outcome was suggested when steroids were used in the context of a cytokine storm[17,22]. However, studies in animal models indicated that long-term use of steroids facilitates viral replication[23]. According to existing (or non-existing evidence-based data), we believe that we should continue using the current steroid protocol that we practice and are familiar with, as no clear evidence proves that avoiding steroids would be of any benefit.

Anti-metabolites, mainly mycophenolate, are used in most maintenance protocols, depleting and interfering with both B and T lymphocytes functions. MPA was shown to inhibit viral replication of 4 different coronaviruses (not including COVID-19) in cell culture[24]. Unfortunately, animal models indicated that MPA worsened disease activity in both common marmosets (significantly higher mortality)[25] and Balb/c mice[26]. MPA together with interferon-b was associated with survival in one clinical report of MER-CoV patients. However, this was significant only in univariate analysis and the greater predictor of survival was disease severity at presentation[27]. Taken together, and in agreement with our common practice during viral infections such as CMV, EBV or HSV, we tend to lower mycophenolate dose and even to hold it. In the case of the

few transplant patients we treated for COVID19 infection, who presented with leukopenia, we stopped mycophenolate. We plan to re-start mycophenolate when 2 consecutive COVID 19 PCR tests are negative.

The calcineurin inhibitors, cyclosporine and tacrolimus are the mainstay of immunosuppression regimens for solid organ transplantation, affecting T cell activation and function. Although there is no doubt regarding their efficacy, calcineurin inhibitors were linked to an increased rate of viral infections. Mammalian target of rapamycin (mTOR) inhibitors were suggested to be beneficial regarding viral infections (refs for BK, HPV viral verrucae). Should we convert the treatment protocol from CNIs to de-novo mTOR inhibitors? Should we use low dose CNI protocols together with low dose mTOR inhibitors? FK binding protein (FKBP) binds the coronavirus non-structural protein (NSP-1), thus explaining the mode of action of Tacrolimus inhibition of human coronavirus replication in cell culture[28]. However, no data are available on CNI effectiveness in inhibiting disease progression in animal models or humans.

MTOR inhibitors were shown to inhibit MERS-CoV replication *in vitro*[29] Another work based on network drug repurposing suggested Sirolimus as a potential treatment for coronavirus infection[30]. Taken together, it is still unclear if CNI should be avoided or minimized, but in low immunological risk patients, mTOR inhibitors-based protocols along with low dose CNI are a reasonable possibility.

In summary, choosing immunosuppressive protocols during the COVID-19 pandemic is challenging. This is true for induction and treatment for newly transplanted patients as well as for maintenance treatment and for infected transplanted patients. Literature is scarce and mostly inconclusive. One should probably use well practiced protocols, avoid over-immunosuppression as much as possible, and minimize it in stable patients. Infected patients should probably be evaluated for severity of symptoms and signs, and if mild, holding the anti-metabolites is acceptable. If moderate or severe, it is possible to hold CNIs, but continue, or even increase the steroids dose.

Contrary to the logic in decreasing immunosuppression during a pandemic, it is important to remember, that at these times ambulatory patients are more difficult to follow. Patients tend to refrain from arriving at the hospital for routine tests, even to outpatient clinics, and community clinics are overloaded. Downgrading the levels of immunosuppression will demand a very tight follow-up protocol that will enable detection of rejections at the earliest time.

MEDICAL STAFF SAFETY

The novel coronavirus has been threatening not only the lives of medical professionals, but also their mental health. This outbreak has caused enormous distress in many health workers who particularly deal with coronavirus patients. This is mainly explained by the various stressful situations including work overload, isolation and relentless fear of infecting patients and family and shortage of medical equipment in some cases[31,32]. In addition to this, the transplantation team is primarily exposed to distress and anxiety due to their stressful work, and this makes the pandemic even more severe. Moreover, it was reported that a number of medical health providers had committed suicide during this pandemic. These facts have caused a serious burden on health systems worldwide.

CONCLUSION

Measurements have been adopted by some governments including creating a telephone line for psychiatric consultations and mental health support to fight depression, suicidal attempts and other psychiatric issues.

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REVIEW

Role of novel biomarkers in kidney transplantation

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Abstract

Clinical application of biomarkers is an integral component of transplant care. Clinicians and scientists alike are in search of better biomarkers than the current serologic (serum creatinine, donor-specific antibodies), urine-derived (urinalysis, urine protein), and histologic ones we now use. The science behind recent biomarker discovery spans across multiple molecular biologic disciplines, including transcriptomics, proteomics, and metabolomics. Innovative methodology and integration of basic and clinical approaches have allowed researchers to unearth molecular phenomena preceding clinical disease. Biomarkers can be classified in several ways. In this review, we have classified them *via* their origin and outcome: Primarily immunologic, *i.e.*, representative of immune regulation and dysfunction and non-immunologic, pertaining to delayed graft function, cardiovascular events/mortality, infection, malignancy, post-transplant diabetes, graft, and patient survival. Novel biomarker uses to guide the diagnosis and management of transplant-related outcomes is a promising area of research. However, the use of biomarkers to predict outcomes after kidney transplantation is not well studied. In this review, we summarize the recent studies illustrating biomarker use and transplant outcomes.

Key Words: Biomarkers; Kidney Transplantation; Rejection; Infection; Mortality; Graft survival

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innovative diagnostic and prognostic adjuncts to current standards of care. This review article aims to summarize the most recent literature describing novel biomarker use in kidney transplantation.

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INTRODUCTION

Kidney transplantation is the optimal renal replacement therapy for patients with end-stage kidney disease (ESKD). Kidney transplant recipients (KTRs) experience survival benefits in all age groups, have improved health-related quality of life, and kidney transplantation is cost-effective compared to hemodialysis or peritoneal dialysis[1-3]. Surveillance of allograft dysfunction is integral to post-transplant management. Ideally, graft injury should be detected and treated before irreversible damage occurs. The gold standard for assessing kidney allografts has been histologic analysis *via* biopsy[4]. Allograft biopsies are imperfect, as they can miss early, reversible pathology. Also, they carry approximately a 1%-2% risk of significant complications[5].

Serial measures of glomerular filtration rate along with qualitative/quantitative measures of urine albumin have been the mainstay of allograft surveillance since they are non-invasive, readily available, and interpretable. Changes in these parameters, however, are often neither sensitive or specific, unpredictive of outcomes, and occur late in the disease[6]. This has led to the need for non-invasive predictive data to allow clinicians to more readily diagnose and manage allograft pathology: Novel biomarkers.

What is a biomarker? The National Institutes of Health Biomarker Definition Working Group provides the subsequent definition: A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic responses, or pharmacological responses to a therapeutic intervention[7]. Another definition per the World Health Organization is the following: Any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease[8].

In this review, our focus is to highlight biomarker use in the context of key kidney transplant outcomes. As such, we classified biomarkers based on immunological and non-immunological related outcomes. With immunological outcomes pertaining primarily to rejection and immune tolerance, this section offered an opportunity to stratify biomarkers further based on their relation to the immune system. The non-immunological section, which was highlighted by biomarkers related to tissue injury primarily, was categorized by meaningful outcomes to emphasize the predictive value of these biomarkers. In cases of the novel, unique pathways, further description is provided accordingly.

Over the past several years, the field of biomarker research has grown exponentially as scientists and physicians alike are searching for novel ways to non-invasively detect allograft perturbations early-to help guide management and prognosticate both allograft and patient outcomes. As seen in a commentary in 2018 regarding the most recent iteration of the Banff classification for rejection from 2017, language regarding "thoroughly validated gene transcripts/classifiers" as adjuncts to diagnose antibody-mediated rejection (ABMR) affirms the emergence of biomarkers as an additional tool to surveil and diagnose post-transplant pathology[9].

In this review, we aim to summarize the most current literature from the past 5 year (2015-present date) on novel biomarkers in kidney transplant recipients and their relevance to fundamental kidney transplant outcomes.

NOVEL BIOMARKER CLASSIFICATION

Novel biomarker use can be classified into 2 main categories: Immunologic and non-immunologic. Immunologic biomarkers are those characterizing immune dysfunction ranging from subclinical to overt rejection. Non-immunologic biomarkers are those

that demonstrate adverse transplant outcomes whereby immune dysfunction is not the sole aberration at play, *e.g.*, delayed graft function, cardiovascular events, infection, malignancy. While an oversimplification, as innate and humoral immunity are rooted in most pathophysiologic responses, these categories provide a logical classification scheme for the myriad types of novel biomarkers.

Immunological

Surveillance and optimization of recipient immune status are vital to prolonged allograft and patient survival. While current practice offers means to risk-stratify patients for poor immunologic outcomes [human leukocyte antigen mismatch, sensitization, calculated panel reactive antibodies, pre-transplant donor-specific antibodies (DSA)], our current surveillance measures (creatinine, urine protein to creatinine ratio) fail to capture clinically unsuspected rejection, which occurs in 20%-25% of patients after kidney transplant[10]. In other words, early molecular level events occur below our current detection thresholds, leading to missed opportunities for intervention, prevention, and management of poor outcomes. Several recent studies offer promising findings to diagnose, treat, and prognosticate adverse immunologic outcomes.

Chemokines: Chemokines are signaling proteins capable of inducing movement of certain cell types to areas of interest. Chemokines arise early in the immune cascade of rejection and thus can act as biomarkers to non-invasively identify deleterious immune events. Both urine and plasma chemokines have been studied extensively to detect immunologic dysfunction.

In one study, Rabant *et al*[11] showed that urinary C-terminal amino acid sequence Cystine-X-Cystine (C-X-C) motif chemokines 9 and 10, interferon gamma (IFN-γ) dependent chemokines secreted by various leukocytes along with renal mesangial and tubular cells, correlated with tubulointerstitial and microvascular inflammation (t + i score; g + peritubular capillaritis score; all $P < 0.001$). The ratio of urinary C-X-C motif chemokine ligand ten (CXCL10) to urine creatinine diagnosed T cell-mediated rejection (TCMR) [area under the curve (AUC) = 0.80, 95% confidence interval (CI): 0.68-0.92; $P < 0.001$] and ABMR [AUC = 0.76 (95%CI: 0.69-0.82); $P < 0.001$]. Furthermore, CXCL10: Creatinine plus DSA improved diagnosis of ABMR [AUC = 0.83 (95%CI: 0.77-0.89); $P < 0.001$] and CXCL10: Creatinine ratio at the time of ABMR predicted risk of graft loss[11]. Similarly, Hricik *et al*[12] in their study from 2015 showed that positive urinary C-X-C motif chemokine ligand nine is predictive of acute rejection (AR) by a median of 15 d before clinical detection[12].

Urinary chemokines (C-X-C motif chemokine ligand nine specifically) were assessed for their predictive value of 5-year graft outcomes in a more recent study, but no clear association was observed[13].

Plasma-derived fractalkine, IFN-γ, and interferon gamma-induced protein ten were evaluated for prediction of AR in a recent study of 87 KTRs; the combined measure of fractalkine on day 0, interferon gamma-induced protein ten and IFN-γ on day 7 was predictive of AR in 1 month (AUC = 0.866) with a sensitivity of 86.8% and a specificity of 89.8%[14]. In a recent study of 65 KTRs, interleukin (IL)-8 was found to predict rejection with higher levels at day 7, day 30 ($P = 0.023$, 0.038), and correlate with serum creatinine (Pearson $r = 0.621$, $P = 0.001$)[15].

Another promising biomarker is soluble cluster of differentiation thirty (CD30), a tumor necrosis factor glycoprotein derived from T cells that regulates the balance between T helper type 1 and T helper type 2 immune responses. Early post-transplant elevations within the first 2 weeks in one study predicted AR (AUC = 0.775; $P = 0.004$) with the sensitivity of 88.8%, specificity of 46.3%[16]. These findings are summarized in Table 1.

In summary, chemokines have potential as novel biomarkers, particularly for predicting acute cellular and antibody-mediated rejection. Prediction of long term outcomes such as graft survival and patient survival, however, were limited. Chemokines may be a useful adjunct to predict early rejection events in kidney transplantation.

Free micro ribonucleic acid: Free micro ribonucleic acid (RNA) are small non-coding RNA segments integral to cellular function. While also present in homeostasis, in certain contexts, they signal perturbations at the molecular level, ergo are linked to disease. Free micro RNA have been studied extensively in renal pathology, both in native and transplanted kidneys. Given their regulatory roles and stability both *in vivo* and in vitro, they exude potential as robust biomarkers. Several recent studies demonstrate the role of free micro RNA as biomarkers[17].

Table 1 Summary of novel biomarker studies of chemokines associated with immunologic outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Rabant *et al*[11], 2015	244	Urine	uCXCL9, uCXCL10	Rejection	CXCL9/10[a] correlated with ti+mvi (i+t; g + ptc) CXCL10: Cr[a] diagnosed TCMR and ABMR (AUC > 0.75); CXCL10: Cr + DSA[a] improved the diagnosis of ABMR (AUC = 0.83)
Hricik *et al*[12], 2015	21	Urine	CXCL9	Rejection	uCXCL9 predicts AR by a median of 15 d before clinical detection
Faddoul *et al*[13], 2018	184	Urine and plasma	IFN-γ ELISpot; CXCL9	ACR	CXCL9 predictive of ACR; IFN-γ predictive of 1 year ↓eGFR; neither predicted 5-yr outcomes
Xu *et al*[14], 2018	87	Plasma	Circulating fractalkine, IFN-γ and IP-10	AR	Fractalkine on day 0, IP-10 at +7 and IFN-γ on +7 had the highest *AUC* (0.866) for predicting AR in 1 mo (sensitivity 86.8%; specificity 89.8%)
Tefik *et al*[15], 2019	65 (9 rejection, 56 stable)	Plasma	IL-2, IL-8	Rejection	IL-2[b] and IL-8[c] predict AR; IL-2[b] and IL-8[d] levels correlated with ↓ 3 mo eGFR in the AR group
de Holanda *et al*[16], 2018	73	Plasma	sCD30	Rejection; Graft survival	Plasma CD30 at +7, +14 associated w AR (P = 0.036). No difference in 5 yr graft survival

[a]P < 0.001 *vs* histology.
[b]P < 0.05 *vs* non-rejection group.
[c]P < 0.02 *vs* non-rejection group.
[d]P <0.01 *vs* non-rejection group.u: Urinary; C-X-C: C-terminal amino acid sequence Cystine-X-Cystine; CXCL9: C-X-C motif chemokine ligand nine; CXCL10: C-X-C motif chemokine ligand ten; ti: Total inflammation; mvi: Microvascular inflammation; i: Interstitial inflammation; t: Tubulitis; g: Glomerulitis; ptc: Peritubular capillaritis; Cr: Creatinine; TCMR: T cell-mediated rejection: ABMR: Antibody-mediated rejection; AUC: Area under the curve; DSA: Donor specific antibodies; AR: Acute rejection; ACR: Acute cellular rejection; IFN-γ: Interferon gamma; eGFR: Estimated glomerular filtration rate; IP-10: Interferon gamma-induced protein ten; IL-2: Interleukin-2; IL-8: Interleukin-8; CD30: Cluster of differentiation thirty; sCD30: Soluble cluster of differentiation 30.

In their 2016 study of 160 patients, Matz *et al*[17] showed that the expression levels of specific serum microRNAs miR-15B, miR-103A, and miR-106A discriminated patients with stable graft function significantly from patients with TCMR (P = 0.001996, 0.0054 and 0.0019 respectively) and from patients with urinary tract infection (P = 0.0001, < 0.0001 and = 0.0001)[17]. This group expounded on these findings with a later study, where they showed that miR-223-3p, miR-424-3p, and miR-145-5p distinguished TCMR and ABMR from stable graft function as well as identifying miR 145-5P as a distinct marker of interstitial fibrosis/tubular atrophy[18].

The utility of urine-derived free microRNA was demonstrated in a study of 80 KTRs from 2017 where urinary miR-155-5P predicted AR (AUC = 0.875; P = 0.046) with an 85% sensitivity and 86% specificity[19].

In a major study of 519 KTRs utilizing microRNA from allograft biopsies, Halloran *et al*[20] showed that use of a centralized microarray algorithm utilizing microRNA, the Molecular Microscope® Diagnostic System, can not only support histology (agreement between Molecular Microscope® Diagnostic System and histology 77% for TCMR, 77% ABMR, 76% no rejection with blinding to histology) but also is more consistent with clinical judgment (87%) than histology (80%) (P = 0.0042) in regards to select cases n = 451 biopsies)[20].

Ledeganck *et al*[21] provided the most comprehensive analysis of microRNAs in the context of kidney transplants in their recent review. They cited 11 studies whereby microRNA upregulation and downregulation were associated with TCMR, ABMR, and chronic ABMR. Across studies, consistently noted biomarkers include the following: miR-142, miR-155, miR-223 (upregulated) and miR-125, miR-30, miR-204 (downregulated)[21].

In their comprehensive review of novel biomarkers, Jamshaid *et al*[22] reported on a high grade study from 2015 by Lorenzen *et al*[23] examining long noncoding RNAs[22,23]. In their study of 93 KTRs (31 stable controls without rejection, 62 patients with AR, plus 10 samples from the rejection cohort after antirejection treatment), they found that RP11-354P17.15-001 (L328) was associated with acute TCMR (AUC = 0.76, P < 0.001; sensitivity 49%, specificity 95%). Moreover, L328 normalized after successful antirejection treatment. Interestingly, 51/62 patients presented with subclinical

rejection, defined as no change in creatinine *i.e.* L328 was able to detect subclinical rejection[23]. A synopsis of these studies can be found in Table 2.

In summary, free microRNA appears to help discriminate rejection from non-rejection as well as subtypes of AR. Interestingly, these biomarkers were durable despite blinding to histology and consistent with clinical judgment as cited by Halloran *et al*[20] Free microRNA, particularly from allograft biopsy tissue, appears to enhance diagnosis of rejection and can supplement histology[20].

Leukocyte subclasses: The predominance and activity of different subclasses of leukocytes can indicate recipient immune status. Leukocyte populations thus can serve as biomarkers to detect and identify immune aberrancy preceding clinical disease.

One such population is donor-reactive memory B cells (mBCs). Donor-reactive memory B cells are a subset of the B cell pool with emerging data supportive of a robust response to alloantigen post-transplant[24]. In a 2018 study, mBCs were associated with rejection; in 85 KTRs who underwent for-cause biopsies, donor reactive mBCs were found in 100% patients with ABMR and *de novo* DSA. They were also present in 72%-80% of patients with chronic ABMR with and without DSA. In the 90 non-sensitized patients, mBC expansion occurred at a higher rate than de novo DSA and independently predicted ABMR [AUC = 0.917 (95%CI: 0.879-0.956); $P < 0.001$][25].

Donor-specific memory CD4 T cells have also been implicated in rejection. In their study from 2016, Gorbacheva *et al*[26] showed that in a murine model, mice sensitized with memory CD4 cells experienced an acute rise in serum creatinine > 1 mg/dL (1.7 ± 0.6 mg/dL by 6–8 d post-transplant) and developed allograft failure at 7 days. At the time of rejection, the recipient mice had high titers of DSA and increased frequencies of donor–reactive T cells producing IFN-γ compared with controls at matching time points[26].

Through the use of genomics in combination with histologic scoring, Yazdani *et al*[27] were able to derive specific immune cell types and demonstrate that the presence of natural killer (NK) cells are predictive of ABMR (AUC = 0.98, $P < 0.001$); ABMR *vs* TCMR (AUC = 0.91, $P < 0.001$) as well as ABMR histology. They found that 22/24 biopsies with microvascular inflammation (g + ptc) had elevated NK levels (AUC = 0.89, $P < 0.0001$). Moreover, activated NK cells had the best predictive capability of graft failure at 1-2 years compared to other leukocytes (AUC = 0.74). Notably, NK cell infiltration predicted graft failure independent of histologic diagnosis ($P = 0.039$)[27].

In their study from 2017, Cortes-Cerisuelo *et al*[28] found that in 23 KTRs receiving belatacept-based immunosuppression, patients with a higher frequency of cluster of differentiation twenty-eight and cluster of differentiation four T-cells experienced more rejection[28]. Though counterintuitive, the authors postulated that this was related to CD28+ cells exhibiting a pro-inflammatory phenotype relative to CD28-subset. With optimal cutoff determination, they were able to discriminate rejectors from non-rejectors with a sensitivity of 80% and specificity of 100%. Therefore, cluster of differentiation twenty-eight and cluster of differentiation four frequencies can act as a biomarker to determine optimal candidates for belatacept therapy. The studies mentioned above are summarized in Table 3.

In summary, leukocyte subclasses offer unique opportunities as biomarkers in that they (1) offer another vantage point into antigen-antibody dynamics that can occur independently of or preceding detectable donor-specific antibodies (2) highlight the role of less understood pathophysiologic mechanisms (NK cells) and their predictability of graft failure and (3) can potentially provide clinicians with an individualized recipient immune profile to guide management in terms of immunosuppression.

Gene expression profiles: Gene expression profiling (GEP) is an approach within the field of molecular biology whereby thousands of genes are analyzed simultaneously *via* messenger RNA to describe cellular function. Differential expression of genes, particularly those associated with immune cells and interleukins, are some of the earliest events leading to immune dysregulation and poor transplant outcomes. Consequently, these gene expression profiles can yield robust, viable biomarkers. Multiple encouraging profiles have been developed recently as cited below.

In their study of 307 KTRs from Clinical Trials in Organ Transplantation-8, Friedewald *et al*[10] created a rejection biomarker for subclinical acute rejection (sc-AR) based on GEP, which had the following characteristics: [sensitivity 64%, specificity 87%, positive predictive value (PPV) 61%, negative predictive value (NPV) 88%]. Moreover, their GEP biomarker was predictive of persistent subclinical AR[10].

A similar study examining the Genomics of Chronic Renal Allograft Rejection cohort led to the development of the Targeted Expression Assay, which allowed for

Table 2 Summary of micro-ribonucleic acid-related novel biomarker studies associated with immunologic outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Matz *et al*[17], 2016	160	Plasma	miR-15B, miR-103A, miR-106A	TCMR	miR-15B[a,b], miR-103A[a,b] and miR-106A[a,b] discriminated patients with stable graft function from patients with TCMR and UTI
Matz *et al*[18], 2018	111	Plasma	miR-223-3p; miR-424-3p; miR-145-5p; miR-15b-5p	ABMR, TCMR, IFTA	miR-223-3p, miR-424-3p and miR-145-5p distinguished TCMR and ABMR from stable graft function; mir-145-5P decreased in IFTA (AUC 0.891) compared to stable graft function
Millán *et al*[19], 2017	80	Urine	miR-142-3p, miR-210-3p and miR-155-5p, CXCL10	Rejection	↑miR-142-3p, ↑miR-155-5p, ↑CXCL10 + ↓miR-210-3p (AUC = 0.875) and CXCL10 (AUC = 0.865) discriminate rejectors and nonrejectors (sensitivity 85%, 84% and specificity 86% and 80% respectively)
Halloran *et al*[20], 2017	519	Allograft biopsy	Molecular Microscope® Diagnostic System (MMDx™)/microRNA	TCMR, ABMR	Agreement between MMDx™ and histology = 77% for TCMR, 77% for ABMR, and 76% for no rejection with blinding to histology, HLA. MMDx™[c] agreed with clinical judgment (87%) more than histology (80%)
Ledeganck *et al*[21], 2019	11 studies	Allograft biopsy	microRNA	TCMR, ABMR, cABMR	↑miR-142, miR-155, miR-223 and ↓miR-125, miR-30, miR-204 predict TCMR, ABMR, cABMR
Lorenzen *et al*[23], 2015	93	Urine	lcRNA; RP11-354P17.15-001 (L328)	TCMR	RP11-354P17.15-001[d] (L328) was associated with acute TCMR (*AUC* = 0.76) sensitivity 49%, specificity 95%; L328 can detect subclinical TCMR

[a]$P < 0.001$ for TCMR *vs* controls.
[b]$P < 0.001$ for UTI *vs* controls.
[c]$P < 0.005$ *vs* histology.
[d]P <0.001 *vs* controls. miR: Mature form of microribonucleic acid; RNA: Ribonucleic acid; TCMR: T cell-mediated rejection; HLA: Human leukocyte antigen; UTI: Urinary tract infection; ABMR: Antibody-mediated rejection; IFTA: Interstitial fibrosis tubular atrophy; AUC: Area under the curve; C-X-C: C-terminal amino acid sequence Cystine-X-Cystine; CXCL10: C-X-C motif chemokine ligand ten; MMDx™: Molecular Microscope® Diagnostic System; cABMR: Chronic antibody-mediated rejection; lcRNA: Long noncoding RNAs.

the prediction of sc-AR at 3 months in 113 KTRs (AUC = 0.830; NPV = 0.98, PPV = 0.79)[29].

A significant development in gene expression assays in kidney transplantation was the development of the Kidney Solid Organ Response Test. This is a 17 gene set created in 2014 that was found to detect AR accurately. Crespo *et al*[30] expanded on this work with the use of Kidney Solid Organ Response Test plus IFN-γ enzyme-linked immunosorbent spot assay in the Evaluation of Sub-Clinical Acute Rejection Prediction trial of 75 KTRs where they found that in combination, these assays synergistically can predict sc-AR, subclinical T cell-mediated rejection and subclinical antibody-mediated rejection (AUC > 0.85, $P < 0.001$)[30].

One of the most promising gene expression profiles is the TruGraf® Molecular diagnostic test, a non-invasive test to surveil patients with a stable renal function that is now reimbursed by Medicare. This test was first validated in 2014 whereby Kurian *et al*[31] showed that the TruGraf® GEP could distinguish patients with rejection from those with non-rejection dysfunction and excellent allograft function[31].

In 2019, First *et al*[32] expanded on these findings with TruGraf® in their study both retrospectively and prospectively. In their retrospective arm, they found that in the evaluation of 192 patients at 7 transplant centers, in 87.5% of the cases, investigators' clinical decisions were influenced by TruGraf® test results. In the prospective arm of 45 patients at 5 centers, TruGraf® supported 87% of the clinical decisions with 93% of investigators stating they would use TruGraf® in subsequent patient care. In these studies, TruGraf® often led to the non-invasive diagnosis, affirming conservative approaches as well as obviating the need for biopsy[32].

Gene expression profiles can also be derived from urine, as demonstrated in a study from 2019, where a common rejection module of 11 genes was analyzed from 150 KTRs. Interestingly, an accurate prediction from 2 genes (Proteasome 20S Subunit Beta 9, CXCL10) was equivalent to the 11-gene model (sensitivity 93.6%, specificity 97.6%)[33]. Table 4 summarizes these studies.

In summary, gene expression profiles are promising biomarkers in surveilling immune status. As seen by their validation, reimbursement from the Centers for

Table 3 Summary of leukocyte subclass related biomarkers associated with immunologic outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Luque *et al*[25], 2019	175	Plasma	donor reactive memory B cells (mBC)	ABMR	For-cause bx: mBC in 100% ABMR/DSA+ and most cABMR, +/- DSA [24/30 (80%) and 21/29 (72.4%)]. Protocol bx: mBC > dnDSA was observed at 6 and 24 mo (8.8% *vs* 7.7% and 15.5% *vs* 11.1%) and identified pts with ongoing subABMR (AUC = 0.917, 0.809)
Gorbacheva *et al*[26], 2016		Plasma	mCD4	Rejection	Murine models with sensitized mCD4 T cells had SCr > 1 mg/dL (1.7 ± 0.6 mg/dL by 6–8 d post-transplant) and developed graft failure. At rejection, these recipients had DSA and ↑ frequencies of donor–reactive T cells producing IFN-γ compared with controls
Yazdani *et al*[27], 2019	95	Plasma	NK gene expression model -> NK cells	Rejection	NK cells predict ABMR[a] *vs* no rejection (AUC = 0.98); ABMR[b] *vs* TCMR (AUC = 0.91) as well as histology: 22/24 biopsies with mvi (g + ptc) had ↑ NK levels (AUC = 0.89) Moreover, activated NK cells had the best predictive capability of graft failure at 1-2 yr (AUC = 0.74). NK cell infiltration[d] predicted graft failure independent of histology
Cortes-Cerisuelo *et al*[28], 2017	23	Plasma	CD28+CD4+	Rejection	CD28+CD4+ T cell frequency is associated with rejection on belatacept based IS

[a]$P < 0.001$ *vs* controls.
[b]$P < 0.001$ *vs* TCMR. [c]$P < 0.0001$ *vs* biopsies w/o mvi.
[d]$P < 0.05$ *vs* controls. mBC: Donor reactive memory B-cells; ABMR: Antibody-mediated rejection; DSA: Donor specific antibodies; cABMR: Chronic antibody-mediated rejection; bx: Biopsy; dnDSA: *De novo* donor specific antibodies; pts: Patients; subABMR: Subclinical ABMR; AUC: Area under the curve; mCD4: Memory cluster of differentiation four; SCr: Serum creatinine; IFN-γ: Interferon gamma; mvi: Microvascular inflammation; NK: Natural killer; TCMR: T cell-mediated rejection; CD28+CD4+: Cluster of differentiation twenty eight and cluster of differentiation four; IS: Immunosuppression.

Medicare and Medicaid Services, and acceptance among investigators, gene expression profiles are helping to pave the way for broader use of biomarkers in kidney transplantation.

Donor-derived cell-free deoxyribonucleic acid: Allograft transplantation can be considered genome transplantation with grafts having a unique allogenomic signature. At baseline, cell-free deoxyribonucleic acid (DNA) is circulating at low levels. However, in the case of injury, including rejection, increased high levels of cell-free DNA are shed into the bloodstream and are thus measurable as a biomarker. Beck *et al*[34] described quantification and reference values for donor-derived cell-free deoxyribonucleic acid (dd-cfDNA) in their study from 2015[34]. Given this recent quantification, dd-cfDNA is a nascent area of research. Donor-derived cell-free DNA has been shown to predict the decline in estimated glomerular filtration rate (eGFR), de novo donor-specific antibody formation, and biopsy-proven rejection in multiple studies. Three recent studies highlight the utility of dd-dfDNA[35].

In their study of 189 KTRs, Oellerich *et al*[35] found that in patients with biopsy-proven rejection, median dd-cfDNA (cp/mL) was 3.3-fold and median dd-cfDNA (%) 2.0-fold higher than medians in stable patients without rejection. Receiver operating characteristic analysis showed superior performance ($P = 0.02$), of measuring dd-cfDNA (cp/mL) (AUC = 0.83) compared to dd-cfDNA (%) (AUC = 0.73). Diagnostic odds ratios were 7.31 for dd-cfDNA (cp/mL), and 6.02 for dd-cfDNA (%) respectively.

Table 4 Summary of gene expression related biomarkers associated with immunologic outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Friedewald *et al*[10], 2019	308	Plasma	Blood based biomarker/gene expression profile	Subclinical acute rejection	GEP AR biomarker predicted sc-AR (sensitivity 64%, specificity 87%, PPV = 61%, NPV = 88%)
Zhang *et al*[29], 2019	113	Plasma	TREx	Rejection at 3 mo, Graft failure	TREx predicts sc-AR at 3 mo in 113 KTRs (AUC = 0.830; NPV = 0.98, PPV = 0.79)
Crespo *et al*[30], 2017	75	Plasma	kSORT™ + ELISpot	Subclinical rejection	kSORT™ + ELISpot predict sc-AR[a], sc-TCMR[a] and sc-ABMR[a] (AUC > 0.85)
First *et al*[32], 2019	192; 45	Plasma	TruGraf® GEP	Surveillance of patients with stable allograft function	In 87.5% of the cases, investigators' clinical decisions were influenced by TruGraf® results. In 45 patients TruGraf® supported 87% of clinical decisions with 93% of investigators stating they would use TruGraf® in subsequent patient care
Sigdel *et al*[33], 2019	150 KTRs (43 stable, 45 AR, 19 borderline AR, 43 BKVN)	Urine	Common rejection module (11 genes)	Rejection	10/11 genes were elevated in AR when compared to stable graft function. Psmb9 and CXCL10 could classify AR versus stable graft function as accurately as the 11-gene model (sensitivity = 93.6%, specificity = 97.6%); uCRM score differentiate AR from stable graft function (AUC = 0.9886)

[a]$P < 0.001$ *vs* controls. GEP: Gene expression profile; AR: Acute rejection; sc-AR: Subclinical acute rejection; PPV: Positive predictive value; NPV: Negative predictive value; TRex: Targeted expression assay; KTRs: Kidney transplant recipients; kSORT™: Kidney Solid Organ Response Test; ELISpot: Enzyme-linked immune absorbent spot; sc-TCMR: Subclinical T cell-mediated rejection; sc-ABMR: Subclinical antibody-mediated rejection; BKVN: BK virus nephropathy; Psmb9: Proteasome 20S Subunit Beta 9; C-X-C: C-terminal amino acid sequence Cystine-X-Cystine; CXCL10: C-X-C motif chemokine ligand ten; uCRM: Urinary common rejection module.

Remarkably, plasma creatinine showed a low correlation (Pearson $r = 0.37$) with dd-cfDNA (cp/mL)[35].

Stites *et al*[36] in examining 79 KTRs with TCMR 1A/borderline rejection found that forty-two patients had elevated dd-cfDNA compared to thirty-seven patients with low levels; elevated levels of dd-cfDNA predicted adverse clinical outcomes, including eGFR decline by 8.5% *vs* 0% in low dd-cfDNA patients ($P = 0.004$), de novo donor-specific antibody formation was seen in 40% (17/42) *vs* 2.7% ($P < 0.0001$), and future or persistent rejection occurred in 9 of 42 patients (21.4%) *vs* 0% ($P = 0.003$)[36].

One of the most important developments in dd-cfDNA technologies has been targeted next-generation sequencing techniques. These techniques allow for the quantification of dd-cfDNA without the need for the prior donor or recipient genotyping[37].

One of the more well-known assays, Allosure®, has been validated in several studies. Notably, Allosure® is commercially available and reimbursed by Medicare. In the study Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients study (ClinicalTrials.gov Identifier: NCT02424227) from 2017, it was shown to discriminate rejection from controls (AUC = 0.74, $P < 0.0001$; PPV = 61%, NPV = 84%); as well as ABMR from non-ABMR [AUC = 0.87 (95%CI: 0.75-0.97)][38]. Ongoing trials using Allosure® (clinical trials NCT04057742, NCT03326076) are being conducted at various transplant centers throughout the country.

In their comprehensive review on dd-cfDNA, Knight *et al*[39] cited 2 recent studies

(Huang *et al*[40] and Whitlam *et al*[41]) of its application in the context of kidney transplantation[39-41]. In their study of 63 KTRs, Huang *et al*[40] found that dd-cfDNA discriminated patients with ABMR (median 1.35%; interquartile range (IQR): 1.10%-1.90%) compared to those with no rejection (median 0.38% (IQR: 0.26% to 1.10%); $P < 0.001$). dd-cfDNA did not distinguish TCMR from no rejection however. Whitlam *et al*[41] in their study of 61 KTRs, found that dd-cfDNA concentration and fraction were predictive of acute antibody-mediated rejection (aAMR) (AUC = 0.92, 0.85) and composite diagnosis of ABMR (AUC = 0.91, 0.89). Graft derived cell free DNA (gd-cfDNA) exhibited modest sensitivity (0.90; 0.85) and specificity (0.88, 0.79) for aAMR and ABMR[41]. These findings are summarized in Table 5.

Donor-derived cell-free DNA is a robust biomarker in predicting rejection outcomes. Moreover, there is evidence supporting its ability to predict longer-term outcomes. The use of dd-cfDNA as a supportive tool for diagnosis and management is already taking place with the implementation of Allosure® and other similar assays.

Immune tolerance: In addition to identifying immune dysfunction, biomarkers can reflect immune quiescence and tolerance in kidney transplant recipients. While this terminology is vague, Mathew *et al*[42] in their review, define immune tolerance nicely as "long-term allograft survival in the absence of immunosuppressive treatment and the presence of stable donor-specific immune responsiveness[42]." In one review, Chan-on *et al*[43] describe biomarker identification *via* differential expression from a tolerance group (stable graft function or healthy non-transplant volunteers) compared to a dysfunction group (acute or chronic rejection). They cite several potential biomarkers, including T cell, B cell, and macrophage populations, as well as genomic signatures from B and T cells along with microRNA[43]. In a recent review, Newell *et al*[44] describe that in 32 tolerant individuals, 31 genes (26 B cell-specific) distinguished tolerant from non-tolerant KTRs[44]. Two promising genes, cited in prior studies are B cell receptor genes immunoglobulin kappa variable 1D-13 and immunoglobulin kappa variable 4-1[44,45].

While less clear of an outcome than others described previously, immune tolerance is one of the primary aims after kidney transplantation. Having tools to validate and reassure clinicians beyond our current insensitive measures and/or detect early perturbations before overt disease manifests can improve patient care.

Non-immunological

The use of biomarkers to identify and predict transplant outcomes applies to non-immune related outcomes. In the following sections, various biomarkers will be discussed in the context of their non-immune outcomes.

Graft quality: Assessing allograft quality/viability is an essential step in kidney transplantation to appropriately allocate organs and predict future outcomes. With the incidence of ESKD increasing and improved transplant outcomes, the demand for donation continues to grow. Refined preservation techniques have helped to broaden the donor pool, giving way to viable donation with higher risk allografts. This in turn has narrowed the margin of error for prognosticating graft quality. In the past five years, biomarker discovery has emerged to help appraise potential allografts. Several robust studies are described below:

Parikh *et al*[46] described in their study of 671 KTRs that perfusate biomarkers of tissue injury were associated with 6-month allograft function *via* eGFR: Each doubling of perfusate neutrophil gelatinase-associated lipocalin (NGAL) and liver fatty acid-binding protein were independently associated with lower 6-month eGFR (1.7 mL/min per 1.73 m^2 ; 1.48 mL/min per 1.73 m^2 respectively)[46].

Moser *et al*[47] in their study of 41 donor kidneys [16 Live donors, 16 donations after brain death (DBD); 9 donations after circulatory death (DCD)] undergoing machine cold perfusion, compared various tissue injury biomarkers. They found that tissue injury markers matrix metalloproteinase-2, lactate dehydrogenase, and NGAL were found in highest perfusate concentrations in DCD kidneys, followed by DBD and living donor allografts (all $P < 0.0001$)[47].

In their unique study comparing modified adenosine and lidocaine (AL) solution to the University of Wisconsin (UW) solution for organ preservation, Hamaoui *et al*[48] utilized perfusate lactate in addition to histology and perfusion dynamics to help compare viability. They found that in 10 DCD porcine kidneys perfused *via* hypothermic machine perfusion with modified AL solution had significantly lower perfusion lactate levels (3.1 mmol/L *vs* 4.1 mmol/L, $P = 0.04$) during reperfusion than those in UW solution. Of note, on histology, UW solution perfused kidneys had a greater degree of tubular dilatation than modified AL kidneys ($P = 0.03$). This

Table 5 Summary of donor-derived cell-free deoxyribonucleic acid biomarkers associated with immunologic outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Oellerich *et al*[35], 2019	189	Plasma	dd-cfDNA	Rejection	In pts with BPR, dd-cfDNA(cp/mL) was 3.3x and dd-cfDNA(%) 2.0x higher (82 cp/mL; 0.57%) than in stable pts w/o rejection (25 cp/mL; 0.29%). dd-cfDNA abs number > dd-cfDNA % (AUC = 0.73). OR = 7.31 for dd-cfDNA (cp/mL)
Stites *et al*[36], 2020	79 KTRs with TCMR 1A/borderline rejection	Plasma	dd-cfDNA	eGFR, dnDSA, Future rejection	↑dd-cfDNA predict adverse outcomes: Among patients with ↑dd-cfDNA[a], eGFR ↓ by 8.5% *vs* 0% in ↓dd-cfDNA pts. dnDSA seen in 40% (17/42) *vs* 2.7%[b] and future or persistent rejection occurred in 9 of 42 pts[a] (21.4% *vs* 0%)
Bloom *et al*[38], 2017	102	Plasma	dd-cfDNA	Rejection	Distinguished any rejection from non-rejection along with ABMR from non-ABMR
Huang *et al*[40], 2019	63	Plasma	dd-cfDNA	ABMR	dd-cfDNA discriminated ABMR[c] [median 1.35%; interquartile range (IQR): 1.10%-1.90%] from no rejection (median 0.38%, IQR: 0.26%-1.10%). dd-cfDNA did not distinguish TCMR from no rejection
Whitlam *et al*[41], 2019	61	Plasma	dd-cfDNA	aABMR cABMR	gd-cfDNA and fraction were predictive of aAMR (AUC = 0.92, 0.85) and composite dx of ABMR (AUC = 0.91, 0.89). gd-cfDNA w/ modest sensitivity (0.90; 0.85) and specificity (0.88, 0.79) for aAMR and ABMR

[a]$P < 0.005$ *vs* low level dd-cfDNA pts.
[b]$P < 0.0001$ *vs* low level dd-cfDNA pts.
[c]$P < 0.001$ *vs* no rejection. dd-cfDNA: Donor derived-cell free deoxyribonucleic acid; Abs: Absolute; BPR: Biopsy proven rejection; AUC: Area under the curve; OR: Odds ratio; KTRs: Kidney transplant recipients; TCMR: T cell-mediated rejection; eGFR: Estimated glomerular filtration rate; dnDSA: *De novo* donor specific antibodies; ABMR: Antibody-mediated rejection; cABMR: Chronic antibody-mediated rejection; IQR: Interquartile range; dx: Diagnosis; aAMR: Acute antibody-mediated rejection; aABMR: Acute antibody mediated rejection; gd-cfDNA: Graft-derived cell-free DNA; Pts: Patients.

demonstrates a potential application of perfusate lactate to detect ischemia-reperfusion injury[48].

A notable recent study is that of van Smaalen *et al*[49] from 2017. The investigators examined cytotoxic extracellular histones, which have been described as markers of cell injury (as seen in inflammation, thrombosis, sepsis namely) in 390 DCD kidney perfusates and sought to determine if their presence was associated with allograft viability. They found extracellular histone concentration was independently associated with 1-year graft failure [hazard ratio (HR) = 1.386 (95%CI: 1.037-1.853)]. Moreover, they observed that 1-year graft survival was improved for the low extracellular histone group (83% *vs* 71%, $P = 0.008$), which was maintained up to 5 years (76% *vs* 65%, $P = 0.014$)[49].

In their recent study from 2019, Weissenbacher *et al*[50] utilized perfusate allograft injury biomarkers NGAL and kidney injury molecule-1 (KIM-1) in addition to histology, urine output, sodium levels to help quantify allograft viability in the context of normothermic kidney perfusion with urine recirculation. While their study was limited in terms of size (11 allografts), lack of organ transplantation, and differing methods (urine recirculation *vs* not), the highest perfusate NGAL level was found in the lowest quality kidney (Kidney 4). In the perfused kidneys without urine recirculation, NGAL and KIM-1 decreased over time, but as the authors conclude, with such a small sample size, it is difficult to assign any predictive value based on this cohort[50].

In their review from 2020, De Beule *et al*[51] nicely summarize the current status of the allograft viability assessment. They illustrate potential roles for different biomarkers in different perfusion contexts *e.g.*, hypothermic, normothermic machine perfusion[51]. In the context of hypothermic machine perfusion, they, in conjunction with a recent meta-analysis performed by Guzzi *et al*[52] report that glutathione S-transferase and its isoforms alpha- and pi-, a family of detoxification enzymes associated with acute kidney injury and renal injury, have moderate predictive ability for delayed graft function (DGF)[52]. In terms of normothermic machine perfusion, few data exist. However, the authors describe potential roles for NGAL and endothelin-1 based on a trial of 56 discarded human kidneys after 1 h of normothermic machine perfusion. In this study, Hosgood *et al*[53] demonstrated that higher levels of urinary NGAL and endothelin-1 correlated with a higher *i.e.* worse *ex vivo* normothermic

kidney perfusion score[53]. They also note that markers of acid-base homeostasis plus lactate and aspartate aminotransferase as demonstrated in the analysis of porcine perfusate after 8 hours of normothermic machine perfusion correlated with posttransplant allograft function[54]. These studies are summarized in Table 6.

The aforementioned research demonstrates potential roles for biomarkers in adjunct with current scoring systems to help classify organs for appropriate allocation. While more research is needed, glutathione S-transferase as well as markers of tissue injury, namely NGAL, appear to show promise on this front.

Delayed graft function: Delayed graft function is a form of acute kidney injury defined by the need for renal replacement therapy in the first week after transplant. DGF is a significant transplant outcome as it is independently associated with AR and graft failure[55]. It is unknown, if biomarkers able to predict the incidence and duration of DGF early, could change management and improve outcomes.

Remarkably, biomarkers detectable within preservation solution during the peri-transplant period offer diagnostic/prognostic information regarding DGF. We will review several notable studies below:

Parikh *et al*[46] in their study cited previously also found that base NGAL concentration was significantly higher in allografts with DGF (P = 0.004). This was also observed in post values of IL-18 (P = 0.005), and base/post perfusate liver fatty acid-binding protein levels (P = 0.029, 0.006). After multivariate adjustment as well as delta concentration (post minus base) however, these biomarkers did not significantly correlate with DGF development[46]. Similarly, in another study, van den Akker *et al*[56] were able to demonstrate that NGAL at day one could predict DGF *vs* immediate graft function, and also NGAL level at day 1, 4 and 7 correlate with the duration of DGF[56].

Van Smaalen *et al*[49] in their study analyzing extracellular histone levels found that extracellular histone concentration was significantly higher in the DGF group (median 0.70 mg/mL (IQR: 0.43 to 0.98) compared to grafts that functioned immediately [median 0.42 mg/mL (IQR: 0.07 to 0.78); P < 0.001][49]. Curiously, there was no significant difference in extracellular histone concentration in grafts with primary non-function *vs* DGF (P = 0.437).

Van Balkom *et al*[57] showed that in 16 DCD kidneys in their discovery cohort, five perfusate proteins [leptin, granulocyte-macrophage colony-stimulating factor granulocyte-macrophage colony-stimulating factor (GM-CSF), periostin, plasminogen activator inhibitor-1 and osteopontin] out of 158 tested in addition to body mass index and dialysis duration predicted DGF. *Via* multivariate analysis, leptin and GM-CSF were found to be the most predictive. Subsequent validation with 40 kidneys found that leptin, GM-CSF + body mass index generated a highly predictive model of DGF [AUC = 0.89 (95%CI: 0.74-1.00)], which performed better than both kidney donor risk index and DGF risk calculator (AUC = 0.55, 0.59)[57].

In a recent study from 2019, Roest *et al*[58] found that in 8 allografts from both DCD and DBD donors, higher levels of perfusate microRNA mir-505-3p correlated with DGF (OR = 1.12, P = 0.028). This was confirmed in a validation cohort of 40 allografts, of which 20 developed DGF (P = 0.011). Interestingly, this predictive capability held true solely for DCD allografts (P = 0.009)[58].

In addition to perfusate markers, plasma and urine-derived biomarkers have been found to predict and prognosticate DGF. These biomarkers are associated with tissue injury. As described in several studies, both urine and plasma-derived NGAL were predictive of DGF development[59-63]. These were directly compared in the review by Li *et al*[64] In their review of 14 studies (8 evaluating urine NGAL, 6 evaluating plasma NGAL), the composite AUC for 24 hours uNGAL was 0.91 (95%CI: 0.89-0.94) and the overall diagnostic OR for 24 hours uNGAL was 24.17(95%CI: 9.94-58.75) with a sensitivity of 0.88 and a specificity of 0.81. The composite AUC for 24 hours blood neutrophil gelatinase-associated lipocalin was 0.95 (95%CI: 0.93-0.97) with an overall diagnostic OR for 24 hours blood neutrophil gelatinase-associated lipocalin = 43.11 (95%CI: 16.43-113.12) with a sensitivity of 0.91 and a specificity of 0.86.

In another study, Bank *et al*[65], showed that urinary tissue inhibitor of metalloproteinases-2 decrease preceded resumption of allograft function and can predict DGF resolution[65].A unique study of DGF utilized microRNA and found that levels of homo sapiens-mature form of microRNA-217 (hsa-miR-217); hsa-miR-125b along with donor age and type of donation predicted DGF with a sensitivity of 61% and specificity of 91%[66]. The aforementioned comprehensive review from Ledeganck *et al*[21] cites 4 studies where biopsy samples of microRNA correlated with DGF. In these studies, the upregulation of miR-21-3P and miR-182-5p were measurable biomarkers[21]. Table 7 highlights these studies.

Biomarkers appear to be predictive of delayed graft function, as early as the peri-

Table 6 Summary of biomarkers associated with graft quality

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Parikh *et al*[46], 2016	671	Perfusate	NGAL, L-FABP	6 mo eGFR	Each doubling of perfusate NGAL and L-FABP were independently associated with ↓6-month eGFR (1.7mL/min per 1.73m^2; 1.48mL/min per 1.73m^2)
Moser *et al*[47], 2017	41	Perfusate	MMP-2, LDH, NGAL	Biomarker levels	MMP-2[a,b], LDH[a,b], and NGAL[a,b] were found in highest perfusate concentrations in DCD kidneys, followed by DBD and living donor allografts
Hamaoui *et al*[48], 2017	10	Perfusate	Perfusate lactate	Perfusion	10 DCD porcine kidneys perfused *via* HMP with modified AL solution[c] had significantly ↓ perfusion lactate levels (3.1 *vs* 4.1 mmol/L) during reperfusion than those in UW solution
van Smaalen *et al*[49], 2017	390	Perfusate	Extracellular histone concentration	1 yr graft survival	(extracellular histone) was associated w/ 1 year graft failure (HR = 1.386) 1 year graft survival was ↑ for the ↓ extracellular histone group[d] (83% *vs* 71%) , maintained up to 5 years[e] (76% *vs* 65%)
Weissenbacher *et al*[50], 2019	11	Perfusate	NGAL, KIM-1	Kidney quality	↑ perfusate NGAL level was found in the lowest quality kidney. In the perfused kidneys w/o urine recirculation, NGAL and KIM-1 ↓ over time. Small sample size; NGAL/ KIM-1 not predictive of kidney quality
Hosgood *et al*[53], 2017	56	Urine	NGAL, endothelin-1	Kidney quality per EVKP score	↑ levels of NGAL and ET-1 were associated with ↑ EVKP score[f] (P < 0.05)

[a]P < 0.0001 *vs* Donation after brain death kidneys.
[b]P < 0.0001 *vs* living donor kidneys.
[c]P < 0.05 *vs* Deceased cardiac death donor kidneys perfused with University of Wisconsin solution.
[d]P < 0.01 *vs* increased extracellular histone group.
[e]P < 0.05 *vs* increased extracellular histone group.
[f]P < 0.05 *vs* EVKP group A. NGAL: Neutrophil gelatinase-associated lipocalin; L-FABP: Liver fatty acid binding protein; eGFR: Estimated glomerular filtration rate; MMP-2: Matrix metalloproteinase-2 LDH: Lactate dehydrogenase; DBD: Donation after brain death; DCD: Deceased cardiac death donor; HMP: Hypothermic machine perfusion; AL: Adenosine lidocaine; UW: University of Wisconsin; HR: Hazard ratio; KIM-1: Kidney injury molecule-1; EVKP: *Ex vivo* normothermic kidney perfusion; ET-1: Endothelin-1.

transplant period as demonstrated by perfusate markers. Urinary and plasma NGAL, among others, show promise and could augment care by changing management before the development of DGF as well as help prognosticate duration.

Cardiovascular events/mortality: Cardiovascular disease is the leading cause of death post-kidney transplantation[67]. Early detection and prediction of outcomes *via* novel biomarkers is a crucial area of research. Several recent studies have explored biomarker use concerning cardiovascular outcomes. Extensive biomarker research has been conducted using KTRs from the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) cohort[68-70].

Bansal *et al*[69] in 2016 examined 1027 KTRs from this cohort and found that each log increase in urine NGAL/creatinine independently associated with a 24% greater risk of cardiovascular events [adjusted hazard ratio (aHR) = 1.24 (95%CI: 1.06-1.45)], a 40% greater risk of graft failure [aHR = 1.40 (95%CI: 1.16-1.68)], and a 44% greater risk of death [aHR = 1.44 (95%CI: 1.26-1.65)]. Urine KIM-1/creatinine and IL-18/creatinine independently associated with a higher risk of death [aHR = 1.29 (95%CI: 1.03-1.61) and 1.25 (95%CI: 1.04-1.49 per log increase, respectively)][69].

In another study of 1184 KTRs, Park *et al*[70] found that higher urine alpha 1 microglobulin (A1M) (HR per doubling of biomarker = 1.40 (95%CI: 1.21-1.62), monocyte chemoattractant protein-1 (MCP-1) [HR = 1.18 (95%CI: 1.03-1.36)], and procollagen type I intact N-terminal peptide [HR = 1.13 (95%CI: 1.03-1.23)] were associated with cardiovascular events, as well as death (HR per doubling A1M = 1.51 (95%CI: 1.32-1.72); HR per doubling MCP1 = 1.31 (95%CI: 1.13-1.51); *HR* per doubling procollagen type I intact N-terminal peptide = 1.11 (95%CI: 1.03-1.20).

Interestingly, a study published in 2020 showed that soluble cardiac biomarker, a member of the IL-1 receptor family, which is predictive of cardiovascular mortality in patients with heart disease as well as those with chronic kidney disease, is associated with cardiovascular events [aHR = 1.31 (95%CI: 1.00-1.73); P = 0.054] and mortality [aHR = 1.61 (95%CI: 1.07-2.41); P = 0.022] in KTRs[71].

Another novel biomarker implicated in cardiovascular mortality is plasma

Table 7 Summary of biomarkers associated with delayed graft function

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Parikh *et al*[46], 2016	671	Perfusate	NGAL, IL-18, L-FABP	DGF	Base (NGAL) was significantly ↑ in allografts with DGF[a]. This was also observed in post values of IL-18[a] and base/post perfusate L-FABP levels[b]. These biomarkers did not significantly correlate with DGF development on multivariate adjustment
van Smaalen *et al*[49], 2017	390	Perfusate	Extracellular histone concentration	DGF	Extracellular histone concentration was significantly ↑ in the DGF group (median 0.70 μg/mL (IQR 0.4 to 0.98) compared to grafts that functioned immediately[c] (median, 0.42 (IQR 0.07 to 0.78). Interestingly there was no significant difference in extracellular histone concentration in grafts with primary non-function *vs* DGF
van Balkom *et al*[57], 2017	40	Perfusate	Leptin, GM-CSF, periostin, plasminogen activator inhibitor-1, osteopontin	DGF	5 perfusate proteins/158 tested predicted DGF. Leptin and GM-CSF -> most predictive. Validation with 40 kidneys found that leptin, GM-CSF + BMI predict DGF (AUC = 0.89 (95%CI: 0.74 to 1.00), which performed better than KDRI and DGF risk calculator (AUC 0.55, 0.59)
Roest *et al*[58], 2019	48	Perfusate	microRNA mir-505-3p	DGF	In 8 DCD and DBD donors, ↑ levels of perfusate microRNA mir-505-3p correlated with DGF[b] (OR 1.12). This was confirmed *via* validation of 40 allografts, of which 20 developed DGF[b]. Interestingly, this predictive capability held true solely for DCD allografts[c]
Truche *et al*[59], 2019	41	Urine and Plasma	uNGAL, uNAG, LDH, UCr	DGF	DGF -UNGAL, UNAG AUC 1, 0.96 (0.84-1.0) , urinary tubular injury biomarker-to-creatinine ratio, and LDH AUC = 1 and 0.92 (95%CI: 0.73 to 1.0)
Pianta *et al*[60], 2015	81	Urine	Urinary clusterin, IL-18, KIM-1, NGAL	DGF	Urinary clusterin predicted DGF at 4 h (AUC = 0.72 (95%CI: 0.57 to 0.97), as did IL-18 , KIM-1 and NGAL; eGFR at 90 d was inversely correlated with urinary clusterin at 12 h[b] (Pearson $r = -0.26$, and 7 d[b] (Pearson $r = -0.25$)
Reese *et al*[61], 2016	1304	Urine	Microalbumin, NGAL, KIM-1, IL-18, L-FABP	AKI, DGF, 6-month eGFR	Microalbumin, NGAL, KIM-1, IL-18, L-FABP from deceased donors at procurement; predictive of AKI; NGAL associated with DGF (RR = 1.21 (95%CI: 1.02 to 1.43), NGAL and L-FABP associated with lower 6 mo eGFR
Nielsen *et al*[62], 2019	225	Plasma and urine	pNGAL, uNGAL uL-FABP, urine cystatin C, urine YLK-40	DGF, 1 yr mGFR/eGFR	pNGAL 1 d after tx -> associated with DGF. Did not correlate to 12-mo eGFR; no relation w L-FABP, cystatin C, and YLK-40
Koo *et al*[63], 2016	94	Urine	Microalbumin, NGAL, KIM-1, IL-18, L-FABP	DGF, 1 yr graft function	NGAL predicts AKI; NGAL + L-FABP predicts DGF (*AUC* 0.758, 0.704); NGAL + L-FABP + Cr better than DGF calculator and KDPI. L-FABP predictive of 1 yr graft function[b]
Li *et al*[64], 2019	1036	Urine and plasma	uNGAL, pNGAL	DGF	Composite AUC for 24 hours uNGAL was 0.91 (95%CI: 0.89 to 0.94) and the overall DOR for 24 hours uNGAL was 24.17; sensitivity 0.88, specificity 0.81. The composite AUC for 24 hours pNGAL was 0.95 (95%CI: 0.93 to 0.97) with an overall DOR for 24 hours pNGAL = 43.11 with sensitivity 0.91 and specificity 0.86
Bank *et al*[65], 2019	74 (DCD KTRs)	Urine	Urinary TIMP-2	DGF	TIMP-2/mOsm on day-1 and day-10 identified patients with DGF (AUC = 0.91) and prolonged DGF (*AUC* = 0.80); Consecutive TIMP-2/mOsm values showed a ↓ in TIMP-2/mOsm before an ↑estimated glomerular filtration rate, predicting resolution of fDGF

McGuinness *et al*[66], 2016	94		hsa-miR-217; hsa-miR-125b	DGF	miRNA + donor age + type donation predicted DGF in 83% of cases (61% sensitivity, 91% specificity)
Ledeganck *et al*[21], 2019	11 studies	Allograft biopsy	microRNA	DGF	Upregulation of miR-21-3P and miR-182-5p associated with DGF

[a]$P < 0.005$ *vs* non-DGF allografts.

[b]$P < 0.05$ *vs* non-DGF allografts.

[c]$P < 0.001$ *vs* immediately functioning grafts. NGAL: Neutrophil gelatinase-associated lipocalin; IL-18: Interleukin eighteen; L-FABP: Liver fatty acid binding protein; DGF: Delayed graft function; IQR: Interquartile range; GM-CSF: Granulocyte-macrophage colony-stimulating factor; BMI: Body mass index; AUC: Area under the curve; KDRI: Kidney donor risk index; RNA: Ribonucleic acid; mir: Pre-microRNA; DCD: Deceased cardiac death donor; DBD: Deceased brain death donor; OR: Odds ratio; u: Urinary; uNGAL: Urinary neutrophil gelatinase-associated lipocalin; uNAG: Urinary N-acetyl-β-glucosaminidase; LDH: Lactate dehydrogenase; UCr: Urine creatinine; KIM-1: Kidney injury molecule-1; CI: Confidence interval; eGFR: Estimated glomerular filtration rate; RR: Relative risk; pNGAL: Plasma neutrophil gelatinase-associated lipocalin; YLK-40: Chitinase-3-like protein mGFR: Measured glomerular filtration rate; KDPI: Kidney donor profile index; DOR: Diagnostic odds ratio; TIMP-2: Tissue inhibitor of metalloproteinases 2; mOsm: Milliosmoles; fDGF: Functional delayed graft function; hsa: Homo sapiens; miR: Mature form of microRNA.

malondialdehyde (MDA), as described in their study published in 2020. In this study, they showed that plasma MDA concentration was significantly associated with the risk for cardiovascular mortality after adjustment for potential confounders, including renal function, immunosuppressive therapy, smoking status, and blood pressure. This association was stronger in KTRs with decreased allograft function [eGFR ≤ 45 mL/min/1.73 m^2; HR = 2.09 (95%CI: 1.45-3.00) per 1-standard deviation increment)][72]. The findings of these studies are summarized in Table 8.

In summary, multiple biomarkers show promise in predicting cardiovascular events and mortality. Analysis of the FAVORIT cohort and others with urinary biomarkers provides some of the most robust data in favor of biomarker use to supplement current standards of care. However, more unique biomarkers utilized in cardiovascular trials, namely cardiac biomarker, as well as other unique markers of inflammation, while needing more research, may also help to prognosticate cardiovascular outcomes.

Infection: Infections, both with common pathogens or opportunistic infections, are commonplace post-transplant due to induction and maintenance immunosuppression. Infection is a crucial outcome, as it is the second leading cause of death for KTRs[67]. Interestingly, novel biomarkers may help to stratify risk after transplant.

Plasma soluble cluster of differentiation 30 at baseline and at 1 mo were demonstrated in a study of 100 KTRs to predict bacterial infection [AUC = 0.633 (95%CI: 0.501-0.765); AUC = 0.846 (95%CI: 0.726-0.966)][73]. Similarly, Sadeghi *et al*[74] demonstrated that patients with post-transplant cytomegalovirus (CMV) were found to have higher levels of IL-23 (8.6 ± 4.4 *vs* 8.0 ± 17; $P = 0.025$) and IL-23/Cr ratios ($P = 0.040$) than patients without CMV disease after transplantation. Moreover, they showed that pre-transplant IL-23 > 7 pg/mL increases the risk for post-transplant CMV [relative risk = 4.50 (95%CI: 1.23 to 16.52); $P = 0.023$][74].

Genetic polymorphisms that modify recipient infection risk can be used as biomarkers. This was demonstrated in a study of 189 KTRs where a genetic polymorphism in the Nuclear Factor kappa-light-chain-enhancer of activated B cells-

Table 8 Summary of biomarkers associated with cardiovascular events and cardiovascular mortality

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Foster *et al*[68], 2017	508	Urine and plasma	Cystatin C, B2M, Cr	CV events, Mortality, Kidney failure	HR $eGFR_{cys}$ and HR $eGFR_{B2M}$ < 30 *vs* 60+ were 2.02[a] (95%CI: 1.09 to 3.76) and 2.56[b] (95%CI: 1.35 to 4.88) for CV events; 3.92[c] (95%CI: 2.11 to 7.31) and 4.09[b] (95%CI: 2.21 to 7.54) for mortality; and 9.49[c] (95%CI: 4.28 to 21.00) and 15.53[b] (95%CI 6.99 to 34.51) for kidney failure
Bansal *et al*[69], 2016	1027	Urine	uNGAL, uKIM-1, IL-18, L-FABP, UCr	CV events, Graft failure, mortality	Each ↑ log in uNGAL/Cr associated with a 24% ↑ risk of CV events (aHR = 1.24 (95%CI: 1.06 to 1.45), graft failure (1.40; 1.16 to 1.68), and risk of death (1.44; 1.26 to 1.65). uKIM-1/Cr and IL-18/Cr associated with higher risk of death (1.29; 1.03 to 1.61 and 1.25; 1.04 to 1.49 per log increase)
Park *et al*[70], 2017	1184 (300 CVD, 371 death, 513 random sub-cohort)	Urine	urine alpha 1 microglobulin [A1M], monocyte chemoattractant protein-1 [MCP-1], procollagen type I [PINP] and type III [PIIINP] N-terminal amino peptide)	CV events, Death	↑uA1M (HR per doubling of biomarker = 1.40 (95%CI: 1.21 to 1.62), MCP-1 [HR 1.18 (1.03 to 1.36)], and PINP [HR = 1.13 (1.03 to 1.23)]were associated with CVD events and death (HR per doubling α1m = 1.51 (95%CI: 1.32 to 1.72); MCP-1 = 1.31 (1.13 to 1.51); PINP = 1.11 (1.03 to 1.20)
Devine *et al*[71], 2020	367	Plasma	ST2	CV events, CV mortality, All-cause mortality	↑ ST2 was associated with CV events (aHR = 1.31 (95% CI: 1.00 to 1.73); significantly for CV mortality[d] (aHR = 1.61; (95%CI: 1.07 to 2.41; *P* = 0.022), The addition of ST2, to risk prediction models for CV mortality/events failed to improve their predictive accuracy
Yepes- Calderón *et al*[72], 2020	604	Plasma	Malondialdehyde	CV mortality	During a follow-up period, 110 KTRs died, with 40% CV death. MDA was significantly associated with the risk for CV mortality. The association between MDA concentration and the risk for CV mortality was stronger in KTRs with ↓ eGFR [HR 2.09 (95%CI: 1.45-3.00) per 1-SD increment]

[a]$P < 0.05$ *vs* $eGFR_{cys} > 60$.
[b]$P < 0.005$ *vs* $eGFR_{B2M} > 60$.
[c]$P < 0.005$ *vs* $eGFR_{cys} > 60$.
[d]$P < 0.05$ *vs* low ST2 group. B2M: Beta-2-microglobulin; Cr: Creatinine; CV: Cardiovascular; HR: Hazard ratio; eGFR: Estimated glomerular filtration rate; $eGFR_{cys}$: Estimated glomerular filtration rate based on cysteine; $eGFR_{B2M}$: Estimated glomerular filtration rate based on beta-2-microglobulin; uNGAL: Urinary neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule 1; IL-18: Interleukin eighteen; L-FABP: Liver fatty acid binding protein; UCr: Urine creatinine; aHR: Adjusted hazard ratio; A1M: Alpha 1 microglobulin; MCP-1: Monocyte chemoattractant protein-1; PINP: Procollagen type I intact N-terminal peptide; PIIINP: Procollagen type III intact N-terminal peptide; ST2: Cardiac biomarker; MDA: Malondialdehyde; SD: Standard deviation.

94ins/delATTG increased the risk of CMV infection; survival free from CMV infection was 54.7% for ins/ins group and 79.4% for deletion carriers one year after transplantation ($P < 0.0001$)[75]. Table 9 highlights the conclusions of these studies.

An important infection in KTRs is BK polyomavirus (BK). BK virus is a double-stranded DNA virus commonly observed in the general population as a commensal organism that can cause disease including ureteral stenosis, allograft nephropathy, and graft loss in kidney allograft recipients[76]. Several studies within the past 5 years have demonstrated the utility of novel biomarkers in identifying BK virus nephropathy (BKVN).

Kim *et al*[77] showed in their cross-sectional study from 385 KTRs that the presence of elevated BK urinary microRNAs bkv-miR-B1-5p and bkv-miR-B1-3p in KTRs with

Table 9 Summary of biomarkers associated with infectious outcomes

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Fernández-Ruiz *et al*[73], 2017	100	Plasma	sCD30	Bacterial infection	sCD30 correlates to bacterial infection at baseline[a] and 1 mo[a], 3 mo[a], and 6 mo[a] after KT. Patients with sCD30 ≥ 13.5 ng/mL had lower 12-mo bacterial infection-free survival[b] (35.0% *vs* 80.0%) Baseline sCD30 levels ≥ 13.5 ng/mL is a risk factor for infection[c] (HR: 4.65; 2.05-10.53)
Sadeghi *et al*[74], 2016	70	Plasma	IL-23	CMV infection	Patients with post-KT CMV disease (n = 13; 150 ± 106 d post-KT range 41–363 d) had higher pre-KT IL-23[d] (8.6 ± 4.4 *vs* 8.0 ± 17) and IL-23/Cr ratios[d] than patients w/o CMV disease post-KT (n = 57). Pre-KT IL-23 plasma level of > 7 pg/mL is a risk factor for post-KT CMV infection/reactivation and symptomatic infection[e] (RR = 4.50, 95%CI: 1.23 to 16.52) *ROC* curve analysis post-KT CMV disease showed a sensitivity of 69% and a specificity of 67%
Leone *et al*[75], 2019	189	Plasma	94ins/delE37delATTG NFKB1 polymorphism	CMV infection	65% of CMV infections occurred in ins/ins group. Survival free from CMV was 54.7% for ins/ins group and 79.4% for del carriers one-year post-KT. A multivariate regression for del carriers showed a ↓ risk of CMV infection[f] and recurrence for ins/ins KTRs[g] (*HR* = 0.224, 0.307)
Kim *et al*[77], 2017	385	Urine	Urine microRNA bkv-miR-B1-5p and bkv-miR-B1-3p	BKVN	↑ bkv-miR-B1-5p and bkv-miR-B1-3p in KTRs w biopsy proven BKVN distinguished them from disease free recipients (AUC = 0.989, 0.985). Only 13 KTRs with BKVN
Abend *et al*[78], 2017	116	Plasma	Donor BK virus antibody, recipient BK virus antibody	Post-transplant BK viremia	Donor BK virus antibody seropositivity correlated to post-transplant BK viremia[h] (OR = 5.0; 95%CI: 1.9-12.7). The authors did not examine for BKVN however
Ho *et al*[79], 2018	107	Urine	CXCL10	BKVN	↑CXCL10 correlated with t+i[i] (uCXCL10/creatinine, 1.23 ng/mmol *vs* 0.46 ng/mmol; *AUC* = 0.69) and mvi, specifically ptc[i] (uCXCL10/creatinine, 1.72 ng/mmol *vs* 0.46 ng/mmol; AUC = 0.69) compared to normal histology. Urinary CXCL10[i] corresponded with BKV, but not CMV viremia. These urine CXCL10 findings were confirmed in the independent validation set

[a]P < 0.05 *vs* kidney transplant recipients without bacterial infection.
[b]P < 0.0001 *vs* kidney transplant recipients with sCD30 < 13.5 ng/mL.
[c]P < 0.001 *vs* kidney transplant recipients with sCD30 < 13.5 ng/mL.
[d]P < 0.05 *vs* kidney transplant recipients w/o CMV disease.
[e]P < 0.05 *vs* kidney transplant recipients with pre-Tx IL 23 < 7 pg/mL.
[f]P < 0.005 *vs* ins/ins carriers.
[g]P < 0.05 *vs* del carriers.
[h]P < 0.0001 *vs* seronegative BK virus antibody donors.
[i]P < *0.05 vs* low CXCL10 KTRs. sCD30: Soluble cluster of differentiation 30; KT: Kidney transplant; HR: Hazard ratio; IL-23: Interleukin twenty three; CMV: Cytomegalovirus; RR: Relative risk; Cr: Creatinine; ROC: Receiver operating characteristic; ins: Insertion; del: Deletion; NFKB1: Nuclear Factor kappa-light-chain-enhancer of activated B cells; KTRs: Kidney transplant recipients; bkv: BK viral; RNA: Ribonucleic acid; miR: Mature form of micro RNA; BKVN: BK virus nephropathy; AUC: Area under the curve; OR: Odds ratio; CI: Confidence interval; C-X-C: C-terminal amino acid sequence Cystine-X-Cystine; CXCL10: C-X-C motif chemokine ligand ten; t: Tubulitis; i: Interstitial inflammation; mvi: Microvascular inflammation; ptc: Peritubular capillaritis.

biopsy-proven BKVN were able to significantly distinguish them from recipients without the disease (AUC = 0.989, 0.985)[77]. While promising, the study was small with only 13 KTRs with BKVN.

Due to its ubiquity in the general population, the determination of the serostatus of the BK virus between donors and recipients is not standard. However, as shown by Abend *et al*[78] in their study of 116 deceased donor kidney transplant recipients, they found that donor BK virus antibody seropositivity correlated to post-transplant BK

viremia (OR = 5.0 (95%CI: 1.9 to 12.7); P = 0.0001)[78]. The authors did not examine for BKVN however.

Serum and urine levels of CXCL10, have been demonstrated as a novel biomarkers in the context of rejection, as stated previously. In their recent study, Ho *et al*[79] demonstrated a further application for CXCL10 in terms of early BKVN. The authors observed elevated urine levels of CXCL10 in patients with subclinical BKVN. Elevated urinary CXCL10 occurred in the context of tubulointerstitial inflammation, peritubular capillaritis and BK viremia (all P < 0.05) They hypothesize that this could be due to either sampling error *vs* early disease preceding histologic phenomena whereby tubulointerstitial inflammation is only identifiable on a molecular level[79].

Upon its emergence in December 2019, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) coronavirus, also known as coronavirus disease (COVID19), has been one of the most impactful pandemics in recent history. Given the high virulence and high transmissibility of SARS-CoV-2 coronavirus, much research has gone into diagnosing and prognosticating coronavirus disease. One such biomarker reported in both KTR and non-KTR literature is IL-6. Ahmadpoor *et al*[80] postulate key mechanisms for COVID 19 infection, noting that when an adaptive immune response is blunted, particularly in populations with low naïve T cells including KTRs, innate-immune mediated inflammation can persist and lead to cytokine storm and severe illness[80]. They refer to the study by Velazquez-Salinas *et al*[81] who described the role of IL-6 in animal and human viral infections (vesicular stomatitis virus, influenza pneumonia, hepatitis B, lymphocyte choriomeningitis virus namely), noting that IL-6 can lead to T-cell inhibition and mitigate cell-mediated antiviral responses potentiating this effect[81]. In light of this, IL-6 is being used as a biomarker and therapeutic target. In their case report describing a patient recovering from COVID19 pneumonia, Lauterio *et al*[82] illustrate the use of IL-6 as a biomarker and therapeutic target *via* the monoclonal antibody tocilizumab[82]. Currently, investigators in Italy are recruiting subjects in clinical trial NCT04317092, TOCIVID-19, examining the efficacy of tocilizumab therapy.

While a smaller area of study, biomarker use to predict infection is an emerging one, particularly in the context of newly surfacing disease *e.g.* COVID19. This could augment current biomarker research as learning about immune-related changes in the context of infection/infection risk will likely bolster our understanding of the immune system and have broad-ranging applications to immune responses after transplantation.

Malignancy: Malignancy is a common complication of kidney transplantation, likely related to the widespread immunologic changes related to induction/maintenance immunosuppression. The development of malignancy after transplant is a crucial outcome as it is the third leading cause of death for KTRs[67]. Biomarkers offer an opportunity for surveillance and prognostication before the development of the evident disease.

Hope *et al*[83] in their study of 82 KTRs (56 with known malignancy, 26 without) found that weak NK cell activity, derived from lactate dehydrogenase and interferon-gamma quantification using reactive T-cell enzyme-linked immunospot, was associated with metastatic cancer, cancer-related death, or septic death [HR = 2.1 (95%CI: 0.97 to 5.00)][83].

IL-27 was shown to discriminate patients with post-transplant neoplasia *vs* KTRs without cancer with a sensitivity of 81% specificity of 80% in a recent study[84].

In their study from 2019, Garnier *et al*[85] examined the pretransplant populations of cluster of differentiation forty five isoform with alternative mRNA splicing of exon (CD45RC) T cells in 89 KTRs. CD45RC expression dictates either a more regulatory (low expression) phenotype or pro-inflammatory (high expression) phenotype. Intriguingly, they found that differences in these populations predicted opposing outcomes: KTRs with a low CD4+CD45RC high population (< 51.9%) carried a 3.7 fold risk of cancer [HR = 3.71 (95%CI: 1.24 to 11.1); P = 0.019] *vs* the high CD4+CD45 high population having a 20-fold higher risk of rejection [HR = 21.7 (95%CI: 2.67 to 176.2); P = 0.0004][85]. The results of these studies are illustrated in Table 10.

While the literature on biomarker predicting malignancy after transplant is limited, these studies provide some interesting insights on immunoregulation and various adverse outcomes. While age-appropriate cancer screening, dermatology follow-up, and appropriate precautions are key tenets of post-transplant care, perhaps adjunctive testing conveying malignancy risk can reiterate their importance to clinicians and patients alike.

Post-transplant diabetes: Post-transplant diabetes mellitus (PTDM) is an adverse

Table 10 Summary of biomarkers associated with post-transplant malignancy

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Hope *et al*[83], 2015	82 (56 KTRs +malignancy, 26 KTRs - malignancy)	Plasma	LDH; IFN-γ; ELISpot	Post- transplant malignancy	Low NK cell function -> HR 2.1 (0.97-5.00) metastatic Ca, Ca-related death, septic death
Pontrelli *et al*[84], 2019	156: 93 KTRs, 34 controls + malignancy, 29 healthy subjects	Plasma	IL-27	Post-transplant malignancy	IL-27 plasma levels were able to discriminate patients with post-transplant neoplasia with a specificity of 80% and a sensitivity of 81%
Garnier *et al*[85], 2019	89	Plasma	CD4+CD45RC	Post-transplant malignancy	KTRs with a low CD4+CD45RChigh population (< 51.9%) carried a 3.7 fold risk of cancer[a] (HR = 3.71 (95%CI: 1.24 to 11.1), CD4+CD45high population having a 20-fold higher risk of rejection[b] (HR = 21.7 (95%CI: 2.67-176.2)

[a]$P < 0.05$ *vs* kidney transplant recipients with a high CD4+CD45R population.
[b]$P < 0.001$ *vs* Kidney transplant recipients with a low CD4+CD45R population. KTR: Kidney transplant recipient; LDH: Lactate dehydrogenase; IFN-γ: Interferon gamma; ELISpot: Enzyme-linked immunosorbent spot assay; NK: Natural killer; HR: Hazard ratio; Ca: Cancer; IL-27: Interleukin twenty seven; CD4+CD45RC: CD45RC – cluster of differentiation four + forty five isoform with alternative mRNA splicing of exon 6; CI: Confidence interval.

outcome after kidney transplantation, stemming from shared disease processes leading to ESKD along with diabetogenic conditions, including immunosuppression and inflammation. PTDM is an important outcome due to decreased allograft and patient survival[86]. Biomarkers have been studied to predict the development of this condition.

In one study, Heldal *et al*[87] studied 20 plasma biomarkers in 852 KTRs and found 6/20 significantly associated with the development of PTDM[87].

Similar to their prior work examining MDA in the context of cardiovascular outcomes, Yepes-Calderon *et al*[88] found that in Cox proportional-hazards regression analyses, MDA was inversely associated with PTDM, independent of immunosuppressive therapy, transplant-specific covariates, lifestyle, inflammation, and metabolism parameters [HR = 0.55 (95%CI: 0.36 to 0.83 per 1- standard deviation increase); $P < 0.01$][88]. The results of these studies are illustrated in Table 11.

Diabetes after transplant is a novel area of research in terms of predictive biomarkers. A need for more sensitive assays besides our current testing is needed to help change management and prevent/treat this disease. As demonstrated by the work from Yepes-Calderón *et al*[88], there is overlap with certain biomarkers and pathways in terms of cardiovascular health, diabetes, inflammation and thus more research in this realm will likely have larger implications in post-transplant disease processes.

Graft survival: With the goal of kidney transplant being to restore kidney function for a recipient's lifespan, graft survival is critical. Unfortunately, transplantation, in most cases, is a form of renal replacement therapy, as allograft failure often precedes death. Novel biomarkers provide a non-invasive strategy to help prognosticate allograft survival.

Several recent studies on novel biomarker use address graft survival[16,63,68,69,89-91]. In their examination of the FAVORIT cohort, Ix *et al*[90] found that in 748 KTRs, urinary injury markers A1M and MCP-1 unadjusted [HR per doubling = 1.73 (95%CI: 1.43 to 2.08); HR per doubling = 1.60 (95%CI: 1.32 to 1.93)] and adjusted [aHR per doubling = 1.76 (95%CI: 1.27 to 2.44)]; aHR per doubling = 1.49 (95%CI: 1.17 to 1.89) were associated with allograft failure[90]. Similarly, Foster *et al*[68] found that in 508 KTRs from the FAVORIT cohort after multivariable adjustment, hazard ratios for eGFR measured by cystatin C and eGFR measured by beta-2-microglobulin < 30 *vs* 60+ were 9.49 (95CI: 4.28 to 21.00) and 15.53 (95%CI: 6.99 to 34.51; both $P < 0.001$) for kidney failure in stable kidney transplant recipients[68].

O'Connell *et al*[89] found that a 13-gene gene expression profile set predicted graft loss in their study of 204 KTRs at 2 (AUC = 0.842) and 3 years (AUC = 0.844), findings that were validated in 2 public data sets[89].

In their study published in 2018, Heylen *et al*[92] showed that ischemia during kidney transplantation leads to DNA hypermethylation, which is a long-lasting effect seen at 1-year post-transplantation and is associated with interstitial fibrosis ($P < 0.001$), vascular intima thickening ($P = 0.003$) and glomerulosclerosis ($P < 0.001$) on the 1-year protocol-specified biopsies[92].

A unique study from 2019 showed that in 133 KTRs, the higher absolute number of

Table 11 Summary of biomarkers associated with post-transplant diabetes mellitus

Ref.	*n*	Sample	Biomarkers	Outcome	Study conclusion
Heldal *et al*[87], 2018	852	Plasma	20 biomarkers	PTDM	6/20 biomarkers associated with PTDM; significant include soluble TNF type 1[a] Pentraxin 3[a] macrophage migration inhibitory factor[a] and endothelial protein C receptor[b]
Yepes-Calderón *et al*[88], 2019	516	Plasma	Malondialdehyde	PTDM	MDA was inversely associated with PTDM, independent of immunosuppressive therapy, transplant-specific covariates, lifestyle, inflammation, and metabolism parameters[a] (HR, 0.55; 95%CI, 0.36-0.83 per 1-SD increase)

[a]$P < 0.05$ *vs* kidney transplant recipients without Post transplant diabetes mellitus.
[b]$P < 0.005$ *vs* kidney transplant recipients without Post transplant diabetes mellitus. PTDM: Post transplant diabetes mellitus; TNF: Tumor necrosis factor; MDA: Malondialdehyde; HR: Hazard ratio; SD: Standard deviation; CI: Confidence interval.

Treg cells 1 year after transplantation was significantly associated with improved 5-year survival (92.5% *vs* 81.4%, Log-rank $P = 0.030$). This finding was preserved after multivariate Cox regression analysis [hazard ratio for death-censored graft loss = 0.961 (95%CI: 0.924 to 0.998); $P = 0.041$], irrespective of 1-year proteinuria, and renal function[93].

Patient survival: In combination with graft survival, patient survival is one of (if not) the primary outcome(s) for kidney transplantation. Multiple studies specifically examined this in terms of cardiovascular mortality, as was mentioned previously[68-70].

One notable study utilizing 2 prospective biomarkers related to the lectin complement pathway, collectin liver-1 and collectin kidney-1 identified the following: High collectin liver-1 and collectin kidney-1 Levels at the time of transplantation were significantly associated with overall mortality in multivariate Cox analyses [HR = 1.50 (95%CI: 1.09-2.07); $P = 0.013$] and [HR = 1.43 (95%CI: 1.02-1.99); $P = 0.038$][91]. The cited studies on patient and graft survival are summarized in Table 12.

Graft and patient survival are the 2 major outcomes of interest after kidney transplantation. As previously stated, transplant across ranging allograft quality and donor/recipient characteristics is the optimal renal replacement strategy for survival. Even after the first year post-transplant, survival for KTRs is inferior to patients without ESKD. Narrowing this gap is a primary objective in transplantation. Perhaps with biomarker prediction/prognostication early (even as soon as hours after transplantation), more aggressive strategies can be undertaken to improve graft and patient survival. Moreover, they can complement current prognostication tools to help communicate impending poor outcomes with patients and prepare patients for next steps albeit graft failure and/or mortality.

FUTURE POTENTIAL BIOMARKERS

In our search, we queried a few particularly unique biomarkers/applications. In this section, we will briefly mention these findings.

In their proteomics study, Moser *et al*[47] described interesting findings in terms of alpha-one-antitrypsin levels across different deceased donor kidneys. They note that in a model of cardiac ischemia, alpha-one-antitrypsin was associated with anti-inflammatory and myocardium protection. As alpha-one antitrypsin is a clinically available therapeutic [Aralast™ (Baxter, United States), Zemeria® (CSL Behring, United States), future studies of either animal models or human subjects could be conducted[47].

In their review, De Beule *et al*[51] postulated a potential biomarker role for flavin mononucleotide (FMN), a subunit of mitochondrial complex I. This molecule has been demonstrated in porcine kidney transplant models and human liver graft perfusion, as markers of mitochondrial, early allograft dysfunction and loss. This has not been studied in the context of human kidney transplantation[51].

DNA hypermethylation in the context of biomarker use in our search was a relatively unique approach, and showed promise, as mentioned earlier[92]. In a recently published review, Yang *et al*[94] combined multiple biomarker modalities, including urine chemokine CXCL10, clusterin, cell free deoxyribonucleic acid, methylated cell free deoxyribonucleic acid, urine protein, and urine creatinine into a comprehensive score, the Q score. In their evaluation of 601 KTRs, they were able to distinguish stable allograft function [median score = 13.1 (95%CI: 8.8-17.9] from AR [median score = 45.2

Table 12 Summary of biomarkers associated with graft survival and/or patient survival

Ref.	*n*	Sample	Biomarkers	Dysfunction	Study conclusion
de Holanda *et al*[16], 2018	73	Plasma	sCD30	Rejection; Graft survival	sCD30 at +7, +14 associated with AR[a]. No difference in 5 yr graft survival
Koo *et al*[63], 2016	94	Urine	microalbumin, NGAL, KIM-1, IL-18, L-FABP	DGF, slow graft function , 1 yr graft function	NGAL predicts AKI; NGAL + L-FABP predicts DGF, slow graft function (AUC 0.758, 0.704); NGAL + L-FABP + Cr better than DGF calculator and KDPI. L-FABP predictive of 1 yr graft function[b]
Foster *et al*[68], 2017	508	Urine and plasma	Cystatin C, B2M, Cr	CV events, Mortality, Kidney failure	HR $eGFR_{cys}$ and HR $eGFR_{B2M}$ < 30 *vs* 60+ were 2.02[c] (1.09-3.76) and 2.56[d] (1.35-4.88) for CV events; 3.92[c] (2.11-7.31) and 4.09[d] (2.21-7.54) for mortality; and 9.49[e] (4.28-21.00) and 15.53[d] (6.99-34.51) for kidney failure
Bansal *et al*[69], 2016	1027	Urine	uNGAL, KIM-1, IL-18, L-FABP, Ucr	CV events, Graft failure, mortality	Each ↑ log in uNGAL/Cr associated with a 24% ↑ risk of CV events (aHR 1.24; 1.06 to 1.45), graft failure (1.40; 1.16 to 1.68), and risk of death (1.44; 1.26 to 1.65). uKIM-1/Cr and IL-18/Cr associated with higher risk of death (1.29; 1.03 to 1.61 and 1.25;1.04 to 1.49 per log increase)
O'Connell *et al*[89], 2016	204	Biopsy	Gene set of 13 genes	IFTA, Graft loss at 2/3 yr	Gene set prediction > clinicopathologic variables (AUC 0.967 > AUC 0.706, AUC 0.806) for IFTA; predicted graft loss at 2 and 3 years (AUC 0.842, 0.844), validated in 2 public datasets
Ix *et al*[90], 2017	748	Urine	Urine A1M, MCP-1, procollagen type III and type I amino-terminal amino pro-peptide	Graft failure	In adjusted models, ↑ concentrations of urine A1M (HR per doubling, 1.73; 1.43-2.08) and MCP-1 (HR per doubling, 1.60; 1.32-1.93) were associated with allograft failure. With the adjustment, urine A1M (HR per doubling, 1.76; 95%CI: 1.27-2.44)] and MCP-1 levels (HR per doubling, 1.49; 95%CI: 1.17-1.89) remained associated with allograft failure
Heylen *et al*[92], 2018	154	Biopsy	DNA methylation	1-yr graft function	↑ methylation risk score[f] at transplant predicted chronic injury at 1 yr (OR 45; 98 to 499; $P < 0.001$; AUC 0.919) *vs* standard baseline clinical risk factors, including age, donor criteria, donor last SCr, CIT, anastomosis time, HLA mismatches (combined AUC 0.743) sensitivity, specificity, and PPV, NPV values of MRS-based ROC curves were 90%, 90%, 95%, and 82%
Park *et al*[70], 2017	1184 (300 CVD, 371 death, 513 random sub-cohort)	Urine	Urine A1M MCP-1, PINP and PIIINP	CV events, Mortality	In adjusted models, higher urine AlM (HR per doubling of biomarker = 1.40 (95%CI: 1.21 to 1.62), MCP-1 [HR = 1.18 (1.03 to 1.36)], and PINP [HR = 1.13 (95%CI: 1.03 to 1.23) were associated with CVD events. These three markers were also associated with death (HR per doubling A1M = 1.51 (95%CI: 1.32 to 1.72); MCP-1 = 1.31 (1.13 to 1.51); PINP = 1.11 (95%CI: 1.03 to 1.20)
Smedbråten *et al*[91], 2017	382	Plasma	CL-L1, CL-K1	CV mortality, Graft survival, Patient survival	↑CL-L1 (≥ 376 ng/mL) and ↑CL-K1 (≥ 304 ng/mL) levels at transplantation were associated with mortality in multivariate Cox analyses[g] [HR = 1.50 (95%CI: 1.09 to 2.07) and HR = 1.43 (95%CI: 1.02 to 1.99)] ↑CL-K1 levels were associated with CV mortality. No association between measured biomarkers and death-censored graft loss was found
San Segundo *et al*[93], 2019	133	Plasma	Abs number peripheral blood Treg cells	Death-censored graft survival	↑ Treg cells 1 yr post-KT[h] showed better DCGL (5-yr survival, 92.5% *vs* 81.4%). 1-yr Treg cells[h] showed a *ROC AUC* of 63.1% (95%CI: 52.9 to 73.2) for predicting DCGL. After multivariate Cox regression analysis, an ↑ number of peripheral blood Treg cells[h] was protective factor for DCGL

	(HR = 0.961 (95%CI: 0.924 to 0.998), irrespective of 1-yr proteinuria and renal function

[a]$P < 0.05$ *vs* grafts without rejection.
[b]$P < 0.05$ *vs* immediate function grafts.
[c]$P < 0.05$ *vs* $eGFR_{cys} > 60$.
[d]$P < 0.005$ *vs* $eGFR_{B2M} > 60$.
[e]$P < 0.005$ *vs* $eGFR_{cys} > 60$.
[f]$P < 0.005$ *vs* low methylation risk score at transplant.
[g]$P < 0.05$ *vs* KTRs with collectin levels below cutoff.
[h]$P < 0.05$ *vs* KTRs with absolute number of peripheral blood Treg cells below threshold. sCD30: Soluble cluster of differentiation thirty; AR: Acute rejection; uNGAL: Urinary neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule 1; IL-18: Interleukin eighteen; L-FABP: Liver fatty acid binding protein; DGF: Delayed graft function; AKI: Acute kidney injury; AUC: Area under the curve; KDPI: Kidney donor profile index; B2M: Beta-2-microglobulin; Cr: Creatinine; CV: Cardiovascular; HR: Hazard ratio; Abs: Absolute; eGFR: Estimated glomerular filtration rate; $eGFR_{cys:}$ Estimated glomerular filtration rate based on cysteine; $eGFR_{B2M}$: Estimated glomerular filtration rate based on beta-2-microglobulin; u: Urine; UCr: Urine creatinine; aHR: Adjusted hazard ratio; IFTA: Interstitial fibrosis tubular atrophy; A1M: Alpha 1 microglobulin; MCP-1: Monocyte chemoattractant protein-1; PINP: Procollagen type I intact N-terminal peptide; PIIINP: Procollagen type III intact N-terminal peptide; DNA: Deoxyribonucleic acid; OR: Odds ratio; SCr: Serum creatinine; CIT: Cold ischemia time; HLA: Human leukocyte antigen; PPV: Positive predictive value; NPV: Negative predictive value; MRS: Methylation risk score; CI: Confidence interval; CL-L1: Collectin liver-1: CL-K1-collectin kidney-1; Treg: Regulatory T cells; KT: Kidney transplant; DCGL: 95% eath censored graft loss; ROC: Receiver operating characteristic.

(95%CI: 40.8-57.9); $P < 0.00001$]. On aggregate, they found the Q score to be accurate [AUC = 9.99 (95%CI: 0.98-0.99); $P < 0.00001$] with a sensitivity of 95.2%, and specificity of 95.9[94]. De Vries *et al*[95] in their study evaluating the tryptophan/kynurenine pathway, one associated with a pro-inflammatory state, showed that in 561 KTRs, serum kynurenine and 3-hydroxykyurenine were independently associated with allograft failure [HR = 1.72 (95%CI: 1.23-2.41)][95].

Another unique study by Kostidis *et al*[96] from 2019 showed that urinary branched-chain amino acids over pyroglutamate and lactate over fumarate were predictive of prolonged delayed graft function (AUC = 0.85) [96].

B cell soluble factors have been implicated in autoimmune diseases such as systemic lupus erythematosus and exert the potential to be nascent biomarkers in the context of kidney transplantation. In their study published in 202, Irure-Ventura *et al*[97] showed that in 109 KTRs, pre-transplant B-cell activating factor (pg/mL) was significantly higher in patients with clinical ABMR during the first year (853.29 pg/mL (IQR: 765.37 to 1545.99 pg/mL) than kidney transplant without clinical rejection (594.60 pg/mL (IQR: 453.21-803.93 pg/mL) or controls ($P = 0.003$ and $P < 0.001$). This corresponded to an AUC = 0.784, with sensitivity 80%, and specificity of 73.3% for predicting ABMR within 12 months of transplantation[97].

Novel biomarker use in kidney transplantation is a vibrant area of research with multiple pioneering approaches and strategies being undertaken to discern the complex pathophysiology after transplantation and improve patient care. As these studies demonstrate, there are myriad pathways and processes implicated in deleterious post-transplant outcomes. As we have described, several nascent biomarkers derived *via* multiple biomolceular disciplines confer similar predictive properties. As we gain understanding and familiarity with biomarkers, one can hope

that scientists and clinicians alike will further incorporate biomarkers in a way analogous to the multi-domain testing inherent to clinical medicine. Perhaps this approach of combining biomarkers across various domains will work synergistically to advance the field of transplant medicine.

CONCLUSION

This article summarizes emerging research about novel biomarker use in kidney transplantation. Further innovation and integration of multiple disciplines/"omics" (transcriptomics, metabolomics, proteomics) will lead to advanced biomarker discovery and implementation, which in turn will augment our current standard of care to predict and enhance post-transplant outcomes.

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MINIREVIEWS

Liver transplantation and aging

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Abstract

An increase in the average life expectancy, paralleled by a demographic shift in the population with end-stage liver disease lies behind the rising demand for liver transplantation (LT) among the elderly. Some of the most common indications for LT including hepatocellular carcinoma, alcohol-related liver disease, chronic hepatitis C and non-alcoholic fatty liver disease tend to affect older patients. Transplant professionals are faced with an increasing demand for LT among elderly patients in an age of organ shortage and it is important that risk and benefits are carefully weighed in order to achieve the optimum use of precious liver grafts.

Key Words: Liver transplantation; Elderly; Hepatocellular carcinoma; Alcohol-related liver disease; Non-alcoholic fatty liver disease; Hepatitis C virus

Core Tip: An increase in the average life expectancy paralleled by a demographic shift in the population with end-stage liver disease raises the demand for liver transplantation (LT) among the elderly. The most common indications for LT such as hepatocellular carcinoma, alcohol-related liver disease, hepatitis C virus and non-alcoholic fatty liver disease tend to affect older patients more and more. However, risks need to be weighed against the benefits since the effects of associated age-related co-morbidities in older individuals may affect transplant outcomes.

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INTRODUCTION

Liver transplantation (LT) is one of the great stories of success of modern surgery and medicine. However, due to the complexity of the procedure and associated complications, LT used to be a therapy saved for patients from younger age groups. Over the years, as the experience has increased and the results have improved, we have seen a shift from the upper age limit around 50 years of age to the present situation where most transplant centers do not have a strict age limit when wait-listing the patients for LT[1].

During the last century a dramatic increase in the average life expectancy has occurred, from 45 to over 80 years[2]. This was paralleled by a demographic shift in the population with end-stage liver disease. Epidemiologic factors lie behind the rising demand for LT among the elderly. Some of the most common indications for LT including hepatocellular carcinoma (HCC), alcoholic cirrhosis and non-alcoholic fatty liver disease (NAFLD) tend to affect older patients[3]. Until recently, hepatitis C virus (HCV) was the leading indication for LT. With the advent of direct acting antiviral (DAA) therapies most of the infected patients can now expect the cure from the virus, however the patients who acquired HCV infection at younger age will continue to present with sequela of hepatitis C infection such as HCC and cirrhosis in years to come[4-7].

Despite the increase in experience and generally excellent results of LT, it is relatively self-evident and universally accepted that the results in the elderly will be inferior to the results in younger patients. This is primarily due to the fact that older individuals will naturally survive for less time, and that the survival is affected in any population as age of the patient increases. There are also the effects of associated age-related co-morbidities in older individuals that may significantly affect transplant outcomes[8,9]. LT definitely has its place in the elderly population with end-stage liver disease. However, with the scarcity of organs for transplantation in mind, careful selection of patients is crucial in order to achieve outcomes that provide the best possible transplant-related benefit to this growing and sensitive part of the population.

CHANGING DEMOGRAPHICS IN THE PRESENT AND IN THE FUTURE

In the recent years we have seen a sharp increase in life expectancy due to a multitude of factors that include advancement of healthcare and improved social and economic conditions. Currently, epidemiological studies show that 11% of the world's population is older than 60. According to the World Health Organization, this percentage is about to double by 2050, amounting to 1500 million people[10]. These developments are a particular problem in the developed countries. Between 2000 and 2030, the percentage of population who are 65 years of age and older is projected to increase from 12.4% to 19.6% in the United States and from 12.6% to 20.3% in Europe[11]. Apart from general increase in morbidity, mortality, disability and healthcare costs, the aging of the population has significant consequences for the care of the patients with liver diseases since the incidence of liver diseases increases with age as well[12].

RISKS AND BENEFITS OF LT IN THE ELDERLY

According to the 2013 Guidelines for the evaluation of pre-transplant candidates issued by the American Association for the Study of Liver Diseases, "in the absence of significant co-morbidities, older recipient age (> 70 years) is not a contraindication to LT"[13]. Accordingly, most transplant centers do not have a strict age limit for LT wait list registrants, and there is a tendency to put more emphasis on the "physiological" than "chronological" age[14]. A number of studies reports very good results of LT in elderly recipients. They include both single center analyses and analyses of registry data, and they all show that the outcomes for the elderly in terms of survival are similar or not much worse than in matched younger patient groups[8,15-19]. Indeed, a recent meta-analysis on LT in the elderly, shows that patient and graft survival rates are not different between younger and elderly LT recipients[1].

When considering medical and ethical aspects of LT in the elderly, it is useful to start from the well-known concepts of urgency, utility and transplant-related benefit in allocation of liver grafts. Most of the current LT prioritization schemes in Europe and the United States are based solely on urgency, considering the risk of death without a

transplant as measured by patients' MELD scores[20]. In an era of organ shortage, transplant-related survival benefit, a measure of impact of transplantation both on the pre-transplant mortality and on the post-transplant survival, needs to be taken into account as well. Increasing age was found to be associated with both increased pre-transplant mortality and an increased risk of post-transplant mortality[21-23]. However, when transplant-related survival benefit is considered, there is no significant difference across different age groups. Elderly patients have decreased survival on the waiting lists compared to the MELD-matched younger registrants. Therefore, in spite of lower post-transplant survival, elderly patients may have transplant-related benefit similar to younger patients if they also have lower survival without transplantation[18]. Achievement of normalization of the expected life span is another way to measure the outcomes of LT in the elderly. Despite the increased risk for post-transplant morbidity and mortality, the elderly recipients of LT regain their anticipated life expectancy as defined by age-equivalent members of the general population. On the other hand, elderly candidates who are denied LT mostly have a short life course and die within a year[15].

Even though age should not be a discriminating criterion for LT candidates, centers still need to be cautious in selection of elderly recipients who would have the most benefit from transplantation. Identification of candidates with a good functional status and without major comorbidities is crucial for good post-transplant outcomes[15,24,25]. Also, increased surgical complexity (reflected in prolonged warm ischemia time and increased transfusion requirements) was found to negatively affect graft and patient outcomes in elderly LT recipients[15].

HCV AND LT IN THE ELDERLY

The increasing life expectancy and the chronic nature of HCV shape the growing trend of advanced age in patients with HCV infection. The prevalence of advanced fibrosis is greater in the elderly than in the younger population, and the proportion of elderly patients with advanced liver disease is expected to rise in the next decade[6,26]. Indeed, since 2002 there has been an increase in number of elderly registrants on the LT lists, aged 65 years or more, with the trend even more prominent in patients with HCV-related liver disease[18].

In the era without efficacious antiviral treatment, recurrent hepatitis C after LT resulted in rapid liver damage especially in elderly grafts, affecting graft and patient survival[27]. Since 2014, the use of DAA therapy has revolutionized the treatment of HCV infection and decreased the burden of chronic infection[28]. However, HCV is still among the leading causes for LT both in males and females[29].

DAA agents are highly efficacious, with sustained virologic response (SVR) rates more than 95% in all HCV genotypes and special populations, including transplant recipients, with excellent safety profiles[28,30]. Achieving SVR leads to short and long-term clinical benefits; reduces the risk of developing liver cirrhosis[31], improves decompensated liver disease[32,33], reduces the need for LT along with liver specific and all-cause mortality[34]. Successful antiviral therapy decreases, but does not eliminate the risk of HCC[7].

Historically, elderly patients have been considered difficult-to-treat, due to higher risk of complications, discontinuation and mortality rates. The concomitant co-morbidities, in particular cardiovascular, renal and metabolic along with hematologic conditions limited the use of interferon treatments[6]. This scenario has changed since interferon-free antiviral therapy regimens with DAAs have been introduced, enabling high efficacy with improved safety profiles also in elderly populations.

The initial results with ledipasvir (LDV)/sofsbuvir (SOF) demonstrated high sustained virologic response (SVR, 97% *vs* 98%) and similar discontinuation rates between patients aged < 65 years and those aged ≥ 65 years, respectively[35]. Similar results have also been demonstrated for SOF/velpatasvir (VEL) in patients aged ≥ 65 years who achieved SVR12 by 100%, compared to 97.8% SVR rate in patients aged < 65 years[36].

Further on, in the real-world setting different DAA-based regimens (SOF + ribavirin, simeprevir/SOF ± ribavirin, LDV/SOF ± ribavirin; daclatasvir/SOF ± ribavirin; paritaprevir/ritonavir-ombitasvir ± dasabuvir ± ribavirin, and ombitasvir/paritaprevir/ritonavir ± ribavirin) showed high efficacy in HCV patients aged ≥ 65 years with advanced fibrosis/cirrhosis with SVR12 of 94.7% and low discontinuation rate (1.4%)[37]. Similarly, high efficacy (SVR 98%) of different combination of DAAs regimens in a real-world setting has been reported for elderly

patients (≥ 65 years) with cirrhosis. However, the elderly are at increased risk for drug-to-drug interaction (DDI) and associated adverse events due to more frequent use of concomitant medications (in particular cardiovascular drugs and diuretics) reflecting the increasing age-related morbidity[38]. However, careful management during antiviral therapy in multi-morbid elderly patients, may effectively prevent DDI-associated adverse events and improve the outcomes. Therefore, as effective and safe therapies are becoming widely more available, the number of treated HCV elderly patients is expected to increase. Also, as the consequence of treatment of higher proportion of elderly patients with more advanced liver disease, greater numbers of HCV-related HCCs might be expected in the future[5].

In the context of donors, grafts from seropositive HCV donors have increased by 20% in recent years, with almost one third of them being non HCV viremic[39,40]. Traditionally, HCV positive grafts were reserved for recipients already infected with HCV, which showed no impact on the severity of HCV-related graft disease, graft or patient survival if younger donors (aged < 50 years) were used[41]. The use of DAAs has dramatically shift our attitude towards HCV positive grafts. As more patients are being treated, the percentage of people who are HCV antibodies positive but HCV RNA negative is likely to increase. Furthermore, the use of DAAs has increased the use of HCV-positive organs in recipients who are infected with HCV, but also in those who are HCV negative[42,43]. Utilization of organs from these donors provides an opportunity to expand the limited organ availability, also for elderly patients who are at higher risk of death or dropout on the liver waiting lists[18].

ALCOHOL-RELATED LIVER DISEASE AND LT IN THE ELDERLY

Alcohol accounts for 3.8% of global mortality and alcohol-related liver disease (ALD) is one of the most disastrous consequences of prolonged alcohol use. ALD encompasses a spectrum of liver pathology including steatosis, steatohepatitis, liver fibrosis and cirrhosis and/or HCC[44,45]. Across all of the adult age groups, ALD is one of the commonest indications for LT both in Europe and in the United States[46,47]. Cirrhosis in ALD patients is often diagnosed at an older age and the referral for LT may be delayed since these patients are primarily managed by primary care physicians as opposed to patients with viral hepatitis or NAFLD who are usually in the care of a hepatologist[48].

Excessive alcohol use is a well-known health risk among elderly people[49]. Despite the statistics showing decreasing alcohol use with age, the number of older adults drinking excessively is expected to rise in the future. This is primarily due to the age cohort born in the 1950s (baby boomers) with heavy drinking habits reaching old age[50,51].

There is a number of factors affecting morbidity and mortality both before and after LT in elderly patients with ALD. Physiological changes associated with aging often lead to more pronounced effects of alcohol in elderly patients compared to their younger counterparts. Old and very old adults are particularly vulnerable to the alcohol-related effects due to metabolic and other changes in their bodies and high rate of concomitant chronic diseases[52-54]. Elderly patients with a history of alcohol use are more likely to suffer from cognitive impairment or dementia resulting from a prolonged alcohol use[55-57]. In the context of LT, the associated metabolic and neurological changes can have profound effects both on the wait-list mortality and on the results of LT in elderly patients undergoing LT for ALD. As the risk of graft rejection is inversely related to age[58], the demands for immune control to prevent rejection lessen with increasing age, especially for non-immune conditions such as ALD. As such, elderly ALD patients after LT represent a lower rejection-risk population in which the reduction or minimization of immunosuppressive regimens is feasible along with the reduction of immunosuppression-related complications[59]. ALD patients have survival rates similar to LT recipients without ALD, however some of the causes of death among ALD patients tend to be especially prevalent among elderly patients[46]. Elderly ALD patients generally suffer from more co-morbidities, including alcohol-induced cardiomyopathy, skeletal myopathy, Wernicke's encephalopathy, chronic pancreatitis and malnutrition. Tobacco use is also more prevalent among ALD patients and associated with cardiovascular deaths and *de novo* cancers among LT recipients. The effect of tobacco tend to accumulate with years of smoking and it is therefore clear that elderly smokers tend to present with the largest health risks affecting the outcomes of LT[60].

HCC AND LT IN THE ELDERLY

HCC is a major health problem being the fifth most common cancer and second most frequent cause of cancer-related deaths worldwide. Most cases of HCC are attributable to chronic liver diseases associated with hepatitis B virus infection, HCV infection or alcohol use[61]. Aging is a well known risk factor for the development of HCC and an age-specific increase in the incidence of HCC among patients 75-years old or older has been shown both in the West and in the East. For instance, in Japan, the average age of HCC patients is increasing as well as the proportion of elderly HCC patients[62,63]. In the United States, the latest estimates suggest that HCC incidence peaks above 70 years of age[64].

Chromosomal changes in the liver associated with aging include shortening of the telomeres and aberrant DNA methylation. These changes are related to carcinogenesis, suggesting that aging alone is a risk factor for the development of HCC[62-64]. HCC in the elderly has been shown to be associated with less advanced liver fibrosis than HCC in younger patients. Also, there are prognostic factors of HCC that tend to be more favorable in the elderly – tumors in the elderly population tend to be encapsulated more frequently, they have better differentiation and there is less vascular invasion than in younger patients[65]. Therefore, some authors speculate that HCC in the elderly is less aggressive and that it may be more amenable to being cured compared with younger patients[65].

Treatments for HCC include surgical resection, LT, transcatheter arterial chemoembolization, percutaneous microwave coagulation, radioembolization, percutaneous ethanol injection and molecular therapies. Treatment decisions in patients with HCC are generally based on tumor-related factors, liver function, performance status and co-morbidities. However, current guidelines do not take the age of the patient into account[66,67].

LT is a well-established curative therapy for patients with HCC. Due to the rising incidence of the disease and excellent results of LT in carefully selected patients, the proportion of recipients with HCC has been increasing over the last years and currently makes up to 18% of all patients undergoing LT in Europe[4]. Patients with HCC are older than patients without HCC and this trend therefore contributes significantly to the overall aging of the population of LT recipients[4].

As for the outcomes of LT in elderly patients, the literature abounds with conflicting evidence. Contrary to the results in elderly patients undergoing liver resections for HCC where survival rates have been shown to be equivalent to those of younger cohorts[68,69], age greater than 60 years correlated negatively with short-term and long-term outcomes in patients with HCC undergoing living donor LT[70-72]. On the other hand, a large retrospective study of OPTN data showed that, while survival of all LT patients older than 70 years yields outcomes inferior to younger cohorts, in the setting of HCC, patients fare no worse than patients with other indications for transplantation[8].

NAFLD AND LT IN THE ELDERLY

The global prevalence of the metabolic syndrome is rapidly rising, given the changes in eating habits and inclination towards sedentary lifestyle. Metabolic syndrome characterized by the morbidity cluster of obesity, type 2 diabetes (T2D), hypertension and dyslipidemia, has become a growing epidemic. As a consequence, its liver manifestation - NAFLD is becoming the most common cause of chronic liver disease[73].

Non-alcoholic fatty liver comprises a spectrum of clinical and pathological entities which may lead to cirrhosis and HCC[74]. In the initial process fat is increasingly stored as triglycerides in hepatocytes. When fat storing capacity of hepatocytes is exceeded, steatosis is accompanied with ballooning cell degeneration and an inflammatory cell infiltrate, resulting in steatohepatitis. Consequent pro-inflammatory signaling and insulin resistance lead to further liver injury, where long-standing liver damage and repair responses result in cirrhosis and the development of HCC[75]. Notably, the whole process is consistently associated with an increased risk of cardiovascular disease. Indeed, NAFLD patients often have one or more components of metabolic syndrome - they are often obese with hyperlipidemia, T2DM and/or hypertension[76].

In the context of aging, changes are reflected in liver morphology, physiology, and oxidative capacity. Aging is associated with an increase in lipid accumulation in non-adipose tissues; heart, skeletal muscle and liver, increasing incidence of disorders such as atherosclerosis, insulin resistance and T2D, hyperlipidemia, hypertension, all of

which increase the chances of developing NAFLD and metabolic syndrome[77,12]. Thus, the prevalence of NAFLD increases with age, mainly affecting individuals in their fourth to sixth decades of life[78]. Even though the NAFLD prevalence increases with age, in the very elderly there is a trend of decline as shown in the Rotterdam study. The prevalence of NAFLD in participants aged < 70 years was 35.8%, aged 70-74 years was 36.6%, aged 75-79 years was 39.6%, aged 80-84 years was 32.1%, and in participants aged older than 85 years was only 21.1%[79].

NAFLD in the elderly is broadly related to the same metabolic risk factors as in the non-elderly, however female gender is no longer protective with the increasing age[80]. It is important to stress out that elderly patients (> 65 years old) have higher prevalence of steatohepatitis and advanced fibrosis, as well as other features of severe liver disease than patients of younger age[80,81]. A recent analysis of the third National Health and Nutrition Examination Survey (NHANES III) showed the high prevalence (40.3%-39.2%) of NAFLD in the elderly with no differences among the age subgroups (60-74 *vs* > 75 years old). NAFLD was associated with increased risk of mortality for 60-74-year-old individuals, but the risk was not increased in those older than 75 years[82].

As HCV burden is decreasing by highly effective antiviral treatments, NAFLD is becoming one of the leading indications for LT based on decompensated cirrhosis with or without HCC[83]. In the context of NAFLD-related HCC, epidemiological evidence show that HCC is rare before the age of 40, and it increases progressively with older age, peaking in incidence around ages 70-75 after which it steadily drops[61,84].

The proportion of patients who are older than 65 years and candidates for LT is increasing in Europe and the United States[18,59]. In general, NAFLD recipients are older than recipients who are listed for autoimmune etiologies or chronic viral hepatitis[76]. Moreover, NAFLD-related cirrhosis has become the most common non-HCC indication for LT in patients aged 65 or older[85].

Additionally, in the context of donors, donor age is considered as one of the variables with the strongest influence on donor risk estimates, associated with worse early outcomes and burdened with complications such as primary graft non-function, hepatic artery thrombosis and biliary complications[86-88]. Severe graft steatosis has been associated with worse outcomes, therefore as a general rule, only grafts with mild (< 30%) and moderate (30-60%) steatosis are accepted[89]. A combination of risk factors, rather than a single one, affects the outcomes of steatotic grafts[90]. As the increasing trend of older donors is likely to continue[4,47], in addition to NAFLD epidemic, more elderly steatotic grafts can be expected in the future.

In conclusion, as the world's elderly population and the prevalence of metabolic syndrome continues to grow at an unprecedented rate, NAFLD, as an indication for LT is projected to increase.

CADEVERIC LIVER GRAFTS FROM ELDERLY DONORS

Significant gap exists between the need for organ transplants and the number of available cadaveric grafts[91]. One of the strategies to deal with this issue is the use of extended criteria grafts[92]. These are the grafts with donor factors that are associated with poor graft function and increased risk for graft loss. Advanced donor age is one of the independent risk factors for graft loss after LT[93,94]. Actually, Feng *et al*[95] showed that donor age > 65 years was the strongest predictor of graft failure. However, a number of case series and registry analyses have shown that the use of elderly and even very old (> 80 years) livers yields good outcomes quite similar to outcomes when using much younger grafts[94,96-99]. It seems that minimization of other risk factors for poor graft function is crucial for achievement of favorable results with elderly grafts. Old liver grafts are highly susceptible to ischemia-reperfusion injury and, therefore, cold ischemia time should be kept to minimum while steatotic elderly grafts should be used very selectively[100-103]. Several studies provide evidence that the use of older livers is associated with an increased incidence of biliary and vascular complications[88,99,104]. Strategies to minimize the incidence of procurement- and transplant-related biliary injuries, including machine perfusion are currently subject of intensive research[92]. Careful donor-to-recipient matching is crucial in obtaining good results using elderly liver grafts. It is generally accepted that elderly grafts should be allocated to the less severe, clinically stable recipients who can tolerate possible delay in graft function[100,105]. In contrast to allocation of kidneys where old grafts are often allocated to old recipients ("old for old"), old liver grafts were shown to have unfavorable outcomes in old recipients so very old and very young recipients are best avoided[92].

In conclusion, elderly grafts are a valuable tool to expand the donor pool and should be used, although cautiously, after careful donor evaluation, selective donor-to-recipient matching and optimization of all aspects of the procurement, transplantation and post-transplantation course[94].

CONCLUSION

LT remains the best available treatment for end-stage liver disease. Once reserved for patients from younger age groups, today LT is increasingly performed in elderly patients. We are witnessing aging of the societies across the world and there is a demographic shift in patients with liver disease as well. Most common indications for LT including HCC, ALD and NAFLD tend to affect older patients. HCV until recently the leading indication for LT, is now amenable to cure using DAAs. However, the patients who acquired HCV infection at a younger age will continue to present with sequela such as cirrhosis and HCC in the years to come.

Results of LT are generally excellent, however, it is self-evident that the results in the elderly will be inferior to the results in younger patients. On the one hand, this reflects the fact that older individuals will naturally survive for less time. Secondly, the effects of associated age-related co-morbidities in older individuals may significantly affect transplant outcomes. However, with careful patient selection and minimization of risk factors, a significant transplant-related benefit can be achieved justifying the use of precious liver grafts in the elderly population.

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MINIREVIEWS

Post-transplant immunosuppression and COVID-19: From a double whammy to a mixed blessing

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Abstract

The coronavirus pandemic (COVID-19) has had an unprecedented effect on various disease processes and their management. COVID-19 is likely to have a complex pathophysiological interplay with the post-transplant patients; one affecting the clinical course and outcome of the other. In the absence of validated data from trials, there is strong dependence on experience based on previous similar epidemics (SARS/MERS), and from consensus based on expert opinions. Despite the fact that our knowledge is rapidly evolving with time, there still is relatively limited objective data on the effect of COVID-19 on the human body. Numerous questions remain unanswered, one of which involves the management of immunosuppression in the post-transplant recipient during this contagion. The core tenet of which continues to be that of establishing an equipoise between infection and rejection. This review summarises the current knowledge on immune interactions of the virus, the immunomodulatory effects that may be at play, and its relation to the art of immunosuppression.

Key Words: COVID-19; Post-transplant immunosuppression; Immunity; Review

Core Tip: As with other pathologies, the coronavirus pandemic is likely to have a complex pathophysiological interplay with the post-transplant recipients; one affecting the clinical course and outcome of the other. These fragile subset of patients, with their immunomodulated state are likely be affected in numerous ways which may not be limited to just a more rapid progression of infection. During this pandemic the need to weigh the benefits of immunosuppression relative to inflammation against its adverse effects remains.

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INTRODUCTION

Notwithstanding the social isolation and restrictions, the coronavirus pandemic (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally. The pandemic has had an unprecedented effect on various disease processes and their management[1]. Especially in the field of transplantation, many questions remain with regard to COVID-19 that need to be addressed. As with other diseases, COVID-19 is likely to have a complex pathophysiological interplay with the post-transplant patients; one affecting the clinical course and outcome of the other[1]. These fragile subset of patients, with their immunomodulated state are likely be affected in numerous ways which may not be limited to just a more rapid progression of infection[2]. The need to weigh the benefits of immunosuppression relative to inflammation against its adverse effects also remains. There also remain the pragmatic concerns of donor-derived COVID-19 infection along with the potential for prolonged shedding by immunocompromised hosts leading nosocomial and community transmission of SARS-CoV-2. Besides, only sparse data exist on the biomarkers which define the risk for disease progression, appropriate therapeutic interventions, and graft rejection on a background of COVID-19. With relatively limited objective knowledge of the effect of COVID-19 on the human body, numerous questions remain unanswered. One of which involves the management of immunosuppression in the post-transplant recipient during this contagion; the core tenet of which continues to be that of establishing an equipoise between infection and rejection. This review summarises the current knowledge on immune interactions of the virus, the immunomodulatory effects that may be at play, and its relation to the art of immunosuppression.

CORONAVIRUS-19 AND THE IMMUNE SYSTEM

While there have been important inroads, a full picture of the critical host immune response and its interplay with the virus remains poorly defined. As an initial step in the infection, the virus binds to its target receptor on the host cell. Based on previous work on SARS-CoV, which demonstrated that this virus principally targets cells which express the angiotensin-converting enzyme 2 (airway epithelial cells, alveolar epithelial cells, vascular endothelial cells and macrophages), it has been postulated that the SARS-CoV-2 uses a similar mode of entry[1,3-6]. Viral infection and replication cause high levels of virus-linked pyroptosis (highly inflammatory form of programmed cell death) with associated vascular leakage[4,5,7].

SARS-CoV-2 triggers both the innate and adaptive immune response synergistically, responses which are essential in controlling viral replication and limiting the spread of virus[3-5,8,9]. Local inflammatory cascades lead to increased secretion of the pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IFNγ, MCP1 and IP-10)[4,5,8,9]. Recruitment of immune cells like monocytes and T lymphocytes from the blood with infiltration of the infected site occurs. This phenomenon contributes to the elevated neutrophil: Lymphocyte ratio and lymphopenia observed in most COVID-19 patients[1,5,10]. In most individuals, this immune response clears the infection and as patients recover, the wave of immune response subsides[1,4,5,8,10].

Nevertheless, sometimes the virus-induced immune response turns dysfunctional, leading to an induction of aberrant production of pro-inflammatory cytokines and an exaggerated recruitment of macrophages and granulocytes. This results in a cytokine storm, an integral component of the macrophage activation syndrome or secondary hemophagocytic lymphohistiocytosis, thus leading to further tissue damage and in severe cases progressing to acute respiratory distress syndrome (ARDS)[1,4,5,11]. Higher plasma levels of IL-2, IL-6, IL-7, IL-8, IL-10, macrophage inflammatory protein-1A, macrophage inflammatory protein-1B, IP-10, MCP1, and tumour necrosis factor have been observed in patients with severe COVID-19 requiring intensive care unit (ICU) admissions[3,5,12]. Patients with severe disease show a significantly higher levels of IL-6, a key cytokine. Higher percentage of CD14+ CD16+ monocytes which secrete

inflammatory cytokines, have also been observed in the sicker patients. This immune dysregulation also involves subsets of T cells, and in the severe cases of COVID-19, levels of helper T cells and cytotoxic suppressor T cells, and regulatory T cells (responsible for immune homeostasis by suppression of activation, proliferation, and function of most lymphocytes) were noted to be significantly lower[5,13,14]. Further immune dysregulation is evident by the disruption of equilibrium between naïve T cells and memory T cells. There is therefore a T cell exhaustion, with a poor effector function, and an increased expression of inhibitory receptors on the cells, the magnitude of which worsens in those who are admitted in the ICU[13-15]. Altogether, this dysfunctional immune response induced by the virus results in further tissue damage. In a small subset of patients, this local inflammatory cascade may become systemic, leading on to organ-system damage and multisystem organ dysfunction syndrome[1,3,11,16].

It is nonetheless important to understand that a simple correlation does not extrapolate to causation. It is equally likely that this cytokine storm is not a straightforward case of an inappropriate host inflammatory response that requires correction, instead is due to an increased viral titre (secondary to failure of the immune response to control infection) which drives the inflammation and its consequent severity. Hence, the decision to pharmacologically immunosuppress a patient with COVID-19 remains a difficult one. The deleterious effects of an impaired immune response must be carefully weighed against the likely benefits of reducing inflammation.

IMMUNOSUPPRESSION AND CORONAVIRUS-19: THE EVIDENCE

A systemic review based of 16 articles on immunosuppressed patients (various causes: Cancer, transplant) showed a milder COVID-19 disease and an overall better outcome as compared to other comorbidities[17]. Two out of 200 heart transplant recipients from China developed COVID-19 infection. While one had a mild disease, the other had a more severe course and required a more intensive care management with high dose corticosteroids and immunoglobulins. Both patients recovered without graft loss and their respective courses were not dissimilar from other immunocompetent patients with COVID-19 in the province. The authors however concluded that immunosuppression may have masked the clinical signs of the infection, and may have delayed their presentation[18].

In a series of 200 transplant recipients from Italy, none of the patients developed COVID-19 pneumonia. There was no increased risk of severe disease or mortality in these patients. This led the author to believe that instead of amplifying the risk of recipients to COVID-19, immunosuppression may actually be protective by dampening the amplified immune response[19]. In a report from China, of 1099 patients with confirmed COVID-19, two were immunosuppressed. Both had an uneventful recovery following a mild disease[19]. Similar such reports of post-transplants patients having a course of COVID-19 not dissimilar from the general population has been reported from across the world[20-22]. Of 1590 patients with COVID-19 included in a series to analyse the influence of comorbidities on the clinical course of the COVID-19, 21 were classified as immunosuppressed. Outcomes analysed included ICU admission, invasive ventilation and mortality. While patients with diabetes, hypertension, co-existing lung disease or malignancy were shown to have a more adverse outcome, immunosuppressed patients met similar endpoints as those of the general population[23]. With the airway being the most common route of entry for the SARS-CoV-2, lung transplantation piques one's interest. Concerns, apart from outcomes would include higher rates and different sources of infection (recipient derived, donor derived or nosocomial) and diagnosis, especially in the early post-transplant period. Non-COVID-19 lung infections or graft dysfunction may have presentations similar to those of a COVID-19 disease, confounding the diagnosis. Nevertheless, anecdotal reports from lung transplant centres across the world suggest a disease presentation and outcome similar to the general population[20,24-26].

Conventionally, immunosuppressants affect humoral immunity and neutrophil action, leading to a higher susceptibility and increased severity of viral infections, often with prolonged shedding. Contrary to other viral illnesses like influenza A and H1N1, SARS-CoV2 does not appear to have a higher predilection towards immunosuppressed hosts[2,17,19]. Immunosuppressed patients may actually have a potential protective effect afforded by a weaker immune response against the pathogen, resulting in a milder course of disease.

IMMUNOSUPPRESSANTS AND CORONAVIRUS-19

The commonly used immunosuppressants in the post-transplant setting include corticosteroids, calcineurin inhibitors, anti-metabolites and biological agents.

Corticosteroids

The use of systemic corticosteroids in COVID-19 remains controversial. On one hand it may worsen viremia by diminishing the immune response and prolong the viral shedding time, on the other with its broad spectrum actions, corticosteroids may suppress the exuberant immune response, and maintain a systemic anti-inflammatory state that can minimize the precipitation of severe pneumonia and ARDS[5,8,16,27].

Studies from the SARS and MERS epidemics have shown deleterious effects of corticosteroids. Apart from a delayed viral clearance, increased rates of secondary infections, steroid related complications and higher mortality rates were observed[3,8,27,28]. Data from two other studies suggested a prolonged SARS-CoV2 shedding and an increased mortality rate with the use of high dose corticosteroids[29,30]. On the contrary, there is some compelling evidence for the use of corticosteroids. Improved outcomes by the suppression of inflammation have been demonstrated, especially in the later stages of ARDS[16,29,30]. Nevertheless, at this point, the potential role of corticosteroids in preventing mild COVID-19 from developing into severe pneumonia remains controversial. While there are recommendations like those from WHO which recommend avoiding the use of corticosteroid, certain other guidelines allow for their usage when there is rapid disease progression on a background of severe inflammation[1,29,31]. There is also no consensus on the dosing of corticosteroids, and these must be individualised for each patient.

Calcineurin inhibitors

Several guidelines variably suggest withdrawal/dose reduction of Calcineurin Inhibitors (CNIs) in transplant patients with severe COVID-19. With evolving data and robust evidence lacking, these guidelines are being updated frequently.

Certain unique features of CNIs are worth mentioning. CNIs are known to inhibit certain viral replication by inhibiting immunophilin pathways[32,33]. Experience with Hepatitis C virus and several coronaviruses suggest that CNIs, especially Cyclosporine can inhibit their replication *in vitro* independent of its immunosuppressive effect. Analyses of virus-host interactions, have shown the SARS-CoV use the host's cyclophilin family of proteins for interaction. *In vitro* tests with tacrolimus and FK506 binding protein knock downs have shown to inhibit viral replication[32,34]. Hence, in principle, CNIs could have the potential to inhibit SARS-CoV-2. Based solely on these studies in related viruses, it would however be imprudent to use CNIs for their purported antiviral properties. Also a withdrawal of CNIs is likely to result in an increased dosing of corticosteroids, which as evidence would suggest may well have deleterious effects[27,35]. Due to their inhibitory action on IL-2 gene transcription, and cell proliferation, CNIs notably Cyclosporine, has been used in the treatment of HLH[16,32,36]. Albeit there is little evidence that they would be capable of attenuating the SARS-CoV-2 CRS, this does suggest that CNIs may not be harmful in the dysregulated immune environment of COVID-19. Hence, current evidence suggests that CNIs remain the preferred maintenance immunosuppressant in post-transplant patients with COVID-19.

Mammalian target of rapamycin inhibitors

As a central regulator of cell metabolism and proliferation, mammalian target of rapamycin (mTOR) pathway affects diverse cellular processes across organisms[37]. Apart being an immunosuppressant, due to its interaction with viral proteases, *in vitro* studies have shown mTOR inhibitors to have a strong antiviral effect on SARS and MERS viruses[13,38,39]. Nonetheless, using these class of drugs purely for their anti-viral properties would be ill-advised. Adverse effects of mTOR inhibitors include interstitial lung disease and mucosal ulcers which could potentially worsen the course of SARS-CoV-2 infection. CNIs and mTOR inhibitors are metabolised *via* the cytochrome P450 enzyme systems (CYP3A4, CYP3A5). These cytochromes are inhibited by anti-viral medication commonly used for COVID-19 pneumonia leading to fluctuations in the levels of both CNIs and mTOR inhibitors. This inhibition is however more intense and unpredictable with mTOR inhibitors[34,37,39-41]. Put together, the recommendations include either stop the drug or reduce to micro-doses in severe cases of COVID-19.

Antimetabolites (mycophenolate mofetil and azathioprine)

Mycophenolate mofetil (MMF) is an inhibitor of inosine-5′-monophosphate, and causes intense immunosuppression by preferentially inhibiting B-cell and T-cell function. In addition to its immunosuppressive properties, several in *vitro* studies have demonstrated its antimicrobial effects against various viruses including vaccinia virus, herpes simplex virus, Coxsackie virus, hepatitis C and influenza virus[42-46]. On the other hand, MMF causes lymphopenia, and is likely to compound the harmful effect of the virus. Hence, despite a potential anti-viral effect, MMF with its powerful suppression of the immune system is likely to be deleterious than beneficial. Another antimetabolite commonly used for immune suppression, especially in renal transplantation is Azathioprine. Its actions are similar to those of MMF, and is also associated with intense lymphopenia. Evidence is lacking as to the true effect of continuing MMF or Azathioprine in post-transplant COVID-19 patients, it is but intuitive to withhold these drugs during severe infection[39,47,48].

Intravenous immunoglobulin

Consisting of pooled polyclonal immunoglobulin G, the exact mechanism of Intravenous immunoglobulin immunomodulatory action is unknown. Proposed mechanisms include apoptosis, expression of pro-inflammatory cytokines, expansion of regulatory T cell population, phagocytosis, antibody dependent cellular cytotoxicity, immune cell differentiation and maturation, and antigen-presentation[8,10]. All of these responses are integral to viral clearance from the body. High dose Intravenous immunoglobulin has been reportedly used successfully in the treatment of severe COVID-19[49-51]. There are several ongoing trials on its application in COVID-19, but its high cost and limited supply is likely to restrict its general use.

Biological agents

There is very little literature evidence regarding the interactions of routinely used biologic agents in the post-transplant setting like Basiliximab and COVID-19. It is nevertheless wise to use them judiciously during this pandemic[47]. Numerous other biological agents acting at various levels of the cytokine cascade are being tested as treatment options for COVID-19[3,5,8,47].

IL-6 is the master-switch of the body's immune response. It acts on various cascades simultaneously and IL-6 receptors are universally expressed on immune cells[52]. Rapid elevations of IL-6 levels are noted in various inflammatory conditions including COVID-19 related cytokine storm. Direct correlations between serum levels of IL-6 and SARS-CoV-2 RNAaemia in severe disease suggest that blocking IL-6 or its receptors could potentially attenuate the dysfunctional immune response induced by the contagion[5,11,30,53]. Several therapeutic agents acting a various stages of the signalling pathway have been developed, these include inhibition of IL-6, inhibition of IL-6 receptors, and/or its postreceptor downstream signalling pathways (JAK/STAT)[3,5,8,41,52]. Under trial IL-6 antagonists include sarilumab, tocilizumab and siltuximab, each with different pharmacologic properties. It is however sobering to note that IL-6 antagonists increase the risk of opportunistic infections, therefore must be used in seriously unwell patients, along with antiviral treatments to reduce the viral load[3,5,8,41,52].

CORONAVIRUS-19 TREATMENT DRUG INTERACTIONS

Remdesivir is a NUC/viral RNA polymerase inhibitor which inhibits SARS-CoV-2 in *vitro*, and there are case reports of its efficacy in COVID-19[54-56]. No relevant drug-interactions with immunosuppressive agents are known and liver toxicity though possible, is rare[54,56]. With contradictory data on their efficacy, treatment of COVID-19 with Chloroquine/Hydroxychloroquine ± Azithromycin has been a subject of intense debate[54-58]. It is remarkable to note that these agents can significantly alter the drug levels of immunosuppressive agents, and a close monitoring of drug levels is required for CNIs and mTOR inhibitors[39,47,54]. It is imperative to exclude G6PD deficiency before starting choloroquine therapy. Despite it being liver-safe, there are reports of clinically apparent acute liver injury[54,57,58]. Lopinavir/ritonavir are approved for Human immunodeficiency virus and have been used in patients with severe acute respiratory syndrome[55,59,60]. Reports of their value in the treatment of COVID-19 exist. They have well known drug-interactions with immunosuppressive drugs and mTOR inhibitors should not be co-administered[54,59,60]. Lopinavir/ritonavir is also a potent inhibitor of CYP3A4 and close monitoring of drug levels are required for CNIs.

CONSENSUS AND RECOMMENDATIONS

The impact of an immunosuppressed state on COVID-19 and vice-versa continues to be unclear, and recommendations extrapolated from the SARS/MERS epidemic remain un-validated. Scientific evidence remains scarce and strategies can only be based on expert opinion. In these uncertain times, based on available evidence, various transplant societies across the world have come up with their recommendations[22,26,39,47,61-68]. Although the management of post-transplant immunosuppression in COVID-19 is largely anecdotal, information from the transplant societies have a high degree of consensus. A summary of their recommendations on post-transplantation immunosuppression during this pandemic include: (1) There is concern that reducing or discontinuing immunosuppressants may cause acute graft rejection, hence dose adjustment of immunosuppressive drugs in transplant recipients without COVID-19 is not warranted; (2) For patients with mild to moderate COVID-19, the current immunosuppressant dosage should be maintained. The patient's condition nevertheless, should be monitored closely; (3) A close watch on drug interactions that may cause large oscillations in plasma CNI concentrations is imperative. Any such fluctuations should be avoided, and such medications should be prescribed only if the benefits greatly outweigh the risks; (4) As low lymphocyte counts in COVID-19 patients is associated with a more severe course of disease, critical reconsideration and a judicious use of lymphocyte depleting therapies must be done; (5) Transplant recipients with severe or rapidly progressive COVID-19 will need a staged approach with reduction of immunosuppression. Stopping of antimetabolites in the early phase and dose reduction of corticosteroids in the late phase, keeping at least a low dose to avoid adrenal insufficiency is recommended; and (6) Corticosteroids or other immunosuppressive therapies should be re-initiated with caution when their potential benefits outweigh the risks of discontinuation.

Unit protocol

Based on the internationally accepted classification of COVID-19, we classify SARS-CoV-2 test (reverse transcription polymerase chain reaction) positive patients into asymptomatic, mild, moderate, severe and critical disease[69-71]. Current immunosuppression is maintained for patients with asymptomatic or mild infections, they are however followed-up closely. Antimetabolites are stopped for those with moderate disease, and their lymphocyte count is monitored. Other immunosuppressants are continued at the usual doses (blood trough Tacrolimus levels 6-8 ng/mL). A low threshold is kept for reducing their immunosuppression, should their clinical condition worsen. For severe and critical disease, immunosuppression is lowered to a bare minimum. CNIs are maintained at low doses and stopped if the patient's condition becomes critical. Low doses of corticosteroids are given to avoid adrenal insufficiency. Drug levels and interactions with anti-viral medication are monitored.

CONCLUSION

In the absence of validated data from trials, there is strong dependence on experience based on previous similar epidemics (SARS/MERS), and from consensus based on expert opinions. However, with time our knowledge is rapidly evolving and this pandemic does come with the silver lining of worldwide collaboration in clinical care and biomedical research. In an endeavour to return to the familiar domain of evidence based medicine, high quality research and accurate documentation remains the need of the hour. Further studies of the host immune response to SARS-CoV-2, including a detailed investigation of the determinants of healthy versus dysfunctional outcomes, will allow for a more evidence based approach to post-transplant immunosuppression with an improved individualization of care.

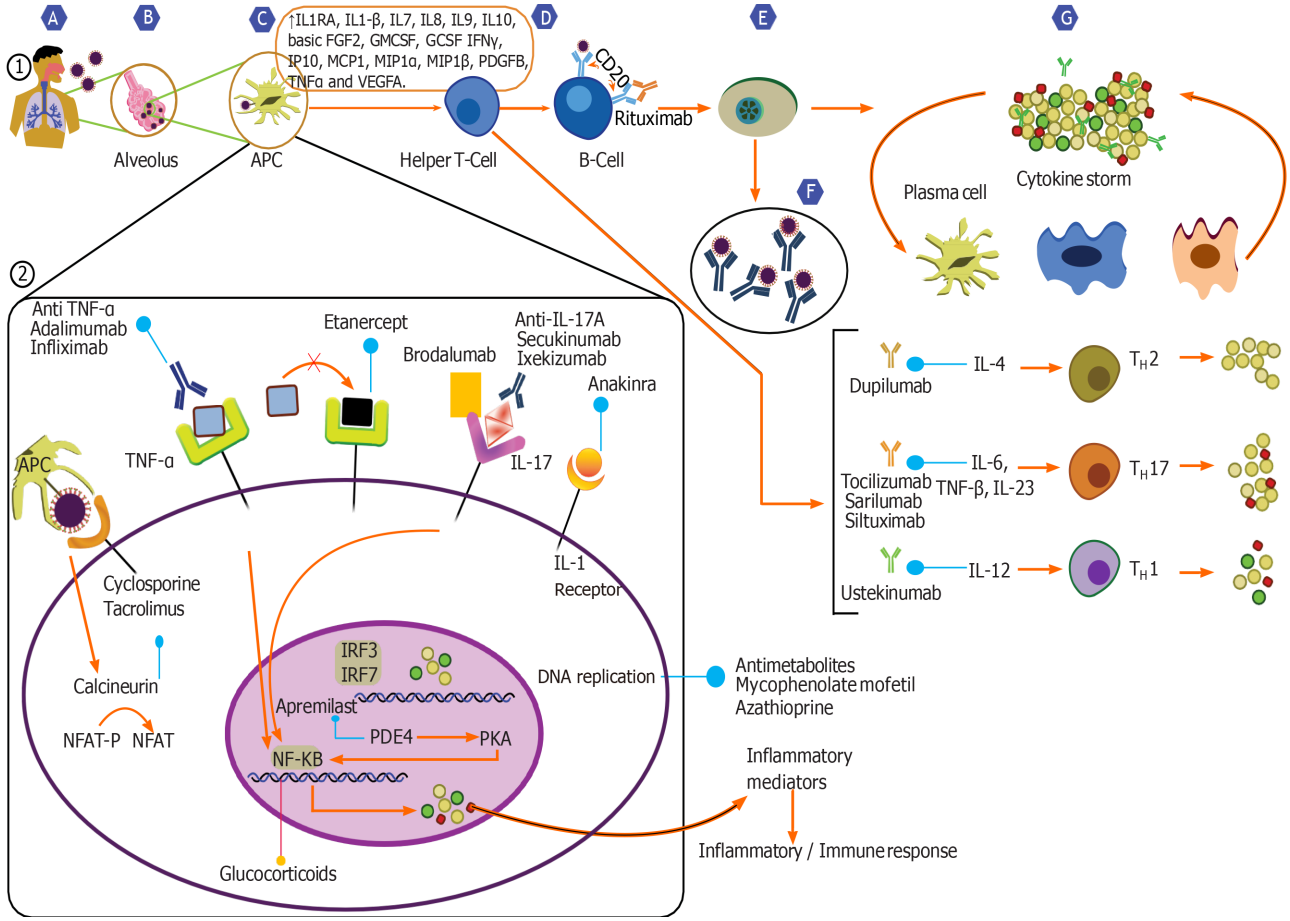

Figure 1 Chronology of events during the severe acute respiratory syndrome coronavirus 2 infection and targets for immunosuppressants and immunomodulators. A and B: Person-to-person transmission of the severe acute respiratory syndrome coronavirus 2 occurs through respiratory secretions of infected individuals; C: The virus infects cells which express surface receptors Angiotensin-converting enzyme 2 leading to intense pyroptosis and release of damage associated molecular patterns, which along with the viral components are recognised by epithelial cells and macrophages; D and E: These antigen presenting cells then trigger the generation of pro-inflammatory cytokines stimulating the immune cascade pathways, leading to a differentiation of T and B cells, followed by activation of B cells into plasma cells to produce viral neutralising antibodies; F: Usually these antibodies block viral infection, and alveolar macrophages recognize neutralized viruses and apoptotic cells and clear them by phagocytosis; and G: However, when unchecked the escalating cascade of the immune system with production of chemokines leads to a cytokine storm. (Inset) Intracellular targets of immunosuppressants and their role in suppressing the inflammatory/immune response. Inhibition: Red line; APC: Antigen presenting cell; CD: Cluster differentiation; MCP1: Monocyte chemoattractant protein 1; GCSF: Granulocyte colony stimulating factor; IL: Interleukin; FGF: Fibroblast growth factor; GMCSF: Granulocyte-macrophage colony stimulating factor; NF-κB: Nuclear factor κB; IP10: Interferon-induced protein 10; VEGFA: Vascular endothelial growth factor A; IRF: Interferon regulatory factor; PDE4: Phosphodiesterase 4; MIP1A: Macrophage inflammatory protein 1A; TNFα: Tumor necrosis factor α; NFAT: Nuclear factor of activated T cells; PDGF: Platelet-derived growth factor; PKA: Protein Kinase A; TH: T-helper cell.

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