CHAPTER 4.4 Immune monitoring after kidney transplantation

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1 Introduction

Post-transplant immune monitoring is a critical component of patient management. It encompasses an array of laboratory assays primarily designed to detect graft rejection and optimise immunosuppressive therapy. In addition to conventional markers of graft function, such as serum creatinine, estimated glomerular filtration rate (eGFR) and proteinuria, both invasive and non-invasive approaches are employed. While graft biopsy remains the gold standard for diagnosing rejection, the risks involved, particularly bleeding, limit its repeated use in children. In recent years, numerous blood- and urine-based biomarkers have been investigated, with donor-specific anti-HLA antibodies (DSAs) now established as a key component of immune surveillance. Other promising candidates include donor-derived cell-free DNA (dd-cfDNA), urinary chemokines, and 'omics'-based signatures (transcriptomics, metabolomics, and proteomics). Immunosuppressive drug monitoring, traditionally based on trough levels, is being refined through emerging metrics such as tacrolimus intra-patient variability (TAC-IPV). Torque Teno Virus (TTV) load and virus-specific T-cell profiling may provide additional information about the recipient's actual immune status and help to refine immunosuppressive dosing strategies. Surveillance for opportunistic viral infections, including cytomegalovirus (CMV), Epstein–Barr virus (EBV) and polyomavirus, remains essential. While some of these approaches have entered routine practice, many are still investigational and require further validation, particularly in paediatric populations. Together, these evolving strategies hold significant promise for advancing personalised, precision-guided post-transplant care.

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This chapter aims to provide an overview of the most well-established immune monitoring tools currently available, and how they are implemented in practice for kidney transplant recipients. For a more comprehensive and in-depth review, readers are referred to recent publications, including those by Peruzzi and Deaglio (2023) and Laroche and Engen (2024) [1, 2]. The post-transplant surveillance of DSAs is addressed in particular by expert consortia such as the "Sensitisation in Transplantation: Assessment of Risk (STAR)" Working Group and the European Society of Organ Transplantation (ESOT) Working Group on Subclinical DSA Monitoring [3, 4]. The "ESOT Working Group on Molecular Biomarkers of Kidney Transplant Rejection" has published recommendations on molecular biology testing for the non-invasive diagnosis of kidney allograft rejection [5]. However, it is important to note that the majority of the existing literature and evidence originates from adult cohorts, with comparatively limited data available for paediatric transplant populations.

2 Human Leukocyte Antigen (HLA) Antibodies

The impact of antibodies directed against donor HLA mismatches (DSAs) on the risk of rejection (particularly antibody-mediated rejection [AMR]) and subsequent graft loss is well established [6, 7]. In paediatric kidney transplantation, approximately 15–45% of patients develop de novo DSAs (dnDSAs) within the first five years post-transplant, depending on the definitions applied and the cohort studied [8, 9]. The current consensus is to use single-antigen bead (SAB) assays for DSA detection [10]. However, interpreting DSA positivity remains challenging in the absence of a universally accepted mean fluorescence intensity (MFI) threshold. Reported laboratory cut-offs vary widely, typically from 500 to 3,000. Based on analyses of inter-laboratory reproducibility and manufacturer-related variability, the STAR consortium recommends an MFI threshold of between 1,000 and 1,500 [3].

The DSA monitoring schedule should be tailored to the individual patient's risk profile, which is mainly constituted by the presence or absence of signs of graft dysfunction and immunological history (e.g. HLA antibody status, re-transplantation with repeated HLA mismatches, immunising events such as blood transfusions), the chosen immunosuppressive regimen (e.g., minimisation of immunosuppression, especially reduction or withdrawal of CNI, as well as non-tacrolimus-based regimens), non-adherence, and comorbidities [11].

Thus, the optimal scheme for routine monitoring of DSA in clinically stable kidney transplant recipients has not yet been established and remains debatable [12]. According to the ESOT consensus, this may include a baseline assessment prior to transplantation, followed by scheduled testing at three to six months post-transplant, and annually thereafter. Intensified monitoring in the first months after transplantation appears beneficial for patients with preformed DSAs due to the increased risk of early AMR [13]. With the emergence of advanced risk-stratification tools, such as molecular mismatch analysis, there is growing evidence that less intensive surveillance protocols may offer a favourable balance between cost-effectiveness and safety [14]. However, younger age within adult transplant cohorts has been associated with an increased risk of developing dnDSA. Together with the tendency towards a higher incidence of dnDSA in paediatric recipients, this observation may argue in favour of a more intensified monitoring schedule in paediatric recipients [9, 15]. The emergence of dnDSA, or a significant rise in MFI (defined as an MFI increase of 25–50% according to the STAR consortium), in combination with clinical and other laboratory parameters, may warrant consideration of a graft biopsy to assess for subclinical rejection [3]. DSA testing is recommended in conjunction with any biopsy, whether protocol-driven or indicated, to support diagnosis and management [16].

The clinical utility of additional assays that evaluate the complement-fixing capacity of DSAs (e.g., C1q, C3d or C4d binding tests) or that delineate IgG subclasses is still unclear. Complement binding assays, especially C1q positivity, are associated with high DSA MFI levels and DSA strength (titres), which increases the risk of rejection or graft loss [17, 18]. However, a recent meta-analysis examined the impact of C1q positivity beyond the mere association with high MFI levels, suggesting that C1q-positive DSAs could predict graft outcomes following therapy [19]. Patients who failed to clear C1q-binding antibodies had poorer outcomes. This suggests that, in certain clinical scenarios, these assays could provide additional information on the potential effectiveness of targeted therapeutic interventions [20, 21]. Nevertheless, these supplementary tests are not yet routinely incorporated into monitoring protocols [4, 22].

3 Non-HLA antibodies

The role of non-HLA antibodies in allograft injury is still being investigated. While several non-HLA targets have been suggested, the majority of exist-

ing studies have been limited by small sample sizes and single-centre designs, with notable exceptions including antibodies directed against the angiotensin II type 1 receptor (AT1R) and MHC class I chain-related gene A (MICA) [24–28]. Although there have been some observations of an association between non-HLA antibodies and poorer graft outcomes (graft survival and rejection), the current evidence is insufficient to justify routine screening for non-HLA antibodies in clinical practice [29]. Heterogeneity in antibody detection methods and threshold definitions further complicates the interpretation of test results [16]. However, as with testing for complement-binding HLA-DSAs, assessing non-HLA antibodies may be a useful diagnostic option in certain cases, such as AMR with incongruent HLA-DSAs [22].

4 Donor-derived cell-free DNA

Donor-derived cell-free DNA (dd-cfDNA) has emerged as a non-invasive biomarker for detecting allograft injury. Elevated dd-cfDNA levels have been consistently associated with biopsy-proven rejection in adult studies, and are quantified as the proportion or absolute amount of circulating donor-derived cell-free DNA in the recipient plasma that originates from the donor organ [30, 31]. When integrated with DSA testing, dd-cfDNA measurement has significant potential to enhance the early detection of alloimmune injury, particularly AMR [32–34]. However, thresholds in paediatric populations remain to be definitively established and prospectively validated. Reflecting this, paediatric-specific research is urgently needed to define the optimal cut-off values, timing and clinical application of dd-cfDNA in the context of long-term graft surveillance [35]. The European Society of Organ Transplantation recently made a 'weak but favourable recommendation' for serial dd-cfDNA monitoring in mainly adult patients with stable graft function as a strategy to exclude subclinical AMR [5]. However, a randomised clinical trial conducted at a single centre reported that dd-cfDNA-guided biopsy in patients with prevalent dnDSA can reduce the time to AMR diagnosis and thereby expedite therapy initiation. This suggests that testing for dd-cfDNA would probably be more meaningful and cost-efficient in a selected cohort of patients, i.e., those with dnDSA and therefore at higher risk of developing AMR [36].

5 Drug level monitoring

Recent studies have demonstrated that tacrolimus intrapatient variability (TacIPV) and the concentration-to-dose ratio (C/D) may be useful markers for predicting graft loss and rejection [37, 38]. In paediatric patients, TacIPV of more than 23% during months 6–12 post-transplant was associated with an increased risk of rejection after 12 months post-transplant. Similarly, a C/D ratio of less than 1.0 (i.e. rapid tacrolimus metabolism) was associated with a higher risk of rejection between months 6 and 12 [39]. Furthermore, high TacIPV has also been reported to be associated with an increased risk of dnDSA development, rejection episodes, and graft failure in both adult and paediatric patients [40–44]. These findings suggest that patients with these risk factors should be closely monitored and their immunosuppressive therapy adjusted accordingly.

6 Monitoring for viral infections and Torque Teno Virus

The reactivation of latent viral infections, particularly EBV, CMV and polyomaviruses (BKPyV and JC viruses), can provide valuable information about the immunological balance after a transplant, albeit indirectly. It is important to note that any reduction in immunosuppression, particularly in managing sustained viral replication, raises concerns about the development of dnDSA [45, 46]. Using virus-specific T cell assays could further improve the personalisation of immunosuppressive management [47]. However, their routine application is currently limited by technical complexity, restricting its use to specialist centres.

Another approach to assessing over- or under-immunosuppression, which is associated with an increased risk of infection or graft rejection, respectively, is Torque Teno Virus (TTV) load monitoring, which has emerged as a promising biomarker. TTV is a non-enveloped, circular, single-stranded DNA virus belonging to the Anellovirus family, and is not known to cause disease in humans. While it is detectable in approximately 90% of healthy individuals, it is present in almost all immunosuppressed transplant recipients [48]. Notably, TTV remains unaffected by conventional antivirals, and TTV plasma loads have been shown to inversely correlate with T-cell count and function, with higher viral loads observed in immunosuppressed patients compared to healthy controls. Conversely, patients experiencing allograft rejection tend to exhibit significantly lower TTV levels [49]. While the directionality of these associations is biologically plausible, the pooled diagnostic performance of TTV-DNA remains

suboptimal for stand-alone clinical use, with a sensitivity of 72% and a specificity of 57% [50]. Despite these limitations, monitoring TTV load may provide a non-invasive adjunct to conventional markers of immune function. In paediatric transplantation, where balancing adequate immunosuppression with the risks of infection and long-term toxicity is particularly challenging, TTV represents a promising area for further research [51].

7 Gene expression profiling (transcriptomics) and protein biomarkers (proteomics)

Peripheral blood gene expression profiling and urinary chemokine assays have the potential to overcome the limitations of conventional monitoring tools. The combination of CXCL9 and CXCL10 in urine, for example, has shown promising results in ruling out subclinical rejection, encompassing both T cell-mediated rejection (TCMR) and AMR [52]. This approach may be particularly useful for paediatric recipients, for whom minimising invasive procedures is of heightened clinical importance [53]. As a 'weak but favourable recommendation' by ESOT, monitoring a combination of urine CXCL9 and CXCL10 can be used to exclude subclinical rejection in stable patients and acute rejection in patients with graft dysfunction. In contrast, the clinical applicability of peripheral blood gene expression profiling remains limited. Although initial studies suggested that such assays, such as the Kidney Solid Organ Response Test (kSORT), might offer a valuable adjunct in the early detection of rejection, most commercial platforms have been withdrawn from the market, primarily due to regulatory challenges and inconsistent performance [54]. Currently, there is no consensus on the implementation of blood gene expression profiling for diagnosing or excluding graft rejection.

8 Summary

In addition to traditional biomarkers, which have long been known to have low sensitivity and specificity, as well as an inability to detect subclinical kidney allograft rejection, a variety of new, non-invasive tests are now available. These tests have the potential to help monitor graft health, evaluate levels of immunosuppression, and reduce the need for biopsies in post-transplant care. However, key parameters for most of these novel biomarkers remain undefined, including

standardised sampling protocols, defined post-transplantation timepoints and validated diagnostic thresholds. These knowledge gaps are particularly significant for paediatric kidney transplant cohorts. While these non-invasive biomarkers represent a promising frontier in transplant immunosurveillance, their translation into standard paediatric practice requires substantial refinement, harmonisation and regulatory clarity, as well as large and rigorous paediatric-specific studies.

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