


Review

Contemporary Biomarkers for Renal Transplantation: A Narrative Overview

Dorin Novacescu ¹, Silviu Constantin Latcu ^{1,2,3,*}, Razvan Bardan ^{2,3}, Liviu Daminescu ²
and Alin Adrian Cumpanas ^{2,3}

¹ Doctoral School, Victor Babes University of Medicine and Pharmacy Timisoara, Eftimie Murgu Square, No. 2, 300041 Timisoara, Romania; novacescu.dorin@umft.ro

² Department of Urology, “Pius Brinzeu” Timisoara County Emergency Hospital, Liviu Rebreanu Boulevard, Nr. 156, 300723 Timisoara, Romania; razvan.bardan@umft.ro (R.B.); daminescu75@gmail.com (L.D.); cumpanas.alin@umft.ro (A.A.C.)

³ Department XV, Discipline of Urology, Victor Babes University of Medicine and Pharmacy Timisoara, Eftimie Murgu Square, No. 2, 300041 Timisoara, Romania

* Correspondence: silviu.latcu@umft.ro; Tel.: +40-741-032-154

Abstract: Renal transplantation (RT) is the preferred treatment for end-stage renal disease. However, clinical challenges persist, i.e., early detection of graft dysfunction, timely identification of rejection episodes, personalization of immunosuppressive therapy, and prediction of long-term graft survival. Biomarkers have emerged as valuable tools to address these challenges and revolutionize RT patient care. Our review synthesizes the existing scientific literature to highlight promising biomarkers, their biological characteristics, and their potential roles in enhancing clinical decision-making and patient outcomes. Emerging non-invasive biomarkers seemingly provide valuable insights into the immunopathology of nephron injury and allograft rejection. Moreover, we analyzed biomarkers with intra-nephron specificities, i.e., glomerular vs. tubular (proximal vs. distal), which can localize an injury in different nephron areas. Additionally, this paper provides a comprehensive analysis of the potential clinical applications of biomarkers in the prediction, detection, differential diagnosis and assessment of post-RT non-surgical allograft complications. Lastly, we focus on the pursuit of immune tolerance biomarkers, which aims to reclassify transplant recipients based on immune risk thresholds, guide personalized immunosuppression strategies, and ultimately identify patients for whom immunosuppression may safely be reduced. Further research, validation, standardization, and prospective studies are necessary to fully harness the clinical utility of RT biomarkers and guide the development of targeted therapies.

Keywords: organ/renal/kidney transplantation; precision/personalized medicine; diagnostic/predictive non-invasive biomarkers; immunopathology of acute allograft rejection (AR); chronic allograft dysfunction (CAD); glomerular vs. tubular nephron damage; clinical operational tolerance (COT); delayed graft function (DGF); ischemia–reperfusion injury (IRI)



Citation: Novacescu, D.; Latcu, S.C.; Bardan, R.; Daminescu, L.; Cumpanas, A.A. Contemporary Biomarkers for Renal Transplantation: A Narrative Overview. *J. Pers. Med.* **2023**, *13*, 1216. <https://doi.org/10.3390/jpm13081216>

Academic Editor: Kenneth P. H. Pritzker

Received: 27 June 2023

Revised: 18 July 2023

Accepted: 28 July 2023

Published: 31 July 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Renal transplantation (RT) is currently the optimal treatment option for patients with end-stage renal disease (ESRD), providing survival benefits, improved health-related quality of life, and cost-effectiveness, compared to dialysis [1]. Despite significant improvements in immunosuppressive therapies and surgical techniques, pervasive challenges still remain unaddressed regarding the complex, multi-modal, clinical management of RT patients. These challenges include early detection of graft dysfunction, timely identification of rejection episodes, personalization of immunosuppressive therapy, and prediction of long-term graft survival. Recently, biomarkers have emerged as valuable tools in addressing these challenges, offering the potential to revolutionize the clinical management of RT patients.

Recent advancements in immunosuppressive therapy have reduced acute rejections (ARs) and improved short-term renal allograft half-life [2]. Even so, late allograft loss still constitutes a major clinical issue post RT [3]. Current monitoring of renal allograft function relies upon serum creatinine measurement and needle-core renal biopsy, both of which have limitations. Creatinine levels rise only in later stages of allograft injury and cannot differentiate between specific injury types or predict chronic injury progression. Needle-core renal biopsy, though considered the gold standard, is invasive, cannot be safely performed repeatedly to monitor injury progression, has potential sampling biases, carries a 1–2% risk of significant complications, and its overall predictive power is poor in RT patients [4,5]. In fact, molecular-level tissue sample examination can detect immune response abnormalities before they become histologically evident [6]. The development of non-invasive, reliable, and predictive biomarkers for early diagnosis and monitoring of clinical conditions post-RT is essential for personalized treatment.

Biomarkers represent measurable objective indicators of normal biological processes, pathogenic visceral responses, and/or therapeutic interventions [7], and thus may also provide critical information about the state of the transplanted organ, i.e., the kidney allograft. Assays for proteomic, metabolomic, transcriptomic, and genomic biomarkers, derived from various biological sources, i.e., donor/recipient peripheral blood/serum/lymphocytes or urine, and tissue biopsy specimens have been extensively explored due to their notable clinical potential in RT, namely, to monitor allograft function, detect early rejection, guide immunosuppressive treatments, and predict long-term allograft survival and RT patient outcomes. The inclusion of validated gene transcripts/classifiers in the Banff classification for rejection highlights the growing importance of biomarkers in post-RT pathology [8]. Thus, it is becoming increasingly clear that further integration of these emerging biomarkers into clinical practice could significantly improve patient care and potentially optimize RT outcomes.

Generally speaking, biomarkers could, at least in theory, play a host of essential clinical roles throughout each step of the entire RT process [4], namely: (1) preoperative donor assessment and kidney allograft retrieval—prediction of short-term outcomes/risk of postoperative complications, i.e., delayed graft function (DGF); (2) in the perioperative setting—assessment, identification and characterization of subacute and/or AR processes, thus enabling more timely interventions; (3) postoperatively, for the crucial differential diagnosis between true chronic rejection (CR) vs. chronic allograft dysfunction (CAD)—similar clinically, yet require completely different treatments, with CR being immunologically mediated, whereas CAD is usually the result of various non-immunological pathogenic factors; (4) long-term monitoring of allograft injury occurrence [4]. Furthermore, biomarkers associated with RT patient immune tolerance are also highly coveted and of great importance for clinical management, as they could potentially allow for the progressive tapering or even complete discontinuation of postoperative immunosuppression, thus further reducing the risk of treatment-associated side effects and complications.

Beyond specific clinical context, RT biomarkers can be classified based on their individual capacity to assess immunological vs. non-immunological outcomes. Immunological outcomes are primarily related to rejection and immune tolerance, whereas non-immunological outcomes are mainly related to tissue injury [9]. Conversely, regarding nephron targeted biomarkers, a further classification based on individual histological nephron component specificity, i.e., glomerular vs. tubular, may also prove useful for the better characterization of pathogenesis and a more nuanced understanding of non-specific patient manifestations [10]. However, non-invasive biomarkers are indeed the primary candidates for clinical application in RT management, due to their inherent practicality, ease of assessment and minimal patient discomfort. Promisingly, non-invasive assessments for RT patients currently include: messenger (m)RNA transcripts; lymphocyte phenotype markers; chemokines; micro(mi)RNA; and donor-specific antibodies (DSA), i.e., antibodies that react specifically to antigens from the organ donor [4].

Notwithstanding the potential benefits of RT biomarkers, their clinical application is not without challenges. Overall, the actual utility of RT biomarkers in real-life patient management is largely dependent on their individual evaluation metrics, such as: sensitivity; specificity; positive predictive value; negative predictive value; receiver operating characteristics (ROC) curves. These metrics help determine the biomarker's precision and reliability in identifying a condition or predicting an outcome, which are critical for guiding clinical decisions [11,12]. Moreover, validation of biomarker assay results can be affected by inter-observational variability (differences in results between evaluators) and inter-laboratory or inter-platform methodological heterogeneity (differences in results due to variations in laboratory methods or testing platforms). These can create discrepancies in biomarker measurements, limiting their predictive power and possibly leading to result misinterpretation [13]. Therefore, before new biomarkers can be confidently integrated into clinical practice, they must undergo thorough validation studies and assay standardization. Validation studies test the biomarker in a large, diverse patient group to ensure its accuracy and reliability across different clinical scenarios. Assay standardization ensures the methods used to detect/measure the biomarker are consistent and reproducible, providing dependable results regardless of where or when the test is conducted.

In summary, while biomarkers hold significant potential for improving patient outcomes in RT, their development, validation, and application involve careful consideration of various factors, including their predictive accuracy, ease of measurement, and consistency across different individuals and testing environments. As research in this area continues, the aim is to overcome these challenges and harness the full potential of biomarkers in guiding personalized care for RT patients, with scientists and physicians seeking non-invasive ways to detect allograft issues early and guide management and prognosis of both allograft and patient outcomes.

The objective of this review is to provide a comprehensive analysis of the contemporary clinical applications of biomarkers in the management of RT patients, focusing on post-RT non-surgical allograft complications. By synthesizing the currently available scientific medical literature, we aim to shed light on the most promising biomarkers, their main biological characteristics and their potential roles in improving clinical decision-making and RT patient outcomes.

2. Immunopathology of Nephron Injury and Allograft Rejection

The recent clinical introduction of more potent immunosuppressive drugs has resulted in a decreased incidence of AR. Nonetheless, about 10% of kidney transplant recipients still experience an AR episode within the first year after RT [14]. Although these episodes can generally be treated with intravenous steroids and/or anti-thymocyte globulin, their occurrence can have a negative impact on graft outcome. Routine immunologic laboratory tests are already being used to determine a patient's immunologic sensitization and to assess the risk of adverse graft outcomes. The complement-dependent cytotoxicity test, performed pre-RT, has significantly reduced the incidence of hyper-acute rejection [15]. Similarly, pre-RT human leukocyte antigen (HLA) alloantibody screening aids in optimizing donor selection. Post-RT HLA alloantibody screening assists in identifying the specific type of AR and potential antibody impact on graft function [16].

In fact, AR episodes, which are most prevalent in the first few weeks after transplantation, can be categorized into T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) [17]. Essentially, TCMR involves lymphocyte infiltration and proliferation within the interstitial space of the kidney allograft, which will subsequently induce cytotoxic effects on renal tubular epithelial cells, causing inflammatory responses, i.e., "tubulitis". Similarly, vascular rejection, a more severe variant of TCMR, involves mononuclear cells invading arteries, leading to arteritis and potentially severe transmural necrosis of allograft vasculature. Conversely, in ABMR, DSA target HLAs or non-HLAs on the donor endothelium, leading to antibody-dependent cellular cytotoxicity and complement activation [16].

DSA refer to the antibodies that a transplant recipient forms against specific HLA antigens found on the donated organ. These antibodies can inflict allograft nephron damage, by inducing multi-lamination of the peritubular capillary basement membranes, or arteriopathy that manifests as intimal fibrosis [18]. The DSA endothelial cell injury can trigger platelet aggregation and leukocyte recruitment, potentially leading to graft failure. Thus, when the allograft is subjected to rapid surges in high-titer DSA, AR occurs, usually either in sensitized recipients, or as de novo responses in non-sensitized patients who do not strictly adhere to their immunosuppressive treatment. Alternatively, CR mediated by DSA occurs in response to a slower emergence of these antibodies, which can be of high or low titer and may be either transient or persistent [18].

While substantial research is being conducted to develop therapeutic strategies aimed at reducing DSA levels [19], our current understanding of how to prevent the initial formation of DSA is still limited. Moreover, risk factors associated with DSA development are not fully defined. Early evidence suggests that specific immunosuppressive treatments could influence DSA formation [20]. Specifically, it appears that treatments based on calcineurin inhibitors are less likely to be associated with DSA formation compared to those based on mTOR inhibitors or lower mycophenolic acid levels [21].

Clinically, microcirculation lesions, C4d deposition in peritubular capillaries, and the presence of DSA in the patient's serum suggest ABMR. However, DSA can be identified in the serum of RT recipients many years before any signs of clinical graft dysfunction appear. Hence, it is crucial to routinely monitor DSA in the follow-up of transplant recipients, even though uniform protocols are not yet in place [22]. Moreover, the onset of de novo DSA (dnDSA) post-RT has been firmly linked to poor graft outcomes in adults as well as children [22,23]. The formation of dnDSA, in general, is associated with lower 10-year graft survival rates, even in pediatric studies [22]. Managing the aftermath of chronic (c)ABMR is typically even more challenging. Given this information, dnDSA are recognized as reliable biomarkers that can predict late acute ABMR, cABMR, transplant glomerulopathy, and graft loss [24]. Even so, their clinical significance is contingent upon certain characteristics of the antibody itself, such as its IgG subclass, which affects its capacity to bind complement cascade components and engage effector cells through Fc receptor binding. For instance, IgG3 subclass dnDSA can bind to complement component (C)1q more efficiently, activate the classical pathway of the complement cascade, and often lead to acute ABMR, whereas IgG4 DSA, which cannot bind Cs, primarily operate through the Fc receptor to magnify alloresponses [25].

Indeed, as detailed in Figure 1, the transplant recipient's adaptive immune system plays a central role in allograft TCMR. Thus, within this process, alloreactive T-lymphocytes, which represent between 1 and 10% of T-lymphocytes overall, interact with mismatched HLAs on donor-derived antigen-presenting cells (APCs) [26]. This interaction, known as direct allorecognition, and the subsequent interaction between recipient APCs and CD4+ T cells, known as indirect allorecognition, promote T cell proliferation and differentiation [27]. Activated CD8+ T cells release perforin and granzyme B, which induce apoptosis of target cells [28], while monocytes and myeloid dendritic cells (DCs) infiltrate the graft and contribute to AR [29,30]. However, innate immunity also plays a role in transplant injury, via intra-allograft complement cascade activation.

Normally, the innate immune system provides a general defense against foreign pathogens by employing the complement system and cellular responses from macrophages and DCs. These cells possess Toll-like receptors (TLRs) that can identify pathogen-related molecular patterns on invading microbes [16]. Importantly, post RT, ischemia reperfusion injury (IRI) is, at least to a certain degree, virtually unavoidable, due to the inherent conceptual and methodological limitations of contemporary surgical strategies [31]. Thus, as detailed in Figure 1, the process of post-RT allograft TCMR damage is initiated by this pervasive associated mechanism of IRI, which determines tubular cellularity apoptosis, causing the subsequent release of damage-associated molecular patterns (DAMPs). These post tubular injury DAMPs, typically concealed within healthy cells, will then bind to TLRs

on DCs, triggering their activation and maturation [32,33]. Furthermore, IRI can also lead to local activation of the complement cascade. The DCs present donor-derived human leukocyte antigen (HLA) target epitopes and co-stimulatory molecules to naïve T-cells, leading to the differentiation of these cells into interferon (IFN) γ -producing T-helper (Th)1 cells. This, in turn, will further stimulate the maturation of other additional recipient DCs, induce macrophage activation and recruitment, and direct the differentiation of CD8+ T-cells. Concurrently, IRI can also induce a local increase in complement component 3 (C3). When C3 is cleaved by the alternative pathway, C3b is deposited on cellular membranes, instigating the activation of the complement cascade. The breakdown of C3 leads to the release of small fragments, i.e., C3a and C5a, during complement activation, both of which have pro-inflammatory effects. The subsequent formation of the membrane attack complex (MAC) results in lysis of the targeted cell and further release of DAMPs [16].

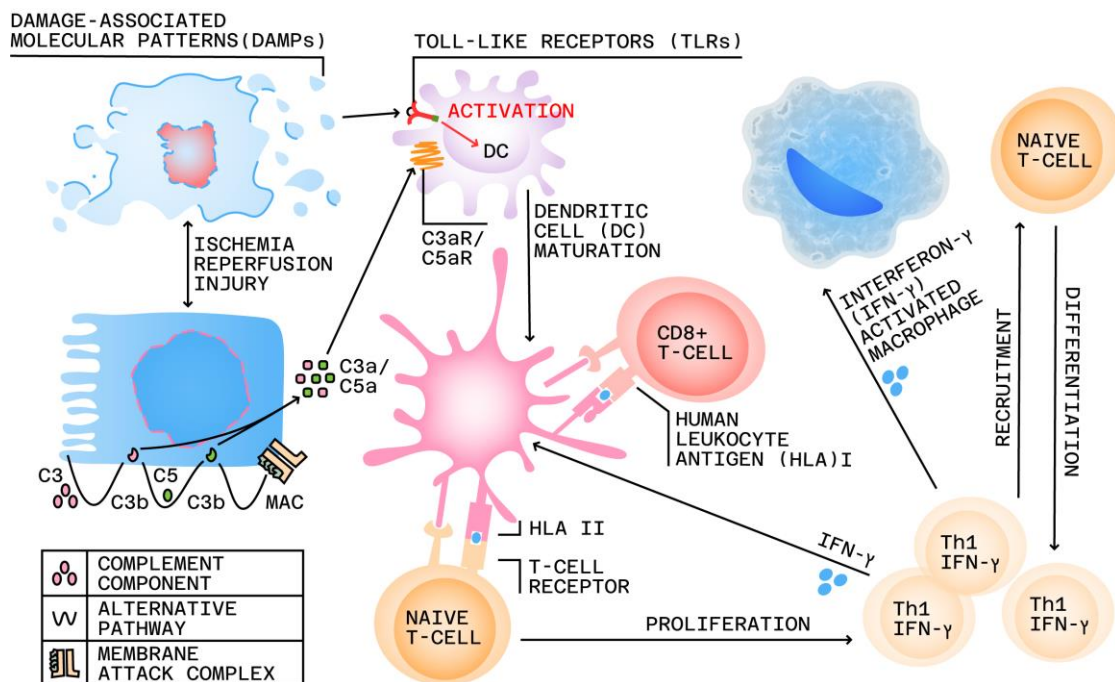


Figure 1. The pathogenic role of innate and adaptive immunity in renal allograft damage. Adapted from Eikmans et al. [16].

3. Glomerular vs. Tubular Biomarkers for Allograft Nephron Damage Assessment

Following RT surgery, the kidney allograft may either immediately resume normal functionality or experience a delay of several days or even weeks, i.e., DGF. A lack of normal kidney transplant function can lead to acute kidney injury (AKI) [34,35], nephrotic syndrome (NS) [36,37], and aggravation of pre-existing chronic kidney disease (CKD) [38]. Thus, post RT, it is crucial to monitor specific biomarkers that can detect disease progression and identify which kidney functions are at risk, facilitating the prompt implementation of appropriate treatments [39–41]. The administration of immunosuppressants to prevent renal graft rejection can, ironically, lead to progressive renal tissue damage (such as interstitial fibrosis, tubular micro calcifications, and renal tubule atrophy), due to the high toxicity of these drugs. The majority of renal pathological changes affect the glomeruli, proximal and distal tubules, as well as the vascular endothelium.

Renal proximal tubular cells (Figure 1), which have the highest metabolic activity and contain large amounts of mitochondria, lysosomes, and peroxisomes, are typically the first to suffer damage. Other sections of the nephron, such as Henle’s loop, distal tubules, and collecting tubules, usually sustain damage later on. There are many biomarkers available to identify injury in different areas of the renal nephron, such as the glomerulus, or the proximal and distal tubules [10]. In Figure 2, we provide a schematic summary of current

conventional non-invasive clinical biomarkers, which have intra-nephron specificities, i.e., glomerular vs. tubular (proximal vs. distal).

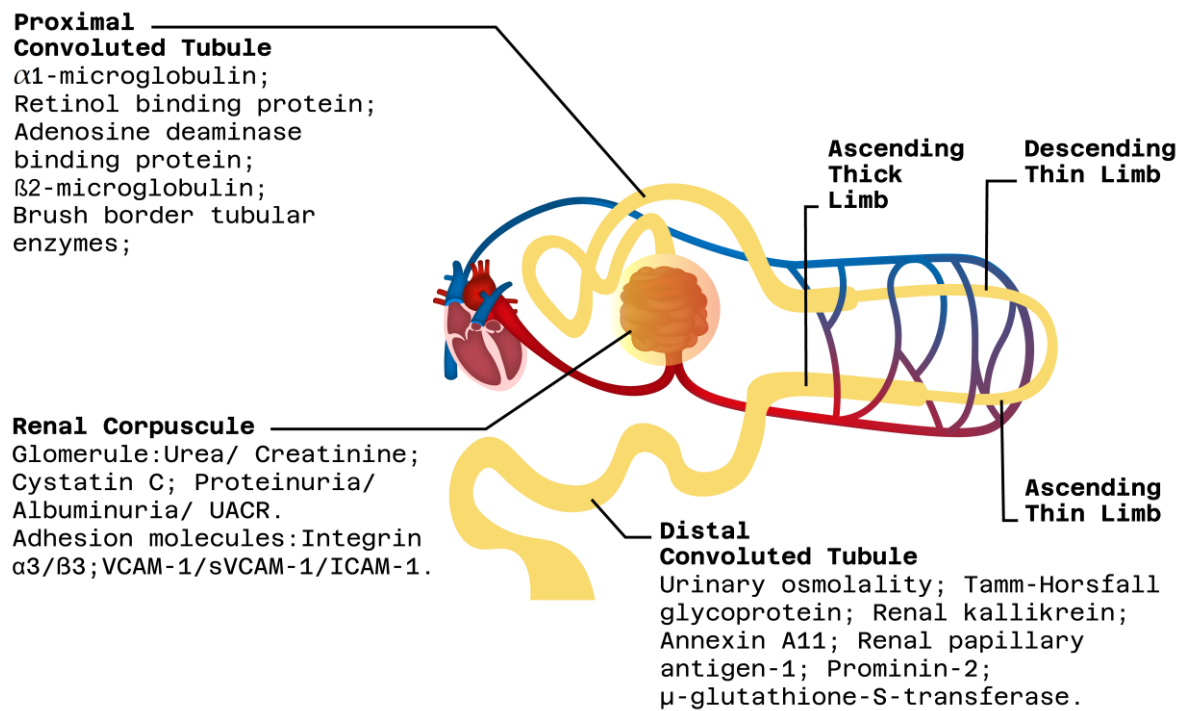


Figure 2. Classification of conventional post-RT allograft injury biomarkers, according to nephron component specificity [10].

All in all, scientific advancements in molecular biology, i.e., novel genomic, transcriptomic, proteomic, and metabolomics experimental data, have revealed an array of new, nephron-segment-specific, post-RT biomarkers for allograft damage. There are high hopes for proteins that present nephron specificities or are locally produced at the site of nephron damage. Traditional biomarkers, particularly enzymuria, still hold diagnostic value in assessing renal tubule function. While this abundance of biomarkers, in particular, may in fact reflect that their individual diagnostic value may be limited, the search for a universal integrative biomarker for allograft assessment remains challenging. Instead, identifying putative biomarker proteins useful in diagnosing key allograft disease features is likely to yield better results [10].

In Table 1, we provide additional data regarding the classification, definitions and currently available supporting data for these nephron-component-specific biomarkers, in hopes of providing clinicians with additional useful evidence regarding the early detection of nephron damage post RT.

Table 1. Biomarkers for monitoring nephron damage post-RT. NB: ↑—increased; ↓—decreased. Adapted after Kepka et al. [10].

	Biomarker	Clinical Evidence
Renal Corpuscle	Urea/ Creatinine	The oldest biomarkers of glomerular injury [42], urea (protein metabolism) and creatinine (muscular metabolism) are both byproducts filtered by the glomeruli and excreted in urine. ↑ serum urea/creatinine, i.e., nitrogen retention, are indicative of ↓ GFR and renal dysfunction.
	Cystatin C (CYC)	CYC = Low-molecular-weight cysteine protease inhibitor [43], secreted by all nucleated cells at a constant rate, freely filtered by the glomerulus and completely reabsorbed by proximal tubule cells, i.e., negligible amounts in final urine [44]. Normal range of 0.05–10.47 mg/L [45]. ↑ urinary levels may indicate proximal tubule dysfunction and tubule-interstitial disease [39].
	Proteinuria	Proteinuria is caused by ↑ filtration of plasma proteins and ↓ proximal tubular reabsorption [46,47]. Levels ≥ 0.5 g/24 h = independent risk factor for progressive tubular-interstitial fibrosis and a strong predictor of ESRD [48–51]. It may indicate established renal damage associated with ↓ GFR [52]. Post-RT, signals poor renal graft function and potential graft failure [53].
	Albuminuria	Urinary albumin (the main plasmatic protein) is a more sensitive marker of GFR than proteinuria [54]. Micro-albuminuria (<200 µg/min) is a better indicator of kidney transplant condition than proteinuria [55]. It predicts renal graft loss and offers early detection of allograft changes [56], but also reflects both glomerular and chronic allograft damage and may be indicative of interstitial inflammation [57]. Thus, it is also considered a predictor of long-term allograft outcomes in RT recipients. Urine albumin-to-creatinine ratios (UACRs) are also recommended for post-RT monitoring [57].
	Adhesion Molecules (between Podocytes & Basal Membranes)	Integrins = transmembranary glycoproteins with two subunits, α and β, which promote cellular attachment/migration/invasion, along the surrounding extracellular matrix (ECM), and are necessary to maintain cellular survival and functions. The β1 subtypes = major class of cell substrate receptors, specifically binding collagens, laminins, and fibronectins [58]. Integrin α3/β3 are particularly recommended for monitoring the function of allografts, both in the early and long-term setting, after RT [58]. Vascular cell adhesion molecule-1 (VCAM-1), Soluble vascular cell adhesion molecule 1 (sVCAM-1)/(CD106), and Anti-intercellular adhesion molecule-1 (ICAM-1), as members of the immunoglobulin (Ig) superfamily, are the chief endothelial cell proteins recognized by white cell integrins [10]. ↑ urinary sVCAM-1, interleukin (IL)6, sIL6R, and tumor necrosis factor (TNF)R1 levels in the first 2 weeks post RT indicate AR [59]. ↑ urinary sICAM-1 levels have also been reported in AR patients [60]. RT patients with proteinuria showed ↑ sVCAM/sICAM urinary levels [61]. Currently, the determination of cell adhesion molecules is recommended as a non-invasive monitoring tool for AR post RT [62].

Table 1. Cont.

	Biomarker	Clinical Evidence
Proximal Tubules	α1-Microglobulin (α1M)	α1M = 27 kDa glycoprotein from the lipocalin family, structurally related to retinol binding protein (RBP), synthesized by liver cells, with various functions, i.e., immunoregulation by binding to T and B lymphocytes, and involvement in heme-complex catabolism [39,62,63]. Stability in acidic urine makes it a sensitive indicator of proximal tubule renal damage, i.e., ↑ urinary levels may be a consequence of GF deterioration. An ↑ α1M/creatinine ratio = an early and sensitive indicator of poor allograft function/prognosis/long-term survival after RT [64], i.e., 6 months post RT, 32% had microalbuminuria.
	β2-Microglobulin (β2M)	β2M = 11.8 kDa protein, part of the major histocompatibility complex (MHC) class I molecules, found on the surface of all nucleated cells [63]. It undergoes GF and then is reabsorbed and catabolized in the proximal tubules. β2M excretion is typically used to evaluate nephrotoxic damage, such as that caused by aminoglycoside antibiotics or heavy metal salts. Urinary β2M can be helpful in evaluating the state of a transplanted kidney, yet the interpretation of results should be done with caution due to the variety of factors that can influence β2M plasmatic/urinary concentration, renal filtration ability, and tubular function, i.e., drugs, ischemia–reperfusion complications, or true renal graft rejection [64].
	Retinol binding protein (RBP)	RBP = 21 kDa protein belonging to the lipocalin family, primarily synthesized in the liver, it mainly transports retinol (vitamin A) from the liver to peripheral tissues [63–66]. RBP is filtered by the glomerulus, and then reabsorbed and catabolized in proximal tubules. ↑ urinary RBP can be a result of impaired GF and/or reabsorption in the renal proximal tubules. Due to its greater stability in acidic urine, RBP is considered a better biomarker for proximal tubule damage than β2M [10].
	Brush Border Tubular Enzymes (↑urinary Excretion = Tubular Brush Border Membrane Damage/ Microvillus Loss [29])	<p>Adenosine deaminase binding protein (ABP) = 120 kDa glycoprotein found in various tissues such as lungs, liver, placenta, and brush border of renal proximal tubules. It is involved in the regulation of adenosine levels and has been implicated in several physiological and pathological processes [67]. ↑ urinary ABP is considered an early indicator of AKI and has been reported in various clinical situations, such as ischemia without sepsis, RT, toxic renal tubular damage, and neonatal sepsis. Some researchers have suggested that ABP may be the best marker of acute renal damage, even better than β2M or α1M [67]. Due to higher ABP excretion in RT recipients compared to those with normal renal function, ABP is a good indicator for detecting graft failure [68].</p> <p>Alkaline phosphatase (AP) = 140 kDa membrane-bound glycoprotein found in various tissues, including renal proximal tubular structures. AP is involved in the metabolism of organic phosphates [69]. One common reason for declining function in allografts post-RT is the nephrotoxicity of chronic immunosuppressive therapy. ↑ urinary AP levels can be a sign of renal damage due to the use of immunosuppressive drugs, i.e., usually Cyclosporine A [69].</p> <p>γ-glutamyl-transferase (GGT) = ubiquitous enzyme found in the cell membranes of numerous tissues such as kidneys, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles. It plays an integral role in amino acid transport across the cell membrane and in the metabolism of leukotrienes. Notably, GGT is also involved in maintaining the balance of oxidative stress within the cell by participating in glutathione metabolism. ↑ urinary GGT provides reliable evidence of nephrotoxicity, such as that caused by prolonged use of anti-rejection drugs in RT patients. An absence of GGT/enzymes in urine suggests a return to normal function of the renal tubules [65].</p>

Table 1. Cont.

	Biomarker	Clinical Evidence
Cytosolic/Lysosomal Tubular Enzymes		Alanyl-aminopeptidase (AAP) , an enzyme that degrades oligopeptides, when ↑ in urine, is associated with severe conditions such as acute renal tubular necrosis, rejection of renal graft, or the toxic effects of immunosuppressive drugs [39,65,70].
		α-/π-Glutathione-S-transferase (α-/π-GST) = a specific cytosolic enzyme of tubular epithelial cells, which consists of two main isoenzymes: α-GST that thrives in alkaline pH, and π-GST which prefers an acidic pH. The α-GST is found in the epithelium of proximal tubular cells, and the π-GST in distal tubules [71]. The determination of α-GST and π-GST in urine is utilized for diagnosing acute renal graft rejection with acute tubular necrosis [65]. A differentiated increase in the urinary excretion of α-GST vs. π-GST may indicate the location of nephron damage [45,71–75].
		N-acetyl-β-D-hexosaminidase (HEX) = a lysosomal renal enzyme and one of the most commonly determined urinary markers for tubular damage, i.e., HEX activity increases early on, prior to the onset of disturbances in renal excretion. Mainly found in proximal tubular cells, HEX is thus specific, i.e., ↑ molecular weight (>130 kDa) prevents glomerular filtration [76,77]. During active kidney disease, HEX activity consistently rises. ↑ urinary activity of HEX/its isoenzyme HEXB indicates damage to renal tubular cellularity. Thus, urinary HEX and, particularly, HEXB, may serve as specific markers for proximal tubular damage post-RT [76,77].
Distal Tubules		Fructose-1,6-bisphosphatase (FBP-1,6) = primarily localized in the convoluted and to a lesser extent in the straight portion of proximal renal tubules. Similar to HEX and GST, it indicates the precise location of allograft nephron damage [73,78]. ↑ urinary FBP-1,6 was observed post-RT. Urinary FBP-1,6 excretion was significantly lower in patients with a median cold ischemia time of <22 h, compared to those with >22 h. Even in the absence of graft dysfunction, if the cold ischemia period is extended, urinary excretion of FBP-1,6 correlates with the extent of damage to the renal tubules [79].
	Urinary Osmolality	Urine osmolality refers to the concentration of solutes in urine, and is regulated by the activity of antidiuretic hormone (ADH) in the distal nephron [80]. An important parameter for evaluating the function of distal renal tubules. ↓ urinary osmolality suggests the presence of distal tubular dysfunction [80].
	Tamm-Horsfall Glycoprotein (THP)	THP , i.e., uromodulin = protein synthesized by renal tubular cells in the thick ascending limb of Henle’s loop and the distal convoluted tubule. THP is the most abundant protein in normal urine, and its concentration is directly proportional to the number of functioning nephrons [81]. A ↓ THP excretion is a sensitive indicator of tubular dysfunction in patients with CKD [81].
	Renal Kallikrein	Renal kallikrein = an enzyme that regulates blood pressure and sodium excretion in the kidney [82]. Urinary kallikrein is considered a sensitive marker of distal tubular dysfunction, and its levels have been shown to decrease in various types of renal disease [82].

Table 1. *Cont.*

Biomarker	Clinical Evidence
Annexin A11 (ANX11)	ANX11 is a calcium-binding protein that is found in high quantities in distal tubular cells and glomerular epithelium. ANX11 has been identified as a useful marker of acute and chronic renal graft rejection [58].
Renal Papillary Antigen (RPA)-1	RPA-1 is a sensitive and specific antigen of renal papillary cells, i.e., a useful marker of damage to renal collecting tubules. RPA-1 has been shown to be a sensitive and specific urinary marker of renal papillary cell injury in both animal models and humans [75].
Prominin-2 (PROM-2)	PROM-2 is a cellular membrane glycoprotein (112 kDa), with peak expression in epithelial cells of fully developed kidneys, i.e., a cholesterol-binding protein, associated with apical and basolateral plasmalemma protrusions in polarized renal epithelial cells that is released into urine [83]. PROM-2 has been identified as a novel biomarker, specific for distal tubules and collecting ducts, in human and murine kidneys, useful biomarker for the functional assessment of distal renal tubules [84].
μ -Glutathione-S-Transferase (μ -GST)	μ -GST is a conjugating glutathione present in tubular epithelial cells, i.e., mainly the ascending part of Henle’s loop [75], alongside π -GST. It represents a nephrotoxicity-specific biomarker. μ -GST is an early biomarker for Henle’s loop and distal tubule damage, and has been shown to be more specific than albuminuria for assessing nephrotoxicity [85]. \uparrow urinary μ -GST levels can be observed in response to treatment with nephrotoxic drugs, such as cisplatin.

Multiple promising biomarkers for kidney damage have been identified, with the most relevant and best-studied being neutrophil gelatinase-associated lipocalin (NGAL), CYC, kidney injury molecule-1 (KIM-1), β 2M, and interleukin-18 (IL-18) [86]. Notably, in kidney allograft recipients, urinary KIM-1 expression provides prognostic information related to the rate of renal function decline, regardless of the underlying kidney pathology [87]. However, validation of these kidney markers in various pathological conditions is still ongoing. High diagnostic value is still held by certain enzymes in diagnosing renal diseases, such as HEX and its isoenzyme HEXB as markers of proximal tubular damage, AAP or GST as markers of the tubular brush border membrane, and cytosolic FBP-1,6 for assessing graft function [10]. A panel of urinary proteins and enzymes may serve as a practical marker for evaluating the nephron function of a transplant kidney and prognosticating the renal allograft’s fate. Future biomarker discoveries and research techniques may change the practical approach to treating patients with renal grafts.

4. Biomarkers for Non-Surgical Renal Allograft Complications

Postoperative monitoring of RT patients is a critical aspect of care management [88]. Currently, the standard of care recommended is quarterly measurements of urinary protein

excretion, within the first year. Moreover, screening for viral infections, i.e., Polyoma and/or Epstein–Barr virus, using plasma nucleic acid testing, should be done monthly, for at least the first three months post RT, and then every three months, until the end of the first year. A percutaneous renal allograft needle biopsy is necessary if there is an unexplained rise in serum creatinine. The Banff classification system provides standardized criteria for histological diagnosis of AR, scoring inflammation in various renal compartments [8]. However, changes in serum creatinine are not specific to graft injury: variations might indicate an intrinsic renal process like AR or graft infection, or a transient process such as the hemodynamic effects of calcineurin inhibitors or pre-renal volume depletion [88]. AR involves various stages, with clinical signs of graft damage appearing late, following a period of subclinical graft damage [89,90]. Thus, serum creatinine levels may remain unchanged despite significant kidney injury.

Moreover, biopsies can also lead to complications for the transplant recipient [91], and being an in-patient procedure, can be quite costly. Other drawbacks of allograft biopsy include potential sampling errors and/or differences in interpretation among pathologists [92]. Therefore, there is a pressing need for alternative, less invasive, yet more sensitive, post-RT biomarkers for diagnosing acute graft rejection, i.e., subclinical allograft nephron damage. Discovering and validating biomarkers that correlate with and/or can predict AR early on, thus capable of enhancing the objectivity, accuracy and overall efficacy of therapeutic decision making for clinicians, are high priorities among most ongoing RT research initiatives [13]. Through regular sampling, the development of rejection might be predicted before tissue injury actually develops. Biomarker information could also help differentiate high-risk patients from low-risk ones, facilitating individualization of immunosuppressive drug therapy.

4.1. Acute Allograft Complications

Post RT, the transplanted renal allograft may be vulnerable to several acute insults, including immunologic injury, IRI, medication related nephrotoxicity, and surgical complications [93]. In the acute context, IRI, in particular, represents, to some degree, an inevitable postoperative occurrence following RT, and can have an impact on both short-term and long-term allograft outcomes [4].

4.1.1. Delayed Allograft Function

The clinical consequences of IRI may include DGF and allograft rejection, i.e., AR, CR, and/or CAD [94]. The severity of IRI is influenced by various donor/recipient-specific factors, as well as associated organ storage conditions [95]. The utilization of extended eligibility criteria for donors and of organs from deceased donors, increases the risk of severe IRI [4]. It is crucial to understand the factors that contribute to severe IRI in order to assess the risk to recipients and diagnose IRI promptly. This enables the implementation of preventive and treatment measures, to diminish the subsequent DGF and prevent rejection. The identification of biomarkers for IRI and IRI derived DGF can aid in these efforts.

Several molecules, indicating allograft tubular and/or vascular damage, have demonstrated associations with the occurrence and severity of IRI [4]. In turn, the severity of IRI influences the occurrence of DGF [96], with graft survival being closely linked to the occurrence of DGF [97]. In Table 2, we provide a summary of the currently available evidence regarding the use of contemporary IRI/DGF-associated biomarkers in various clinical settings.

Table 2. Summary of evidence regarding emerging predictive biomarkers for IRI/DGF [98–117].

		Pre-RT Applications	Post-RT Applications
Ischemia–reperfusion Injury (IRI)/ Delayed Graft Function (DGF)	Proteomic Data	<p>Donor urinary biomarkers:</p> <p>→ NGAL, KIM-1, and fatty acid binding protein (FABP) levels have predictive value for DGF [98–100];</p> <p>Recipient cytokines:</p> <p>→ Plasma levels of soluble interleukin 6 receptor (sIL-6R) and low soluble gp130 correlate with DGF [101];</p> <p>Recipient circulating regulatory T-cells:</p> <p>→ Expressing tumor necrosis factor receptor 2 (TNFR-2), as a peripheral blood DGF predictor [102].</p>	<p>Recipient urinary biomarkers</p> <ul style="list-style-type: none"> • NGAL, KIM-1, IL-18 and FABP are specific for AKI and/or IRI [103,104], and are related to renal allograft dysfunction [105–109]; • NGAL/KIM-1 correlate with DGF severity [76]; • NGAL is associated with long-term graft dysfunction, and is predictive of DGF [110]; • IL-18 can predict DGF within 4 h post RT [111].
	Genomic/Transcriptomic Data	<p>Predictive of DGF on pre-RT allograft biopsy samples:</p> <ul style="list-style-type: none"> • Adhesion molecules: ICAM-1 upregulation in tubular epithelial cells (only cadaveric grafts) [112]; • Anti-apoptotic genes, i.e., B-cell lymphoma 2 (Bcl-2)/extra-large (Bcl-xl): lack of upregulation in donor renal tissue [113]; • Complement system, metabolic and immune pathway genes: allograft upregulation [114]; • ↑ lipocalin-2 (LCN)/NGAL [115]; • ↑ cyclin-dependent kinase inhibitor 2A (CDKN2A) [116]. 	<p>MicroRNAs (miRNAs) = short endogenous non-coding RNAs that inhibit gene expression; miR-182-5p and mi-21-3p, have been found to play a role in the pathogenesis of DGF [117].</p> <p>Secretory Leukocyte Peptidase Inhibitor (SLPI): ↑ urine and serum transcript expression levels were reported in post-RT AKI cases [117].</p>

NB: ↑—increased.

4.1.2. Acute Allograft Rejection

To this day, AR still constitutes a major cause of early allograft loss and remains a significant clinical hurdle in post-RT patient management. Current gold-standard methods for diagnosing AR rely on histological examination of renal allograft biopsies, which are invasive and subject to sampling variability. Therefore, numerous studies have focused on identifying non-invasive biomarkers that can predict, preoperatively, the risk of AR occurrence later on, and/or accurately detect AR postoperatively, thus potentially reducing the need for allograft biopsies.

In the pre-transplant setting, serum biomarkers have mainly been explored for their potential to predict AR. Most investigated among them, soluble CD30 (sCD30), is a glycoprotein found on human CD4+ /CD8+ Th lymphocytes, that produce Th2-type cytokines [118]. sCD30 helps identify recipients who may generate an immune response against a transplanted kidney, acting as a predictor of poor graft outcomes [119], often due to a higher incidence of AR [120–124]. Conversely, Th1 immune response is associated with IFN-γ-producing cells and IFN-induced chemokines, i.e., C-X-C motif chemokine ligand (CXCL) 9/10. Several studies have found that the pre-RT frequency of donor-specific IFN-γ-producing cells correlates with AR among recipients of cadaveric kidney allografts [125–128]. Increased serum levels of CXCL10 in recipients have been linked to higher transplant failure due to increased AR incidence [129,130].

As reported in Table 3, post-RT urinary CXCL9 mRNA levels were found to be predictive of AR, with lower levels indicative of low risk for immunological events [131,132]. Several urinary biomarkers were correlated with post-RT allograft injury, including CXCL9,

CXCL10, C-C motif chemokine ligand 2 (CCL2), NGAL, IL-18, CYC, KIM1, and T-cell immunoglobulin/mucine domains-containing protein 3 (TIM3) [133]. Urinary CXCR3 chemokine receptor is emerging as a promising candidate for detecting subclinical inflammation [134]. Furthermore, certain genes in peripheral blood lymphocytes and kidney graft biopsies have been shown to identify patients with AR. These genes relate to immune inflammation, transcription factors, cell growth, and DNA metabolism. Moreover, T lymphocytes and IFN γ -producing Th1 cells are being studied as cellular markers of AR [135,136]. Finally, donor-derived cell-free (ddcf)DNA has been detected in the recipient's blood and urine during AR episodes [137,138].

Table 3. Summary of evidence regarding emerging post-RT biomarkers for AR [4,131,132,139–163].

Postoperative Biomarkers Specific for Acute Renal Allograft Rejection	
Proteomic Evidence	<p>Plasmatic samples:</p> <ul style="list-style-type: none"> ➤ Twelve diagnostic proteins, with a fold-change in ≥ 1.15, in RT patients with biopsy-confirmed AR, were identified: <ul style="list-style-type: none"> - Seven proteins with \uparrow levels: titin (TTN), lipopolysaccharide-binding protein (LBP), peptidase inhibitor 16 (PI16), complement factor D (CFD), mannose-binding lectin 2 (MBL2), protein Z-dependent protease, and $\beta 2M$; - Five proteins with \downarrow levels: kininogen-1, afamin, serine protease inhibitor, phosphatidylcholine-sterol acyltransferase, and sex hormone-binding globulin [139]; ➤ \uparrow Nuclear Factor Kappa B (NFκB), Signal Transducer and Activator of Transcription 1 (STAT1), STAT3, and 63 other proteins showed ≥ 2-fold differences in expression levels between 13 RT patients, with vs. without AR [140]; ➤ \uparrow α-1 antitrypsin (A1AT), α-2 antiplasmin (A2AP) and serum amyloid A (SAA) expression in confirmed AR cases (31 RT cases in total), and apo-lipoprotein CIII (APOC3) expression was exclusive to AR cases [141].
	<p>Urinary samples:</p> <ul style="list-style-type: none"> ➤ Uromodulin (THP), Pigment Epithelium-Derived Factor (PEDF/SERPINF1), and CD44 were indicative of AR in a 60 RT case cohort [142]; ➤ Insulin-Like Growth Factor Binding Protein 7 (IGFBP7), Vasorin (VASN), Epidermal Growth Factor (EGF), and Galectin-3-Binding Protein (LG3BP) levels were found to be \uparrow in 12 RT patients with AR [143]; ➤ Actin β (ACTβ), Dipeptidyl-Peptidase 4 (DPP4), Fibrinogen α Chain Precursor (FGα), Fibrinogen β Chain Precursor (FGβ), Fibrinogen γ (FGγ), Histone Cluster 1 H4 Family Member B (HIST1H4B), HLA class II protein HLA-DRB1 (HLA-DRB1), Keratin 7 (KRT7), KRT14 levels were highly specific for AR, with significant differences within this subgroup, i.e., a fold-increase > 1.5, as compared to all others (154 RT cases in total) [144].
Transcriptomic Evidence	<p>Messenger (m)RNAs:</p> <ul style="list-style-type: none"> ➤ \uparrow Granzyme B (GZMB), perforin (PRF1) and Fas Ligand (FASLG) mRNA levels, in peripheral blood samples and graft tissue biopsies, among 25 AR cases [145]; ➤ \uparrow urinary GZMB/PRF1 mRNA levels in 22 post-RT patients with AR [146]; ➤ \uparrow OX40, OX40L, programmed cell-death (PD)-1 and Forkhead box P3 (FOXP3) mRNA levels in urinary cellularity strongly predict acute kidney transplant rejection, i.e., upregulated AR-specific gene signature in 21 RT cases. Additionally, OX40, OX40L, and Foxp3 mRNA levels predicted reversal of AR, while OX40 alone predicted graft loss post-AR [147]; ➤ \uparrow Foxp3 mRNA urinary levels were found to be AR-specific, when comparing 36 confirmed AR cases with CAN/normal biopsy cohorts [148]; ➤ The Clinical Trials in Organ Transplantation (CTOT)-04 multicenter trial (485 post-RT patients): a three-gene AR-specific urinary signature was reported for biopsy-confirmed AR cases, i.e., \uparrow CD3ϵ mRNA, IFN-inducible protein 10 (IP-10/CXCL10) mRNA, and 18S ribosomal RNA [149]; ➤ CTOT-01 multicenter study (280 post-RT patients): \uparrow urinary CXCL9 mRNA represents the best predictor of AR [131], whereas \downarrow levels may be indicative of a proportionally \downarrow risk of immunological allograft complications [132].
	<p>MicroRNAs (miRNAs):</p> <ul style="list-style-type: none"> ➤ \uparrow miR-181a, miR-483-5p, and miR-557 expression levels, in the serum of 15 post-RT patients, were found to be significant predictive factors for AR [150]; ➤ Urinary miR-210 expression is \downarrow during AR, but normalizes after successful treatment (62 AR cases) [151]; ➤ Serum levels of miR-223 and miRNA10a were shown to be significantly \downarrow during AR (12 RT cases) [152]; ➤ Inhibition of miR-155 and miR-221 is associated with T-cell proliferation, whereas miR-142-3p is associated with tolerant kidney allograft recipients [153].

Table 3. Cont.

Postoperative Biomarkers Specific for Acute Renal Allograft Rejection	
Gene signatures (array technology on multicenter graft biopsies and paired peripheral blood samples):	
Genomic Evidence	<ul style="list-style-type: none"> ➤ SNSO1 clinical trial dataset (367 pediatric RT patients) [154]—A five-gene panel proved to accurately identify patients affected by AR, using microarray with quantitative polymerase chain reaction (qPCR) for confirmation, namely: DUSP1, NKTR, MAPK9, PBEF1 and PSEN1 [155,156]; ➤ AART study (436 adult/pediatric RT patients) [157]—An integrative AR screening panel of 43 rejection-related genes was compiled and assessed in RT recipients, through whole-genome microarray analysis. These genes were selected based on the various principles [155,158], and showed the following results: <ul style="list-style-type: none"> ○ An initial 10-gene panel (CFLAR, DUSP1, IFNGR1, ITGAX, MAPK9, NAMPT, NKTR, PSEN1, RNF130, RYBP), which had previously been found to be significantly associated with AR in pediatric RT patients, i.e., cross-validated by the SNSO1 randomized multicenter trial [154], correctly predicted AR in 87.4% of adult samples, with 93.8% specificity and 74.5% sensitivity; ○ Out of the 43 genes tested, 31 were differentially expressed in adult RT recipients with AR. Eight of these genes were part of the initial 10-gene panel [159]. In the adult dataset, a subset of 15 out of the 43 genes accurately classified 91.6% of the AR and non-AR samples [157]. However, this approach failed to identify AR in a purely pediatric SNSO1 data subset [154,159]. To ensure the test could identify AR regardless of patient age, the original panel of 10 genes was retained, and 7 additional genes (CEACAM4, EPOR, GZMK, RARA, RHEB, RXRA, SLC25A37) were added to optimize the gene set's performance for discriminating AR in both adult and pediatric samples [157]. ○ The resulting optimized 17-gene panel, constituting the Kidney Solid Organ Response Test (kSORT), was used successfully to detect RT patients at high risk for AR, demonstrating better diagnostic accuracy compared to the initial 10-gene panel [157] and apparently being able to predict rejection episodes up to 3 months prior to their clinical detection by the current gold standard methods (biopsy) [160]. ➤ CRM meta-analysis [161]: A common rejection module (CRM), consisting of 11 AR-specific genes, was identified through a meta-analysis of eight independent transplant patient datasets, i.e., 236 graft biopsy tissue samples, from four different organs (heart, kidney, liver, and lung), namely: BASP1, CD6, CXCL10, CXCL9, INPP5D, ISG20, LCK, NKG7, PSMB9, RUNX3, TAP1. These genes were found to be significantly overexpressed in AR-confirmed cases, across all the transplanted organs within the study population. Importantly, this initial high diagnostic capability for AR reported for the CRM, was further validated within this meta-analysis, on 5 different additional transplantation cohorts (945 samples), demonstrating both high specificity and sensitivity when tested. Moreover, in these additional cohorts, CRM genes showed a correlation with the extent of graft injury and also displayed predictive capabilities regarding future graft injury occurrence, as determined by protocol biopsies [161]. Furthermore, external validation initiatives have corroborated the aforementioned results on post-RT patients, as follows: <ul style="list-style-type: none"> ○ The tissue 11-gene CRM (tCRM) qPCR score was found to be significantly ↑ in AR, with the greatest significance for CXCL9/10. It was also correlated with the extent of AR lesions and was predictive of CAD [162]. ○ A recent study evaluated the CRM gene set in the urine of RT patients with acute allograft dysfunction, and found that only 5 out of the 11 genes were highly significant at the time of rejection [163]. However, the urinary common rejection module (uCRM) score was also found to be elevated in other kidney injuries, such as acute tubular necrosis and BK virus nephropathy (BKVN) [163].

NB: ↑—increased; ↓—decreased; AART = Assessment of Acute Rejection in Renal Transplantation; BASP1 = Brain abundant membrane attached signal protein 1-5p15.1; CD6 = CD6 molecule-11q12.2; CEACAM4 = Carcinoembryonic antigen-related cell adhesion molecule 4-19q13.2; CFLAR = CASP8 and FADD-like apoptosis regulator gene-2q33.1; DUSP1 = Dual-specificity phosphatase 1-5q35.1; EPOR = Erythropoietin receptor encoding gene-19p13.2; GZMK = Granzyme K encoding gene-5q11.2; IFNGR1 = Ligand binding chain of the gamma interferon receptor gene-6q23.3; INPP5D = Inositol polyphosphate-5-phosphatase D-2q37.1; ISG20 = IFN-stimulated exonuclease gene 20-15q26.1; ITGAX = Integrin α-X-chain protein-16p11.2; LCK = LCK proto-oncogene, SRC family tyrosine kinase-1p35.2; MAPK9 = Mitogen-activated protein kinase 9-5q35.3; NAMPT = Nicotinamide phosphoribosyl-transferase-7q22.3; NKG7 = Natural killer cell granule protein 7-19q13.41; NKTR = Natural killer cell triggering receptor-3p22.1; PSMB9 = Proteasome subunit beta 9-6p21.32; PSEN1 = Presenilin 1-14q24.2; RARA = Retinoic acid receptor-17q21.2; RHEB = Ras homolog enriched in brain-7q36.1; RNF130 = Ring finger motif-5q35.3; RUNX3 = Runt related transcription factor 3-1p36.11; RYBP = RING1 and YY1 binding protein-3p13; RXRA = Retinoic X receptor α-9q34.2; SLC25A37 = Solute carrier family 25 number 37-8p21.2; TAP1 = Transporter 1, ATP binding cassette subfamily B member-6p21.32.

Overall, as is the case for emerging biomarkers in contemporary renal oncology [164–169], while numerous potential AR biomarkers have been identified through the plethora of recent studies published, their specificity and sensitivity in clinical practice remains to be determined. For instance, ddcfDNA has been found to be increased in confirmed AR cases [138], yet it is also present in other kidney injuries, such as pyelonephritis, making it less specific as a marker for AR [170]. Similarly, the kidney solid organ response test (kSORT) has shown high sensitivity in predicting AR and subclinical AR, but these findings need further validation [157,160]. The common rejection module (CRM), a set of 11 genes found to be overexpressed in AR across different organ transplants, is another promising development, but again, further studies are needed to validate the existing results and determine their clinical utility [161–163]. Most

recently, capitalizing on longstanding, sustained scientific efforts aimed at the molecular characterization of the mechanisms involved in graft rejection after solid organ transplantation, the Molecular Microscope Diagnostic System (MMDx), i.e., a clinical tool which uses mRNA to differentiate between specific AR subtypes, has been investigated in the context of RT. A 77% agreement with histology was shown for TCMR and ABMR, and a 76% agreement for no rejection, when assessed without prior knowledge of histology and HLA profiles. Interestingly, the MMDx showed an 87% agreement with clinical judgment, which is higher than the agreement with histology at 80%. This suggests that the MMDx may offer additional or more nuanced information beyond traditional histology in diagnosing transplant rejection [171].

4.2. Chronic Allograft Rejection vs. Dysfunction

Even though, broadly speaking, the field of RT patient management has seen significant sustained advancements throughout recent decades, the progress achieved was inhomogeneous, i.e., persistently disproportionate outcomes and incidence rates between early acute complications and latent chronic allograft dysfunction. Overall, long-term renal allograft survival rates have been notably lagging behind, as opposed to the ever increasing short-term (1 year) renal allograft survival rates currently reported, and the significantly declining occurrence of AR [3]. As postoperative survival intervals increase, the primary reason for latent renal allograft loss in post-RT patients is a clinical condition known traditionally as Chronic Allograft Nephropathy (CAN), characterized by the gradual non-specific deterioration of kidney transplant function. Despite numerous efforts, CAN's origins remain complex and unattributable to a single cause. This appears to result from a variety of interconnected processes between the host and the transplanted organ, leading to ongoing kidney tissue damage, through both immune and non-immune-mediated mechanisms [172].

Thus, in recent times, CAN has been replaced clinically with the more modern, wider term chronic allograft dysfunction (CAD), facilitating the more accurate identification of true CR cases and allowing a finer distinction between immunological CR and other non-immunological causes of chronic dysfunction, such as drugs and viruses. The most recent genomic and proteomic data highlight the similarity in molecular injury patterns between AR and CAN. There is a so-called "threshold effect" for AR, and during its clinical phase, the molecular injury mirrors what is observed in CAN, albeit at a more intense level. Conversely, the continuous, low-grade immune activation in allograft tissues increases gradually post RT, independently driving the progression of CAN, without requiring overt AR episodes [4]. These findings are further validated by urinary proteomic studies [14,173].

Similarly, from a morphopathology perspective, CAN's clinical manifestation has been redefined and renamed as Interstitial Fibrosis and Tubular Atrophy (IFTA) of unknown origin [174,175]. Histological examination of biopsies shows that IFTA occurs in ~50% of renal allografts, at 1-year post RT, ~70% at 2 years, and virtually all cases after 10 years [176,177]. In corroboration, further data clearly demonstrate a correlation between IFTA's progression and renal function decline [178,179]. However, IFTA's progression is not always linear or predictable, suggesting that aspects of the condition are dynamic. Consequently, there is an urgent need for developing new strategies to disentangle the intricate mechanisms of tissue injury that culminate in the development of CAN/IFTA, allowing for the identification and clinical implementation of a sensitive and reliable biomarker, or panel of biomarkers, able to distinguish AR from other forms of CAD.

In fact, current proteomic data suggest that non-invasive biomarkers may soon play a crucial clinical role in the identification of chronic allograft injury (CAI) and CR post RT. In order to identify relevant biomarkers for CAD, a plethora of proteomic investigations, using variable research platforms, on tissue biopsy, peripheral blood and, most frequently, urinary samples, have already been conducted, analyzing thousands of potential targets [172,180]. Even so, for the most part, these large-scale proteomic efforts have thus far failed to offer reliable genomic validation for the wide array of potentially impactful CAD-specific biomarkers identified, mainly due to inherent study design limitations. For

instance, the study conducted by Quintana et al. [180] relied on single urine samples from 50 subjects: 32 CAD patients (14 with IF and 18 with chronic active-antibody-mediated rejection—caABMR) and 18 controls (8 stable post-RT patients and 10 healthy individuals). Even though >2000 protein signals were assessed, using modern mass spectrometry (MS), and subjected to unsupervised hierarchical cluster analysis, only 14 protein signals were reported as capable of distinguishing between samples from patients with IF vs. true CR, i.e., caABMR. However, these 14 protein ions were only identified by their mass/charge ratio and no further attempt was made to identify the actual proteins. Shortly after, the same author, using a different MS platform, reported on 6000 polypeptide ions assessed in post-RT urinary samples, i.e., 39 CAD patients vs. 32 controls, and found specific uromodulin and kininogen-1 derived peptides were notably more abundant in controls than in CAD patients, marking them as potential diagnostic biomarkers for CAD [181].

Thereafter, in an effort to find urinary proteomic profiles that could predict and distinguish/stratify CAD/IFTA, similar proteomic approaches, derived from the same conceptual premises, i.e., MS assessment of post-RT urinary samples, have been further explored fruitfully. Among urinary samples from 70 post-RT cases, 34 with confirmed IFTA vs. 36 controls with normal renal function, a 11.7 kDa protein, identified as β 2M, has emerged as a highly reliable IFTA detection/screening/diagnostic biomarker, i.e., consistently \uparrow β 2M urinary levels in the confirmed IFTA cohort vs. control [182]. Corroborating these findings, in a seminal study, with a limited cohort (36 cases in total), 2D Fluorescence Difference Gel Electrophoresis (2DE-DIGE) managed to establish the normal urinary proteomic map of stable post-RT patients, while also identifying 21 potential urinary biomarkers, specific for different stages of IFTA, such as: A1AT, α 1-B-glycoprotein, angiotensinogen (AGT), anti-TNF α antibody light chain, β 2M, brevin, heparin-sulfate proteo-glycan, leucine-rich α 2-glycoprotein-1 (LRG1), and transferrin [183].

Moreover, in a very recent investigation of urinary proteomics focused specifically on caABMR-specific biomarkers, urinary extracellular vesicle (EV) changes were assessed, using a combined approach, i.e., label-free liquid chromatography and tandem MS, with Western blot confirmation, in post-RT patients (26 cases with confirmed caABMR, 57 with long-term allograft survival and 10 rejection-free controls). After selecting only high-significance proteins, i.e., with a fold-change \geq 1.5, the study reported six proteins, i.e., apolipoprotein A-1 (APOA1), zinc- α 2-glycoprotein (AZGP1), ceruloplasmin (CP), hemopexin (HPX), polymeric immunoglobulin receptor (PIGR), and transthyretin (TTR), as potential biomarkers for caABMR, able to discriminate between caABMR vs. long-term allograft survival subgroups. Among these proteins, AZGP1 showed specificity for caABMR and was distinguishable from the rejection-free control group, with matching age at transplant, time since transplantation, and graft function [184].

In fact, currently, by analyzing pooled urinary proteins from AR, BKVN, and CAN cohorts, in comparison to stable transplant urinary samples, while using control fold-change criteria of >1.5 for each transplant injury phenotype (AR vs. BKVN vs. CAN/IFTA), proteomic analysis of post-RP patients has already managed to reveal specific proteins associated with each condition, potentially aiding differential diagnosis immensely [144]. As previously noted (see Table 3), in patients with AR, increases in ACT β , DPP4, FGA, FGB, FGG, HIST1H4B, HLA-DRB1, KRT7, and KRT14 proteins were found. For BKVN, there were increases in Complement Factor H Related 2 (CFHR2), Family with Sequence Similarity 3 Member C (FAM3C), Histone Cluster 1 H2B Family Member A (HIST1H2BA), KRT8, KRT18, KRT19, KRT75, Ribosomal Protein L18 (rPL18), Stathmin1 (STMN1), Small Ubiquitin-like Modifier 2 (SUMO2). Lastly, in the case of CAN patients, increased levels of AGT, Calreticulin (CALR), Dystroglycan 1 (DAG1), FABP4, Family with Sequence Similarity 151 Member A (FAM151A), FAM3C, KIT Ligand (KITLG), LRG1, Lumican (LUM), and Serpin Family A Member 2 (SERPINA2P) were observed [4,144]. These specific proteins can therefore be considered as potential discriminatory proteomic biomarkers for different types of transplant injury phenotypes. However, further investigation and clinical trials

would be required to confirm these findings and to evaluate their practical utility in a clinical setting.

Conversely, a particularly promising target discovery investigational strategy appears to be the combination of proteomics and genomics, i.e., proteogenomics [172,185]. Herein, a proteogenomic approach by Kurian et al. focused on discovering CAN/IFTA-specific biomarkers, in peripheral blood samples, collected from 77 post-RT patients, with significant clinical differences among themselves, in the hopes of developing a practical model for post-RT monitoring, based on serial, prospective measurements of the identified target signatures, throughout the lifespan of the renal allograft. Ultimately, this development would allow for optimal immunosuppressive drug management, with the potential to introduce personalized medicine to RT. Several hundred mRNAs and proteomic biomarkers were identified as potentially useful in the differential diagnosis and clinical staging of IFTA. Specifically, 302 proteins unique to mild CAI and, respectively, 509 proteins unique to moderate/severe CAI were reported. Despite the diversity and heterogeneity of patient samples, the predictive accuracy of these biomarkers was quite high, i.e., 80% for mild CAI vs. 92% for moderate/severe CAI [185].

Recent studies have utilized molecular tools such as miRNAs and gene expression analyses to better understand CAD/IFTA. One study identified five specific miRNAs (miR142-3p, miR-32, miR204, miR-107, and miR-211) that were differentially expressed in both allograft tissue biopsies and urine samples of post-RT patients affected by IFTA vs. control [186]. Another set of miRNAs (miR99a, miR-140-3p, miR-200b, and miR-200) were found to be differentially expressed at different time points post RT in relation to graft outcome, being useful for monitoring [187]. Notably, urinary miRNA profiles varied in IFTA patients based on whether they received a kidney from a living or cadaveric donor [188]. Furthermore, relevant for monitoring kidney allograft function in patients affected by IFTA, a study on IFTA renal biopsies showed significant upregulation of miR-142-5p and miR-142-3p, and downregulation of miR-211, as compared to controls (stable graft) [189]. Interestingly, the same results were observed in peripheral blood cells from the same IFTA cohort, suggesting that peripheral blood cells could provide an additional non-invasive method for monitoring graft function [189]. Lastly, in another post-RT study, miR-486-5p was found to be significantly over-expressed in patients who produced DSA and/or had biopsy-confirmed caABMR [190]. This suggests that specific miRNAs might serve as potential biomarkers for graft rejection. The discovery of these miRNA profiles in different patient groups suggests a promising avenue for non-invasive diagnosis and monitoring of post-RT complications.

Genomic studies have also uncovered promising evidence regarding specific CAI biomarkers. Microarray analysis has identified upregulation of 10 genes (fold-change > 6.00) related to fibrosis, extracellular matrix deposition, and immune response, in renal allograft tissues of 11 patients with biopsy confirmed CAD/IFTA, as compared to controls [191]. Using PCR, the markers identified through the microarray analysis in these CAD/IFTA patients, such as transforming growth factor beta (TGF- β), epidermal growth factor receptor (EGFR), and AGT, were examined in urinary/peripheral blood samples, retrieved at the time of biopsy, and shown to be statistically different in urinary, but not in blood samples, when compared to controls [191]. On a larger scale, in the aforementioned CTOT-04 trial, besides the validated three-gene signature (CD3 ϵ mRNA, CXCL10 mRNA, and 18S rRNA) predictive of AR (see Table 3) [149], an additional four-gene signature (vimentin, NKCC2, E-cadherin, and 18S rRNA) in urinary mRNA was reported as diagnostic for IFTA, providing a potential non-invasive biomarker for this condition [192]. Similarly, within another computational gene expression score, the tCRM, a subset of seven genes (CD6, INPP5D, ISG20, NKG7, PSMB9, RUNX3, and TAP1) demonstrated a higher predictive value for the development of IFTA over time [162].

Conversely, the international Genomics of Chronic Allograft Rejection (GoCAR) study, a prospective microarray analysis of gene expression profiles in allograft tissue samples from 159 RT recipients, with stable graft function at 3 months post RT, identified a set of

13 genes that were independently predictive of allograft fibrosis at 12 months after RT. This gene signature, i.e., Ankirin repeat and SOCS box-containing 15 (ASB15), Coiled-coil-helix-coiled-coil helix domain containing 10 (CHCHD10), Four jointed box 1 (FJX1), Kelch-like family member 13 (KLHL13), Kidney-associated antigen 1 (KAAG1), Met proto-oncogene (MET), Retinoid X receptor alpha (RXRA), Ring finger protein 149 (RNF149), Serine incorporator 5 (SERINC5), Sprouty homolog 4 (SPRY4), Suppressor of tumorigenicity 5 (ST5), TGF- β -induced factor homeobox 1 (TGIF1), and Wingless-type MMTV integration site family member 9A (WNT9A), was found to have a superior predictive value for allograft fibrosis development, outperforming clinical and pathological variables [193].

Lastly, a very recent meta-analysis of molecular datasets identified a robust distinctive transcriptional response in IFTA allografts, as compared to non-IFTA cases, i.e., 85-gene signature significantly associated with IFTA. In a novel approach, genomics was thereafter used to identify novel potential therapeutic agents for IFTA. Through computational repurposing analysis of the aforementioned 85-gene signature, besides validation of azathioprine, an already established treatment for AR and pulmonary fibrosis, two promising novel drugs were identified: Kaempferol, which attenuates TGF- β 1, and Esculetin, which inhibits the Wnt/ β -catenin pathway. Preclinical models demonstrated the effectiveness and safety of these drugs, suggesting their potential for therapeutic intervention in IFTA [194]. All in all, these studies highlight the significant potential of molecular tools for diagnosis, prognosis, and treatment of CAD/IFTA.

5. Immune Tolerance and Therapeutic Drug Monitoring

Drug level monitoring is an important biomarker for assessing the proper use of immunosuppressive drugs in transplant recipients. It is commonly performed for drugs such as tacrolimus, cyclosporine, everolimus, and sirolimus [195]. However, monitoring mycophenolic acid (MPA) using single-sample drug concentrations in the recipient's blood immediately before the next dose is administered may not accurately reflect the overall drug exposure. To overcome this limitation, MPA area under the curve estimation has been introduced as a more effective clinical tool. However, it requires multiple concentration samplings, which can be less practical, especially in pediatric patients [195,196].

In the case of tacrolimus, intra-patient variability (IPV) refers to fluctuations in blood levels over time in individual patients receiving a fixed dose. High IPV of tacrolimus has been associated with the development of DSA, allograft dysfunction, rejection, transplant glomerulopathy, and late graft loss in adult studies [197]. In pediatric studies, tacrolimus IPV has been correlated with de novo DSA development, but its correlation with rejection, decline in graft function, and graft loss is weaker. This may be due to differences in defining cut-off values, cohort size, and methodological variations [198,199].

Future perspectives in drug monitoring advocate the use of expert systems to estimate drug exposure [200], the development of novel techniques for simultaneous evaluation of multiple drugs, and a shift towards the concept of "time in therapeutic range" [201]. This concept, already employed in other medical fields, can provide more precise predictors of under-suppression and the potential risk of allograft rejection. Advancements in drug monitoring techniques and the use of more comprehensive predictors of drug exposure hold promise for improving individualized immunosuppressive therapy and optimizing transplant outcomes.

Global immunosuppression markers are important for assessing the overall intensity of immunosuppression in transplant recipients. Albeit still subject to scientific scrutiny and clinical exploration, various techniques, including flow cytometry and pathogen-specific T-cell response assays, show promise, but still require further validation and standardization [14]. These biomarkers have the potential to improve individualized immunosuppressive therapy and identify patients who can safely reduce their immunosuppression levels. Simple numeric quantitative measurements of lymphocytes have not proven to be reliable indicators, even for determining the dosage of immunosuppressive agents used for depletion induction. AR can occur even in patients with profound T-cell depletion and

without additional immunosuppression [202]. One potential measure of global immunosuppression is the quantification of CD4+ T-cell adenosine triphosphate (ATP) production after polyclonal antibody stimulation in vitro [203]. This assay has only been assessed in a non-controlled trial thus far, and still lacks validation and substantial evidence of its utility, yet it has been marketed commercially as a clinical tool for post-RT monitoring [14].

Indirect assessment of global immunosuppression can be performed by quantifying biomarkers of pre-existing protective immunity. Techniques such as PCR, enzyme-linked immunosorbent spot (ELISPOT), and flow cytometry have been developed to detect pathogen-specific T-cell responses against common viral pathogens like cytomegalovirus (CMV), Epstein–Barr virus, and BK virus [204–206]. However, these techniques are labor intensive and lack standardization across transplant centers. The detection of IFN γ production in response to CMV peptides, using currently available, well-validated CMV immune assays, i.e., ELISPOT and/or QuantiFERON, might help standardize monitoring for this viral infection [207,208], but further characterization of the correlations between immunosuppression degree, viremia risk, and allograft rejection risk is needed. Most recently, multiple potentially impactful, novel experimental applications for immune monitoring post RT have been developed, centered around the most abundant virus of the commensal human virome, the non-pathogenic Torque Teno Virus (TTV), i.e., an anellovirus that does not cause disease directly, but rather replicates based on the immune status of its host [209]. Thus, as TTV viremia has already previously been shown to correlate with the overall level of immunosuppression, while also predicting the occurrence of viral infections, graft rejection, and antibody response after COVID-19 vaccination in lung transplant recipients, it has now been proposed and investigated as a biomarker of functional immunity in RT patients [210]. Apparently, monitoring TTV viremia could be an additional tool for predicting CMV reactivation. However, while these TTV methods have potential in risk prediction, they have not been explicitly tested in drug titration protocols and have not clearly documented a direct drug-infection relationship [209,210].

Flow-cytometry-based assessment of lymphocyte phenotypes has been investigated as a means of gauging immunosuppression intensity. Interestingly, while T-cell phenotypes have not provided significant insights, three studies have observed a B-cell phenotype signature associated with spontaneously immuno-tolerant RT patients [211–213]. This unexpected association suggests that transplant recipients may have altered peripheral blood lymphocyte repertoires that warrant further investigation. If validated, an assay based on flow cytometry could be easily adopted in clinical laboratories to prospectively identify tolerant patients, allowing clinicians to reduce immunosuppression and avoid unnecessary adverse drug effects [14].

Even so, clinically stable allograft function, within acceptable parameters, under the long-term absence of immunosuppressive therapy, i.e., operational tolerance (OT), post RT, represents an exceedingly rare phenomenon, with only ~100 cases hitherto reported [214]. However, some studies have identified specific genes that are upregulated in OT patients. In different patient cohorts and using various microarrays, 39 genes were found to be elevated in OT, with 24 of them being B-cell related. CD79b and prepronociceptin were among the most highly expressed OT-related genes [211,212,215]. Furthermore, miR-142-3p was also found to be upregulated in B cells of OT patients [216].

Genomic studies have revealed gene expression changes associated with tolerance. Membrane-spanning 4-domains A1 (MS4A1/CD20), T-cell leukemia/lymphoma 1A (TCL1A), CD79b, tolerance-associated gene 1 (TOAG1), and FOXP3 genes were found to be upregulated in peripheral B cells [217]. A multicenter study reviewed a cohort of kidney transplant recipients to identify an immunosuppression-independent gene signature for predicting tolerance. They identified nine genes, including Ataxin 3 (ATXN3), BCL2-related protein A1 (BCLA1), Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), Gem-associated protein 9 (GEMIN7), Immunoglobulin lambda constant 1 (IGLC1), Membrane-spanning 4-domains A4A (MS4A4A), Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha (NF κ BIA), RAB40C-member of RAS oncogene family, and TNF,

α -induced protein 3 (TNFAIP3) [218]. Additionally, the kidney spontaneous operational tolerance test (kSPOT) program identified 21 genes involved in OT [219]. Among them, Kruppel-Like Factor 6 (KLF6), Basonuclin 2 (BNC2), and Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1) were used to develop a three-gene assay with high accuracy for detecting OT [213,219,220].

Overall, the pursuit of a tolerance signature in RT remains challenging due to the small number of OT patients. Biomarker studies are primarily focused on identifying OT in post-RT patients, i.e., screening applications. Various large-scale approaches, such as kSORT, tCRM, uCRM, and kSPOT, may assist in reclassifying transplant recipients based on immune risk threshold and determining which patients can benefit from immunosuppression withdrawal or minimization [14].

6. Conclusions

Biomarkers have emerged as valuable tools in addressing the challenges associated with the clinical management of RT patients. Despite improvements in immunosuppressive therapies, there are still pervasive challenges in early detection of graft dysfunction, timely identification of rejection episodes, personalization of immunosuppressive therapy, and prediction of long-term graft survival. Serum creatinine measurements and needle-core renal allograft biopsy, the current methods for evaluating allograft function, have important limitations. Serum creatinine levels are non-specific and unable to differentiate between specific types of injury, while renal allograft biopsy is invasive and cannot be performed repeatedly. Non-invasive biomarkers offer the potential to revolutionize the clinical management of RT patients by providing early diagnosis and monitoring applications of allograft function, i.e., timely detection of complications. Biomarkers associated with AR, CAD, and immune tolerance have shown promise in various studies. Serum and urinary biomarkers, as well as gene signatures and miRNAs, have been identified as potential clinical indicators of allograft injury and rejection. These biomarkers provide valuable insights into the immunopathology of nephron injury and have the potential to improve overall outcomes in post-RT patients. However, the clinical application of biomarkers faces challenges such as sensitivity, specificity, and inter-observational variability. Extensive validation studies and assay standardization are necessary before biomarkers can be confidently integrated into clinical practice. Furthermore, statistical limitations and the variability of transplant recipients' clinical course must be addressed to generate robust evidence. For now, more scientific research is needed to fully harness the potential of biomarkers in guiding personalized care for RT patients.

Author Contributions: Conceptualization, D.N., S.C.L. and A.A.C.; methodology, D.N.; software, R.B.; validation, S.C.L., R.B. and L.D.; formal analysis, D.N.; investigation, L.D.; resources, R.B.; data curation, S.C.L.; writing—original draft preparation, D.N.; writing—review and editing, D.N.; visualization, A.A.C.; supervision, A.A.C.; project administration, A.A.C.; funding acquisition—not applicable. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the main author.

Acknowledgments: We the authors would like to thank our very talented graphic designer, Marius Filip, for his invaluable contributions to the current paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yang, F.; Liao, M.; Wang, P.; Yang, Z.; Liu, Y. The Cost-Effectiveness of Kidney Replacement Therapy Modalities: A Systematic Review of Full Economic Evaluations. *Appl. Health Econ. Health Policy* **2021**, *19*, 163–180. [[CrossRef](#)] [[PubMed](#)]

2. Peddi, V.R.; First, M.R. Recent Advances in Immunosuppressive Therapy for Renal Transplantation. *Semin. Dial.* **2001**, *14*, 218–222. [[CrossRef](#)] [[PubMed](#)]
3. Meier-Kriesche, H.-U.; Schold, J.D.; Srinivas, T.R.; Kaplan, B. Lack of Improvement in Renal Allograft Survival despite a Marked Decrease in Acute Rejection Rates over the Most Recent Era. *Am. J. Transpl.* **2004**, *4*, 378–383. [[CrossRef](#)]
4. Salvadori, M.; Tsalouchos, A. Biomarkers in Renal Transplantation: An Updated Review. *World J. Transpl.* **2017**, *7*, 161–178. [[CrossRef](#)] [[PubMed](#)]
5. Redfield, R.R.; McCune, K.R.; Rao, A.; Sadowski, E.; Hanson, M.; Kolterman, A.J.; Robbins, J.; Guite, K.; Mohamed, M.; Parajuli, S.; et al. Nature, Timing, and Severity of Complications from Ultrasound-guided Percutaneous Renal Transplant Biopsy. *Transpl. Int.* **2016**, *29*, 167–172. [[CrossRef](#)]
6. Naesens, M.; Khatri, P.; Li, L.; Sigdel, T.K.; Vitalone, M.J.; Chen, R.; Butte, A.J.; Salvatierra, O.; Sarwal, M.M. Progressive Histological Damage in Renal Allografts Is Associated with Expression of Innate and Adaptive Immunity Genes. *Kidney Int.* **2011**, *80*, 1364–1376. [[CrossRef](#)]
7. Biomarkers Definitions Working Group. Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. *Clin. Pharmacol. Ther.* **2001**, *69*, 89–95. [[CrossRef](#)]
8. Loupy, A.; Haas, M.; Solez, K.; Racusen, L.; Glotz, D.; Seron, D.; Nankivell, B.J.; Colvin, R.B.; Afrouzian, M.; Akalin, E.; et al. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am. J. Transplant.* **2017**, *17*, 28–41. [[CrossRef](#)]
9. Swanson, K.J.; Aziz, F.; Garg, N.; Mohamed, M.; Mandelbrot, D.; Djamali, A.; Parajuli, S. Role of Novel Biomarkers in Kidney Transplantation. *World J. Transpl.* **2020**, *10*, 230–255. [[CrossRef](#)]
10. Kepka, A.; Waszkiewicz, N.; Chojnowska, S.; Zalewska-Szajda, B.; Ładny, J.R.; Wasilewska, A.; Zwierz, K.; Szajda, S.D.; Kepka, A.; Waszkiewicz, N.; et al. Utility of Urinary Biomarkers in Kidney Transplant Function Assessment. In *Current Issues and Future Direction in Kidney Transplantation*; IntechOpen: London, UK, 2013. [[CrossRef](#)]
11. Mao, S.; Wang, C.; Dong, G. Evaluation of Inter-Laboratory and Cross-Platform Concordance of DNA Microarrays through Discriminating Genes and Classifier Transferability. *J. Bioinform. Comput. Biol.* **2009**, *7*, 157–173. [[CrossRef](#)]
12. Sato, F.; Tsuchiya, S.; Terasawa, K.; Tsujimoto, G. Intra-Platform Repeatability and Inter-Platform Comparability of MicroRNA Microarray Technology. *PLoS ONE* **2009**, *4*, e5540. [[CrossRef](#)]
13. Menon, M.C.; Murphy, B.; Heeger, P.S. Moving Biomarkers toward Clinical Implementation in Kidney Transplantation. *J. Am. Soc. Nephrol.* **2017**, *28*, 735. [[CrossRef](#)]
14. Lo, D.J.; Kaplan, B.; Kirk, A.D. Biomarkers for Kidney Transplant Rejection. *Nat. Rev. Nephrol.* **2014**, *10*, 215–225. [[CrossRef](#)] [[PubMed](#)]
15. Patel, R.; Terasaki, P.I. Significance of the Positive Crossmatch Test in Kidney Transplantation. *N. Engl. J. Med.* **1969**, *280*, 735–739. [[CrossRef](#)]
16. Eikmans, M.; Gielis, E.M.; Ledeganck, K.J.; Yang, J.; Abramowicz, D.; Claas, F.F.J. Non-Invasive Biomarkers of Acute Rejection in Kidney Transplantation: Novel Targets and Strategies. *Front. Med.* **2019**, *5*, 358. [[CrossRef](#)]
17. Naesens, M.; Kuypers, D.R.J.; De Vusser, K.; Evenepoel, P.; Claes, K.; Bammens, B.; Meijers, B.; Sprangers, B.; Pirenne, J.; Monbaliu, D.; et al. The Histology of Kidney Transplant Failure: A Long-Term Follow-Up Study. *Transplantation* **2014**, *98*, 427. [[CrossRef](#)] [[PubMed](#)]
18. Chong, A.S. Mechanisms of Organ Transplant Injury Mediated by B Cells and Antibodies: Implications for Antibody-Mediated Rejection. *Am. J. Transplant.* **2020**, *20*, 23–32. [[CrossRef](#)] [[PubMed](#)]
19. Ding, Y.; Francis, J.; Gautam, A.; Pelletier, L.; Sancharawala, V.; Quillen, K. Durable Renal Response after Combination of Bortezomib, Corticosteroids, Rituximab, and Plasmapheresis for Late Antibody-Mediated Renal Transplant Rejection. *Clin. Nephrol.* **2018**, *89*, 252–259. [[CrossRef](#)] [[PubMed](#)]
20. O’Leary, J.G.; Samaniego, M.; Barrio, M.C.; Potena, L.; Zeevi, A.; Djamali, A.; Cozzi, E. The Influence of Immunosuppressive Agents on the Risk of De Novo Donor-Specific HLA Antibody Production in Solid Organ Transplant Recipients. *Transplantation* **2016**, *100*, 39–53. [[CrossRef](#)]
21. Filler, G.; Todorova, E.K.; Bax, K.; Alvarez-Elías, A.C.; Huang, S.-H.S.; Kobrzynski, M.C. Minimum Mycophenolic Acid Levels Are Associated with Donor-Specific Antibody Formation. *Pediatr. Transplant.* **2016**, *20*, 34–38. [[CrossRef](#)]
22. Ginevri, F.; Nocera, A.; Comoli, P.; Innocente, A.; Cioni, M.; Parodi, A.; Fontana, I.; Magnasco, A.; Nocco, A.; Tagliamacco, A.; et al. Posttransplant De Novo Donor-Specific HLA Antibodies Identify Pediatric Kidney Recipients at Risk for Late Antibody-Mediated Rejection. *Am. J. Transplant.* **2012**, *12*, 3355–3362. [[CrossRef](#)] [[PubMed](#)]
23. Kim, J.J.; Balasubramanian, R.; Michaelides, G.; Wittenhagen, P.; Sebire, N.J.; Mamode, N.; Shaw, O.; Vaughan, R.; Marks, S.D. The Clinical Spectrum of De Novo Donor-Specific Antibodies in Pediatric Renal Transplant Recipients. *Am. J. Transplant.* **2014**, *14*, 2350–2358. [[CrossRef](#)] [[PubMed](#)]
24. Sellarés, J.; de Freitas, D.G.; Mengel, M.; Reeve, J.; Einecke, G.; Sis, B.; Hidalgo, L.G.; Famulski, K.; Matas, A.; Halloran, P.F. Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence. *Am. J. Transplant.* **2012**, *12*, 388–399. [[CrossRef](#)] [[PubMed](#)]
25. Loupy, A.; Lefaucheur, C.; Vernerey, D.; Prugger, C.; van Huyen, J.-P.D.; Mooney, N.; Suberbielle, C.; Frémeaux-Bacchi, V.; Méjean, A.; Desgrandchamps, F.; et al. Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival. *N. Engl. J. Med.* **2013**, *369*, 1215–1226. [[CrossRef](#)] [[PubMed](#)]

26. Macedo, C.; Orkis, E.A.; Popescu, I.; Elinoff, B.D.; Zeevi, A.; Shapiro, R.; Lakkis, F.G.; Metes, D. Contribution of Naïve and Memory T-Cell Populations to the Human Alloimmune Response. *Am. J. Transplant.* **2009**, *9*, 2057–2066. [CrossRef]
27. Wood, K.J.; Goto, R. Mechanisms of Rejection: Current Perspectives. *Transplantation* **2012**, *93*, 1. [CrossRef]
28. Nankivell, B.J.; Alexander, S.I. Rejection of the Kidney Allograft. *N. Engl. J. Med.* **2010**, *363*, 1451–1462. [CrossRef] [PubMed]
29. Segerer, S.; Cui, Y.; Eitner, F.; Goodpaster, T.; Hudkins, K.L.; Mack, M.; Cartron, J.-P.; Colin, Y.; Schlondorff, D.; Alpers, C.E. Expression of Chemokines and Chemokine Receptors during Human Renal Transplant Rejection. *Am. J. Kidney Dis.* **2001**, *37*, 518–531. [CrossRef]
30. Zuidwijk, K.; de Fijter, J.W.; Mallat, M.J.K.; Eikmans, M.; van Groningen, M.C.; Goemaere, N.N.; Bajema, I.M.; van Kooten, C. Increased Influx of Myeloid Dendritic Cells during Acute Rejection Is Associated with Interstitial Fibrosis and Tubular Atrophy and Predicts Poor Outcome. *Kidney Int.* **2012**, *81*, 64–75. [CrossRef]
31. Requião-Moura, L.R.; de Durão, M.S.; de Matos, A.C.C.; Pacheco-Silva, A. Ischemia and Reperfusion Injury in Renal Transplantation: Hemodynamic and Immunological Paradigms. *Einstein* **2015**, *13*, 129–135. [CrossRef]
32. Leemans, J.C.; Kors, L.; Anders, H.-J.; Florquin, S. Pattern Recognition Receptors and the Inflammasome in Kidney Disease. *Nat. Rev. Nephrol.* **2014**, *10*, 398–414. [CrossRef] [PubMed]
33. O'Neill, L.A.J.; Golenbock, D.; Bowie, A.G. The History of Toll-like Receptors—Redefining Innate Immunity. *Nat. Rev. Immunol.* **2013**, *13*, 453–460. [CrossRef] [PubMed]
34. Devarajan, P. Neutrophil Gelatinase-Associated Lipocalin: A Promising Biomarker for Human Acute Kidney Injury. *Biomark. Med.* **2010**, *4*, 265–280. [CrossRef] [PubMed]
35. Acute Kidney Injury (AKI)—KDIGO. Available online: <https://kdigo.org/guidelines/acute-kidney-injury/> (accessed on 27 May 2023).
36. Hull, R.P.; Goldsmith, D.J.A. Nephrotic Syndrome in Adults. *BMJ* **2008**, *336*, 1185–1189. [CrossRef] [PubMed]
37. Grahmmer, F.; Schell, C.; Huber, T.B. The Podocyte Slit Diaphragm—From a Thin Grey Line to a Complex Signalling Hub. *Nat. Rev. Nephrol.* **2013**, *9*, 587–598. [CrossRef]
38. Ahmad, A.; Roderick, P.; Ward, M.; Steenkamp, R.; Burden, R.; O'Donoghue, D.; Ansell, D.; Feest, T. Current Chronic Kidney Disease Practice Patterns in the UK: A National Survey. *QJM* **2006**, *99*, 245–251. [CrossRef]
39. Lisowska-Myjak, B. Serum and Urinary Biomarkers of Acute Kidney Injury. *Blood Purif.* **2010**, *29*, 357–365. [CrossRef]
40. Alachkar, N.; Rabb, H.; Jaar, B.G. Urinary Biomarkers in Acute Kidney Transplant Dysfunction. *Nephron Clin. Pract.* **2011**, *118*, c173–c181; discussion c181. [CrossRef]
41. Metzger, J.; Kirsch, T.; Schiffer, E.; Ulger, P.; Menten, E.; Brand, K.; Weissinger, E.M.; Haubitz, M.; Mischak, H.; Herget-Rosenthal, S. Urinary Excretion of Twenty Peptides Forms an Early and Accurate Diagnostic Pattern of Acute Kidney Injury. *Kidney Int.* **2010**, *78*, 1252–1262. [CrossRef]
42. Finney, H.; Newman, D.J.; Thakkar, H.; Fell, J.M.; Price, C.P. Reference Ranges for Plasma Cystatin C and Creatinine Measurements in Premature Infants, Neonates, and Older Children. *Arch. Dis. Child.* **2000**, *82*, 71–75. [CrossRef]
43. Filler, G.; Bökenkamp, A.; Hofmann, W.; Le Bricon, T.; Martínez-Brú, C.; Grubb, A. Cystatin C as a Marker of GFR—History, Indications, and Future Research. *Clin. Biochem.* **2005**, *38*, 1–8. [CrossRef] [PubMed]
44. Campo, A.; Lanfranco, G.; Gramaglia, L.; Goia, F.; Cottino, R.; Giusto, V. Could Plasma Cystatin C Be Useful as a Marker of Hemodialysis Low Molecular Weight Proteins Removal? *Nephron Clin. Pract.* **2004**, *98*, c79–c82. [CrossRef] [PubMed]
45. Herget-Rosenthal, S.; Feldkamp, T.; Volbracht, L.; Kribben, A. Measurement of Urinary Cystatin C by Particle-Enhanced Nephelometric Immunoassay: Precision, Interferences, Stability and Reference Range. *Ann. Clin. Biochem.* **2004**, *41*, 111–118. [CrossRef]
46. Haraldsson, B.; Sörensson, J. Why Do We Not All Have Proteinuria? An Update of Our Current Understanding of the Glomerular Barrier. *News Physiol. Sci.* **2004**, *19*, 7–10. [CrossRef]
47. Halbesma, N.; Kuiken, D.-S.; Brantsma, A.H.; Bakker, S.J.L.; Wetzels, J.F.M.; De Zeeuw, D.; De Jong, P.E.; Gansevoort, R.T. Macroalbuminuria Is a Better Risk Marker than Low Estimated GFR to Identify Individuals at Risk for Accelerated GFR Loss in Population Screening. *J. Am. Soc. Nephrol.* **2006**, *17*, 2582–2590. [CrossRef]
48. Abbate, M.; Zoja, C.; Remuzzi, G. How Does Proteinuria Cause Progressive Renal Damage? *J. Am. Soc. Nephrol.* **2006**, *17*, 2974–2984. [CrossRef]
49. Eddy, A.A. Proteinuria and Interstitial Injury. *Nephrol. Dial. Transplant.* **2004**, *19*, 277–281. [CrossRef]
50. Tryggvason, K.; Pettersson, E. Causes and Consequences of Proteinuria: The Kidney Filtration Barrier and Progressive Renal Failure. *J. Intern. Med.* **2003**, *254*, 216–224. [CrossRef]
51. Zoja, C.; Morigi, M.; Remuzzi, G. Proteinuria and Phenotypic Change of Proximal Tubular Cells. *J. Am. Soc. Nephrol.* **2003**, *14* (Suppl. 1), S36–S41. [CrossRef] [PubMed]
52. Ofstad, J.; Iversen, B.M. Glomerular and Tubular Damage in Normotensive and Hypertensive Rats. *Am. J. Physiol.-Ren. Physiol.* **2005**, *288*, F665–F672. [CrossRef]
53. Kang, N.R.; Lee, J.E.; Huh, W.; Kim, S.J.; Kim, Y.-G.; Kim, D.J.; Oh, H.Y. Minimal Proteinuria One Year after Transplant Is a Risk Factor for Graft Survival in Kidney Transplantation. *J. Korean Med. Sci.* **2009**, *24* (Suppl. S1), S129–S134. [CrossRef]
54. Remuzzi, G.; Benigni, A.; Remuzzi, A. Mechanisms of Progression and Regression of Renal Lesions of Chronic Nephropathies and Diabetes. *J. Clin. Investig.* **2006**, *116*, 288–296. [CrossRef] [PubMed]

55. Ramesh Prasad, G.v.; Bandukwala, F.; Huang, M.; Zaltzman, J.S. Microalbuminuria Post-Renal Transplantation: Relation to Cardiovascular Risk Factors and C-Reactive Protein. *Clin. Transplant.* **2009**, *23*, 313–320. [[CrossRef](#)]
56. Erman, A.; Rahamimov, R.; Mashraki, T.; Levy-Drummer, R.S.; Winkler, J.; David, I.; Hirsh, Y.; Gafter, U.; Chagnac, A. The Urine Albumin-to-Creatinine Ratio: Assessment of Its Performance in the Renal Transplant Recipient Population. *Clin. J. Am. Soc. Nephrol.* **2011**, *6*, 892. [[CrossRef](#)] [[PubMed](#)]
57. Nauta, F.L.; Bakker, S.J.L.; van Oeveren, W.; Navis, G.; van der Heide, J.J.H.; van Goor, H.; de Jong, P.E.; Gansevoort, R.T. Albuminuria, Proteinuria, and Novel Urine Biomarkers as Predictors of Long-Term Allograft Outcomes in Kidney Transplant Recipients. *Am. J. Kidney Dis.* **2011**, *57*, 733–743. [[CrossRef](#)] [[PubMed](#)]
58. Srivastava, M.; Eidelman, O.; Torosyan, Y.; Jozwik, C.; Mannon, R.B.; Pollard, H.B. Elevated Expression Levels of ANXA11, Integrins B3 and A3, and TNF- α Contribute to a Candidate Proteomic Signature in Urine for Kidney Allograft Rejection. *PROTEOMICS-Clin. Appl.* **2011**, *5*, 311–321. [[CrossRef](#)]
59. Reinhold, S.W.; Straub, R.H.; Krüger, B.; Kaess, B.; Bergler, T.; Weingart, C.; Banas, M.C.; Krämer, B.K.; Banas, B. Elevated Urinary SVCAM-1, IL6, SIL6R and TNFR1 Concentrations Indicate Acute Kidney Transplant Rejection in the First 2weeks after Transplantation. *Cytokine* **2012**, *57*, 379–388. [[CrossRef](#)]
60. Teppo, A.M.; von Willebrand, E.; Honkanen, E.; Ahonen, J.; Grönhagen-Riska, C. Soluble Intercellular Adhesion Molecule-1 (SICAM-1) after Kidney Transplantation: The Origin and Role of Urinary SICAM-1? *Transplantation* **2001**, *71*, 1113–1119. [[CrossRef](#)]
61. van Ree, R.M.; Oterdoom, L.H.; de Vries, A.P.J.; Homan van der Heide, J.J.; van Son, W.J.; Navis, G.; Gans, R.O.B.; Bakker, S.J.L. Circulating Markers of Endothelial Dysfunction Interact with Proteinuria in Predicting Mortality in Renal Transplant Recipients. *Transplantation* **2008**, *86*, 1713–1719. [[CrossRef](#)]
62. Gwinner, W. Renal Transplant Rejection Markers. *World J. Urol.* **2007**, *25*, 445–455. [[CrossRef](#)]
63. Guder, W.G.; Hofmann, W. Clinical Role of Urinary Low Molecular Weight Proteins: Their Diagnostic and Prognostic Implications. *Scand. J. Clin. Lab. Investig.* **2008**, *68*, 95–98. [[CrossRef](#)] [[PubMed](#)]
64. Câmara, N.O.S.; Williams, W.W., Jr.; Pacheco-Silva, A. Proximal Tubular Dysfunction as an Indicator of Chronic Graft Dysfunction. *Braz. J. Med. Biol. Res.* **2009**, *42*, 229–236. [[CrossRef](#)]
65. Kuźniar, J.; Marchewka, Z.; Krasnowski, R.; Boratyńska, M.; Długosz, A.; Klinger, M. Enzymuria and Low Molecular Weight Protein Excretion as the Differentiating Marker of Complications in the Early Post Kidney Transplantation Period. *Int. Urol. Nephrol.* **2006**, *38*, 753–758. [[CrossRef](#)] [[PubMed](#)]
66. Uchida, K.; Gotoh, A. Measurement of Cystatin-C and Creatinine in Urine. *Clin. Chim. Acta* **2002**, *323*, 121–128. [[CrossRef](#)]
67. Bagshaw, S.M.; Langenberg, C.; Haase, M.; Wan, L.; May, C.N.; Bellomo, R. Urinary Biomarkers in Septic Acute Kidney Injury. *Intensive Care Med.* **2007**, *33*, 1285–1296. [[CrossRef](#)] [[PubMed](#)]
68. Iglesias, J.H.; Richard, G.A. Urinary Adenosine Deaminase Binding Protein as a Predictor of Renal Transplant Rejection in Children. *Transpl. Proc.* **1994**, *26*, 75–76.
69. Refaie, M.O.I.; Abo-Zaid, H.; Gomma, N.A.; Aboul-Enein, H.Y. Determination of Urinary and Serum β -Glucuronidase and Alkaline Phosphatase in Various Renal Disease and Kidney Rejection Transplanted Patients. *Prep. Biochem. Biotechnol.* **2000**, *30*, 93–106. [[CrossRef](#)]
70. Santos, C.; Marcelino, P.; Carvalho, T.; Coelho, J.; Bispo, M.; Mourão, L.; Perdigo, R.; Barroso, E. The Value of Tubular Enzymes for Early Detection of Acute Kidney Injury After Liver Transplantation: An Observational Study. *Transplant. Proc.* **2010**, *42*, 3639–3643. [[CrossRef](#)]
71. Branten, A.J.W.; Mulder, T.P.J.; Peters, W.M.; Assmann, K.J.M.; Wetzels, J.F.M. Urinary Excretion of Glutathione S Transferases Alpha and Pi in Patients with Proteinuria: Reflection of the Site of Tubular Injury. *Nephron* **2000**, *85*, 120–126. [[CrossRef](#)]
72. Westhuyzen, J.; Endre, Z.H.; Reece, G.; Reith, D.M.; Saltissi, D.; Morgan, T.J. Measurement of Tubular Enzymuria Facilitates Early Detection of Acute Renal Impairment in the Intensive Care Unit. *Nephrol. Dial. Transplant.* **2003**, *18*, 543–551. [[CrossRef](#)]
73. Trof, R.J.; Di Maggio, F.; Lemreis, J.; Groeneveld, A.B.J. Biomarkers of acute renal injury and renal failure. *Shock* **2006**, *26*, 245. [[CrossRef](#)]
74. Polak, W.P.; Kosieradzki, M.; Kwiatkowski, A.; Danielewicz, R.; Lisik, W.; Michalak, G.; Paczek, L.; Lao, M.; Wałaszewski, J.; Rowiński, W.A. Activity of Glutathione S-Transferases in the Urine of Kidney Transplant Recipients during the First Week after Transplantation. *Ann. Transpl.* **1999**, *4*, 42–45.
75. Gautier, J.-C.; Riefke, B.; Walter, J.; Kurth, P.; Mylecraïne, L.; Guilpin, V.; Barlow, N.; Gury, T.; Hoffman, D.; Ennulat, D.; et al. Evaluation of Novel Biomarkers of Nephrotoxicity in Two Strains of Rat Treated with Cisplatin. *Toxicol. Pathol.* **2010**, *38*, 943–956. [[CrossRef](#)]
76. Liangos, O.; Perianayagam, M.C.; Vaidya, V.S.; Han, W.K.; Wald, R.; Tighiouart, H.; MacKinnon, R.W.; Li, L.; Balakrishnan, V.S.; Pereira, B.J.G.; et al. Urinary N-Acetyl- β -(D)-Glucosaminidase Activity and Kidney Injury Molecule-1 Level Are Associated with Adverse Outcomes in Acute Renal Failure. *J. Am. Soc. Nephrol.* **2007**, *18*, 904. [[CrossRef](#)]
77. Holdt-Lehmann, B.; Lehmann, A.; Korten, G.; Nagel, H.-R.; Nizze, H.; Schuff-Werner, P. Diagnostic Value of Urinary Alanine Aminopeptidase and N-Acetyl- β -d-Glucosaminidase in Comparison to A1-Microglobulin as a Marker in Evaluating Tubular Dysfunction in Glomerulonephritis Patients. *Clin. Chim. Acta* **2000**, *297*, 93–102. [[CrossRef](#)] [[PubMed](#)]
78. Kotanko, P.; Keiler, R.; Knabl, L.; Aulitzky, W.; Margreiter, R.; Gstraunthaler, G.; Pfaller, W. Urinary Enzyme Analysis in Renal Allograft Transplantation. *Clin. Chim. Acta* **1986**, *160*, 137–144. [[CrossRef](#)] [[PubMed](#)]

79. Kotanko, P.; Margreiter, R.; Pfaller, W. Graft Ischemia Correlates with Urinary Excretion of the Proximal Marker Enzyme Fructose-1,6-Bisphosphatase in Human Kidney Transplantation. *Nephron* **2008**, *77*, 62–67. [[CrossRef](#)]
80. Mazloun, M.; Jouffroy, J.; Brazier, F.; Legendre, C.; Neuraz, A.; Garcelon, N.; Prié, D.; Anglicheau, D.; Bienaimé, F. Osmoregulation Performance and Kidney Transplant Outcome. *J. Am. Soc. Nephrol.* **2019**, *30*, 1282–1293. [[CrossRef](#)]
81. Kaden, J.; Groth, J.; May, G.; Liedvogel, B. Urinary Tamm-Horsfall Protein as a Marker of Renal Transplant Function. *Urol. Res.* **1994**, *22*, 131–136. [[CrossRef](#)]
82. Thongboonkerd, V.; Malasit, P. Renal and Urinary Proteomics: Current Applications and Challenges. *Proteomics* **2005**, *5*, 1033–1042. [[CrossRef](#)]
83. Florek, M.; Bauer, N.; Janich, P.; Wilsch-Braeuninger, M.; Fargeas, C.A.; Marzesco, A.-M.; Ehniger, G.; Thiele, C.; Huttner, W.B.; Corbeil, D. Prominin-2 Is a Cholesterol-Binding Protein Associated with Apical and Basolateral Plasmalemmal Protrusions in Polarized Epithelial Cells and Released into Urine. *Cell Tissue Res.* **2007**, *328*, 31–47. [[CrossRef](#)]
84. Jászai, J.; Farkas, L.M.; Fargeas, C.A.; Janich, P.; Haase, M.; Huttner, W.B.; Corbeil, D. Prominin-2 Is a Novel Marker of Distal Tubules and Collecting Ducts of the Human and Murine Kidney. *Histochem. Cell Biol.* **2010**, *133*, 527–539. [[CrossRef](#)] [[PubMed](#)]
85. Tonomura, Y.; Tsuchiya, N.; Torii, M.; Uehara, T. Evaluation of the Usefulness of Urinary Biomarkers for Nephrotoxicity in Rats. *Toxicology* **2010**, *273*, 53–59. [[CrossRef](#)]
86. Ting, Y.-T.; Coates, P.T.; Walker, R.J.; McLellan, A.D. Urinary Tubular Biomarkers as Potential Early Predictors of Renal Allograft Rejection. *Nephrology* **2012**, *17*, 11–16. [[CrossRef](#)] [[PubMed](#)]
87. Szeto, C.-C.; Kwan, B.C.-H.; Lai, K.-B.; Lai, F.M.-M.; Chow, K.-M.; Wang, G.; Luk, C.C.-W.; Li, P.K.-T. Urinary Expression of Kidney Injury Markers in Renal Transplant Recipients. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, 2329. [[CrossRef](#)]
88. Josephson, M.A. Monitoring and Managing Graft Health in the Kidney Transplant Recipient. *Clin. J. Am. Soc. Nephrol.* **2011**, *6*, 1774. [[CrossRef](#)]
89. Colvin, R.B. Antibody-Mediated Renal Allograft Rejection: Diagnosis and Pathogenesis. *J. Am. Soc. Nephrol.* **2007**, *18*, 1046. [[CrossRef](#)]
90. Nankivell, B.J.; Chapman, J.R. The Significance of Subclinical Rejection and the Value of Protocol Biopsies. *Am. J. Transplant.* **2006**, *6*, 2006–2012. [[CrossRef](#)]
91. Schwarz, A.; Gwinner, W.; Hiss, M.; Radermacher, J.; Mengel, M.; Haller, H. Safety and Adequacy of Renal Transplant Protocol Biopsies. *Am. J. Transplant.* **2005**, *5*, 1992–1996. [[CrossRef](#)] [[PubMed](#)]
92. Furness, P.N.; Taub, N. International Variation in the Interpretation of Renal Transplant Biopsies: Report of the CERTPAP Project1. *Kidney Int.* **2001**, *60*, 1998–2012. [[CrossRef](#)]
93. Hernández, D.; Rufino, M.; Armas, S.; González, A.; Gutiérrez, P.; Barbero, P.; Vivancos, S.; Rodríguez, C.; de Vera, J.R.; Torres, A. Retrospective Analysis of Surgical Complications Following Cadaveric Kidney Transplantation in the Modern Transplant Era. *Nephrol. Dial. Transplant.* **2006**, *21*, 2908–2915. [[CrossRef](#)]
94. Salvadori, M.; Rosso, G.; Bertoni, E. Update on Ischemia-Reperfusion Injury in Kidney Transplantation: Pathogenesis and Treatment. *World J. Transpl.* **2015**, *5*, 52–67. [[CrossRef](#)]
95. Cheung, K.P.; Kasimsetty, S.G.; McKay, D.B. Innate Immunity in Donor Procurement. *Curr. Opin. Organ. Transpl.* **2013**, *18*, 154–160. [[CrossRef](#)] [[PubMed](#)]
96. Mueller, T.F.; Solez, K.; Mas, V. Assessment of Kidney Organ Quality and Prediction of Outcome at Time of Transplantation. *Semin. Immunopathol.* **2011**, *33*, 185–199. [[CrossRef](#)] [[PubMed](#)]
97. Ojo, A.O.; Wolfe, R.A.; Held, P.J.; Port, F.K.; Schmouder, R.L. Delayed Graft Function: Risk Factors and Implications for Renal Allograft Survival. *Transplantation* **1997**, *63*, 968–974. [[CrossRef](#)] [[PubMed](#)]
98. Hollmen, M.E.; Kyllönen, L.E.; Inkinen, K.A.; Lalla, M.L.T.; Merenmies, J.; Salmela, K.T. Deceased Donor Neutrophil Gelatinase-Associated Lipocalin and Delayed Graft Function after Kidney Transplantation: A Prospective Study. *Crit. Care* **2011**, *15*, R121. [[CrossRef](#)] [[PubMed](#)]
99. Reese, P.P.; Hall, I.E.; Weng, F.L.; Schröppel, B.; Doshi, M.D.; Hasz, R.D.; Thiessen-Philbrook, H.; Ficek, J.; Rao, V.; Murray, P.; et al. Associations between Deceased-Donor Urine Injury Biomarkers and Kidney Transplant Outcomes. *J. Am. Soc. Nephrol.* **2016**, *27*, 1534–1543. [[CrossRef](#)]
100. Koo, T.Y.; Jeong, J.C.; Lee, Y.; Ko, K.-P.; Lee, K.-B.; Lee, S.; Park, S.J.; Park, J.B.; Han, M.; Lim, H.J.; et al. Pre-Transplant Evaluation of Donor Urinary Biomarkers Can Predict Reduced Graft Function After Deceased Donor Kidney Transplantation. *Medicine* **2016**, *95*, e3076. [[CrossRef](#)]
101. Sadeghi, M.; Daniel, V.; Naujokat, C.; Mehrabi, A.; Opelz, G. Association of High Pretransplant SIL-6R Plasma Levels with Acute Tubular Necrosis in Kidney Graft Recipients. *Transplantation* **2006**, *81*, 1716–1724. [[CrossRef](#)]
102. Nguyen, M.-T.J.P.; Fryml, E.; Sahakian, S.K.; Liu, S.; Cantarovich, M.; Lipman, M.; Tchervenkov, J.L.; Paraskevas, S. Pretransplant Recipient Circulating CD4+CD127lo/- Tumor Necrosis Factor Receptor 2+ Regulatory T Cells: A Surrogate of Regulatory T Cell-Suppressive Function and Predictor of Delayed and Slow Graft Function After Kidney Transplantation. *Transplantation* **2016**, *100*, 314–324. [[CrossRef](#)]
103. Haase, M.; Bellomo, R.; Devarajan, P.; Schlattmann, P.; Haase-Fielitz, A.; NGAL Meta-analysis Investigator Group. Accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diagnosis and Prognosis in Acute Kidney Injury: A Systematic Review and Meta-Analysis. *Am. J. Kidney Dis.* **2009**, *54*, 1012–1024. [[CrossRef](#)]

104. Siew, E.D.; Ware, L.B.; Ikizler, T.A. Biological Markers of Acute Kidney Injury. *J. Am. Soc. Nephrol.* **2011**, *22*, 810–820. [[CrossRef](#)] [[PubMed](#)]
105. Fonseca, I.; Oliveira, J.C.; Almeida, M.; Cruz, M.; Malho, A.; Martins, L.S.; Dias, L.; Pedroso, S.; Santos, J.; Lobato, L.; et al. Neutrophil Gelatinase-Associated Lipocalin in Kidney Transplantation Is an Early Marker of Graft Dysfunction and Is Associated with One-Year Renal Function. *J. Transpl.* **2013**, *2013*, 650123. [[CrossRef](#)] [[PubMed](#)]
106. Mishra, J.; Ma, Q.; Kelly, C.; Mitsnefes, M.; Mori, K.; Barasch, J.; Devarajan, P. Kidney NGAL Is a Novel Early Marker of Acute Injury Following Transplantation. *Pediatr. Nephrol.* **2006**, *21*, 856–863. [[CrossRef](#)]
107. Sureshkumar, K.K.; Marcus, R.J. Urinary Biomarkers as Predictors of Long-Term Allograft Function after Renal Transplantation. *Transplantation* **2010**, *90*, 688–689. [[CrossRef](#)] [[PubMed](#)]
108. Pajek, J.; Škoberne, A.; Šosterič, K.; Adlešič, B.; Leskošek, B.; Bučar Pajek, M.; Osredkar, J.; Lindič, J. Non-Inferiority of Creatinine Excretion Rate to Urinary L-FABP and NGAL as Predictors of Early Renal Allograft Function. *BMC Nephrol.* **2014**, *15*, 117. [[CrossRef](#)] [[PubMed](#)]
109. Malyszko, J.; Koc-Zorawska, E.; Malyszko, J.S.; Mysliwiec, M. Kidney Injury Molecule-1 Correlates with Kidney Function in Renal Allograft Recipients. *Transpl. Proc.* **2010**, *42*, 3957–3959. [[CrossRef](#)]
110. Lacquaniti, A.; Caccamo, C.; Salis, P.; Chirico, V.; Buemi, A.; Cernaro, V.; Noto, A.; Pettinato, G.; Santoro, D.; Bertani, T.; et al. Delayed Graft Function and Chronic Allograft Nephropathy: Diagnostic and Prognostic Role of Neutrophil Gelatinase-Associated Lipocalin. *Biomarkers* **2016**, *21*, 371–378. [[CrossRef](#)]
111. Pianta, T.J.; Peake, P.W.; Pickering, J.W.; Kelleher, M.; Buckley, N.A.; Endre, Z.H. Clusterin in Kidney Transplantation: Novel Biomarkers versus Serum Creatinine for Early Prediction of Delayed Graft Function. *Transplantation* **2015**, *99*, 171–179. [[CrossRef](#)]
112. Schwarz, C.; Regele, H.; Steininger, R.; Hansmann, C.; Mayer, G.; Oberbauer, R. The Contribution of Adhesion Molecule Expression in Donor Kidney Biopsies to Early Allograft Dysfunction. *Transplantation* **2001**, *71*, 1666–1670. [[CrossRef](#)]
113. Schwarz, C.; Hauser, P.; Steininger, R.; Regele, H.; Heinze, G.; Mayer, G.; Oberbauer, R. Failure of BCL-2 up-Regulation in Proximal Tubular Epithelial Cells of Donor Kidney Biopsy Specimens Is Associated with Apoptosis and Delayed Graft Function. *Lab. Invest.* **2002**, *82*, 941–948. [[CrossRef](#)] [[PubMed](#)]
114. Hauser, P.; Schwarz, C.; Mitterbauer, C.; Regele, H.M.; Mühlbacher, F.; Mayer, G.; Perco, P.; Mayer, B.; Meyer, T.W.; Oberbauer, R. Genome-Wide Gene-Expression Patterns of Donor Kidney Biopsies Distinguish Primary Allograft Function. *Lab. Invest.* **2004**, *84*, 353–361. [[CrossRef](#)] [[PubMed](#)]
115. Kamińska, D.; Kościńska-Kasprzak, K.; Drulis-Fajdasz, D.; Hałoń, A.; Polak, W.; Chudoba, P.; Jańczak, D.; Mazanowska, O.; Patrzalek, D.; Klinger, M. Kidney Ischemic Injury Genes Expressed after Donor Brain Death Are Predictive for the Outcome of Kidney Transplantation. *Transpl. Proc.* **2011**, *43*, 2891–2894. [[CrossRef](#)]
116. McGuinness, D.; Leierer, J.; Shapter, O.; Mohammed, S.; Gingell-Littlejohn, M.; Kingsmore, D.B.; Little, A.-M.; Kerschbaum, J.; Schneeberger, S.; Maglione, M.; et al. Identification of Molecular Markers of Delayed Graft Function Based on the Regulation of Biological Ageing. *PLoS ONE* **2016**, *11*, e0146378. [[CrossRef](#)]
117. Wilflingseder, J.; Sunzenauer, J.; Toronyi, E.; Heinzel, A.; Kainz, A.; Mayer, B.; Perco, P.; Telkes, G.; Langer, R.M.; Oberbauer, R. Molecular Pathogenesis of Post-Transplant Acute Kidney Injury: Assessment of Whole-Genome mRNA and miRNA Profiles. *PLoS ONE* **2014**, *9*, e104164. [[CrossRef](#)]
118. Del Prete, G.; De Carli, M.; Almerigogna, F.; Daniel, C.K.; D’Elios, M.M.; Zancuoghi, G.; Vinante, F.; Pizzolo, G.; Romagnani, S. Preferential Expression of CD30 by Human CD4+ T Cells Producing Th2-Type Cytokines. *FASEB J.* **1995**, *9*, 81–86. [[CrossRef](#)]
119. Weimer, R.; Zipperle, S.; Daniel, V.; Carl, S.; Staehler, G.; Opelz, G. Pretransplant CD4 Helper Function and Interleukin 10 Response Predict Risk of Acute Kidney Graft Rejection. *Transplantation* **1996**, *62*, 1606–1614. [[CrossRef](#)] [[PubMed](#)]
120. Rajakariar, R.; Jivanji, N.; Varaganam, M.; Rafiq, M.; Gupta, A.; Sheaff, M.; Sinnott, P.; Yaqoob, M.M. High Pre-Transplant Soluble CD30 Levels Are Predictive of the Grade of Rejection. *Am. J. Transpl.* **2005**, *5*, 1922–1925. [[CrossRef](#)] [[PubMed](#)]
121. Cinti, P.; Pretagostini, R.; Arpino, A.; Tamburro, M.L.; Mengasini, S.; Lattanzi, R.; De Simone, P.; Berloco, P.; Molajoni, E.R. Evaluation of Pretransplant Immunologic Status in Kidney-Transplant Recipients by Panel Reactive Antibody and Soluble CD30 Determinations. *Transplantation* **2005**, *79*, 1154–1156. [[CrossRef](#)]
122. Sengul, S.; Keven, K.; Gormez, U.; Kutlay, S.; Erturk, S.; Erbay, B. Identification of Patients at Risk of Acute Rejection by Pretransplantation and Posttransplantation Monitoring of Soluble CD30 Levels in Kidney Transplantation. *Transplantation* **2006**, *81*, 1216–1219. [[CrossRef](#)]
123. Altermann, W.; Schlaf, G.; Rothhoff, A.; Seliger, B. High Variation of Individual Soluble Serum CD30 Levels of Pre-Transplantation Patients: SCD30 a Feasible Marker for Prediction of Kidney Allograft Rejection? *Nephrol. Dial. Transpl.* **2007**, *22*, 2795–2799. [[CrossRef](#)] [[PubMed](#)]
124. Shooshtarizadeh, T.; Mohammadali, A.; Ossareh, S.; Ataipour, Y. Relation between Pretransplant Serum Levels of Soluble CD30 and Acute Rejection during the First 6 Months after a Kidney Transplant. *Exp. Clin. Transpl.* **2013**, *11*, 229–233. [[CrossRef](#)] [[PubMed](#)]
125. Augustine, J.J.; Siu, D.S.; Clemente, M.J.; Schulak, J.A.; Heeger, P.S.; Hricik, D.E. Pre-Transplant IFN-Gamma ELISPOTs Are Associated with Post-Transplant Renal Function in African American Renal Transplant Recipients. *Am. J. Transpl.* **2005**, *5*, 1971–1975. [[CrossRef](#)] [[PubMed](#)]
126. Bendjelloul, F.; Desin, T.S.; Shoker, A.S. Donor Non-Specific IFN- γ Production by Primed Alloreactive Cells as a Potential Screening Test to Predict the Alloimmune Response. *Transpl. Immunol.* **2004**, *12*, 167–176. [[CrossRef](#)]

127. Heeger, P.S.; Greenspan, N.S.; Kuhlenschmidt, S.; DeJelo, C.; Hricik, D.E.; Schulak, J.A.; Tary-Lehmann, M. Pretransplant Frequency of Donor-Specific, IFN- γ -Producing Lymphocytes Is a Manifestation of Immunologic Memory and Correlates with the Risk of Posttransplant Rejection Episodes. *J. Immunol.* **1999**, *163*, 2267–2275. [[CrossRef](#)]
128. Bellisola, G.; Tridente, G.; Nacchia, F.; Fior, F.; Boschiero, L. Monitoring of Cellular Immunity by Interferon-Gamma Enzyme-Linked Immunosorbent Spot Assay in Kidney Allograft Recipients: Preliminary Results of a Longitudinal Study. *Transpl. Proc.* **2006**, *38*, 1014–1017. [[CrossRef](#)]
129. Rotondi, M.; Rosati, A.; Buonamano, A.; Lasagni, L.; Lazzeri, E.; Pradella, F.; Fossombroni, V.; Cirami, C.; Liotta, F.; La Villa, G.; et al. High Pretransplant Serum Levels of CXCL10/IP-10 Are Related to Increased Risk of Renal Allograft Failure. *Am. J. Transpl.* **2004**, *4*, 1466–1474. [[CrossRef](#)]
130. Lazzeri, E.; Rotondi, M.; Mazzinghi, B.; Lasagni, L.; Buonamano, A.; Rosati, A.; Pradella, F.; Fossombroni, V.; La Villa, G.; Gacci, M.; et al. High CXCL10 Expression in Rejected Kidneys and Predictive Role of Pretransplant Serum CXCL10 for Acute Rejection and Chronic Allograft Nephropathy. *Transplantation* **2005**, *79*, 1215–1220. [[CrossRef](#)]
131. Hricik, D.E.; Nickerson, P.; Formica, R.N.; Poggio, E.D.; Rush, D.; Newell, K.A.; Goebel, J.; Gibson, I.W.; Fairchild, R.L.; Riggs, M.; et al. Multicenter Validation of Urinary CXCL9 as a Risk-Stratifying Biomarker for Kidney Transplant Injury. *Am. J. Transpl.* **2013**, *13*, 2634–2644. [[CrossRef](#)]
132. Srinivas, T.R.; Kaplan, B. Urinary Biomarkers and Kidney Transplant Rejection: Fine-Tuning the Radar. *Am. J. Transpl.* **2013**, *13*, 2519–2521. [[CrossRef](#)]
133. Kim, S.C.; Page, E.K.; Knechtle, S.J. Urine Proteomics in Kidney Transplantation. *Transpl. Rev* **2014**, *28*, 15–20. [[CrossRef](#)] [[PubMed](#)]
134. Hirt-Minkowski, P.; De Serres, S.A.; Ho, J. Developing Renal Allograft Surveillance Strategies—Urinary Biomarkers of Cellular Rejection. *Can. J. Kidney Health Dis.* **2015**, *2*, 28. [[CrossRef](#)] [[PubMed](#)]
135. Augustine, J.J.; Hricik, D.E. T-Cell Immune Monitoring by the ELISPOT Assay for Interferon Gamma. *Clin. Chim. Acta* **2012**, *413*, 1359–1363. [[CrossRef](#)]
136. Bestard, O.; Crespo, E.; Stein, M.; Lúcia, M.; Roelen, D.L.; de Vaal, Y.J.; Hernandez-Fuentes, M.P.; Chatenoud, L.; Wood, K.J.; Claas, F.H.; et al. Cross-Validation of IFN- γ Elispot Assay for Measuring Alloreactive Memory/Effector T Cell Responses in Renal Transplant Recipients. *Am. J. Transpl.* **2013**, *13*, 1880–1890. [[CrossRef](#)] [[PubMed](#)]
137. Gielis, E.M.; Ledeganck, K.J.; De Winter, B.Y.; Del Favero, J.; Bosmans, J.-L.; Claas, F.H.J.; Abramowicz, D.; Eikmans, M. Cell-Free DNA: An Upcoming Biomarker in Transplantation. *Am. J. Transpl.* **2015**, *15*, 2541–2551. [[CrossRef](#)]
138. García Moreira, V.; Prieto García, B.; Baltar Martín, J.M.; Ortega Suárez, F.; Alvarez, F.V. Cell-Free DNA as a Noninvasive Acute Rejection Marker in Renal Transplantation. *Clin. Chem.* **2009**, *55*, 1958–1966. [[CrossRef](#)] [[PubMed](#)]
139. Freue, G.V.C.; Sasaki, M.; Meredith, A.; Günther, O.P.; Bergman, A.; Takhar, M.; Mui, A.; Balshaw, R.F.; Ng, R.T.; Opushneva, N.; et al. Proteomic Signatures in Plasma during Early Acute Renal Allograft Rejection. *Mol. Cell Proteom.* **2010**, *9*, 1954–1967. [[CrossRef](#)]
140. Wu, D.; Zhu, D.; Xu, M.; Rong, R.; Tang, Q.; Wang, X.; Zhu, T. Analysis of Transcriptional Factors and Regulation Networks in Patients with Acute Renal Allograft Rejection. *J. Proteome Res.* **2011**, *10*, 175–181. [[CrossRef](#)]
141. Perez, J.D.; Sakata, M.M.; Colucci, J.A.; Spinelli, G.A.; Felipe, C.R.; Carvalho, V.M.; Cardozo, K.H.M.; Medina-Pestana, J.O.; Tedesco-Silva, H.; Schor, N.; et al. Plasma Proteomics for the Assessment of Acute Renal Transplant Rejection. *Life Sci.* **2016**, *158*, 111–120. [[CrossRef](#)]
142. Sigdel, T.K.; Kaushal, A.; Gritsenko, M.; Norbeck, A.D.; Qian, W.-J.; Xiao, W.; Camp, D.G.; Smith, R.D.; Sarwal, M.M. Shotgun Proteomics Identifies Proteins Specific for Acute Renal Transplant Rejection. *Proteom. Clin. Appl.* **2010**, *4*, 32–47. [[CrossRef](#)]
143. Loftheim, H.; Midtvedt, K.; Hartmann, A.; Reisæter, A.V.; Falck, P.; Holdaas, H.; Jenssen, T.; Reubsæet, L.; Åsberg, A. Urinary Proteomic Shotgun Approach for Identification of Potential Acute Rejection Biomarkers in Renal Transplant Recipients. *Transpl. Res.* **2012**, *1*, 9. [[CrossRef](#)]
144. Sigdel, T.K.; Salomonis, N.; Nicora, C.D.; Ryu, S.; He, J.; Dinh, V.; Orton, D.J.; Moore, R.J.; Hsieh, S.-C.; Dai, H.; et al. The Identification of Novel Potential Injury Mechanisms and Candidate Biomarkers in Renal Allograft Rejection by Quantitative Proteomics. *Mol. Cell Proteom.* **2014**, *13*, 621–631. [[CrossRef](#)] [[PubMed](#)]
145. Vasconcellos, L.M.; Schachter, A.D.; Zheng, X.X.; Vasconcellos, L.H.; Shapiro, M.; Harmon, W.E.; Strom, T.B. Cytotoxic Lymphocyte Gene Expression in Peripheral Blood Leukocytes Correlates with Rejecting Renal Allografts. *Transplantation* **1998**, *66*, 562–566. [[CrossRef](#)]
146. Li, B.; Hartono, C.; Ding, R.; Sharma, V.K.; Ramaswamy, R.; Qian, B.; Serur, D.; Mouradian, J.; Schwartz, J.E.; Suthanthiran, M. Noninvasive Diagnosis of Renal-Allograft Rejection by Measurement of Messenger RNA for Perforin and Granzyme B in Urine. *N. Engl. J. Med.* **2001**, *344*, 947–954. [[CrossRef](#)]
147. Afaneh, C.; Muthukumar, T.; Lubetzky, M.; Ding, R.; Snopkowski, C.; Sharma, V.K.; Seshan, S.; Dadhania, D.; Schwartz, J.E.; Suthanthiran, M. Urinary Cell Levels of mRNA for OX40, OX40L, PD-1, PD-L1 or PD-L2 and Acute Rejection of Human Renal Allografts. *Transplantation* **2010**, *90*, 1381–1387. [[CrossRef](#)] [[PubMed](#)]
148. Muthukumar, T.; Dadhania, D.; Ding, R.; Snopkowski, C.; Naqvi, R.; Lee, J.B.; Hartono, C.; Li, B.; Sharma, V.K.; Seshan, S.V.; et al. Messenger RNA for FOXP3 in the Urine of Renal-Allograft Recipients. *N. Engl. J. Med.* **2005**, *353*, 2342–2351. [[CrossRef](#)] [[PubMed](#)]

149. Suthanthiran, M.; Schwartz, J.E.; Ding, R.; Abecassis, M.; Dadhania, D.; Samstein, B.; Knechtle, S.J.; Friedewald, J.; Becker, Y.T.; Sharma, V.K.; et al. Urinary-Cell mRNA Profile and Acute Cellular Rejection in Kidney Allografts. *N. Engl. J. Med.* **2013**, *369*, 20–31. [[CrossRef](#)] [[PubMed](#)]
150. Sui, W.; Yang, M.; Li, F.; Chen, H.; Chen, J.; Ou, M.; Zhang, Y.; Lin, H.; Xue, W.; Dai, Y. Serum MicroRNAs as New Diagnostic Biomarkers for Pre- and Post-Kidney Transplantation. *Transpl. Proc.* **2014**, *46*, 3358–3362. [[CrossRef](#)]
151. Lorenzen, J.M.; Volkmann, I.; Fiedler, J.; Schmidt, M.; Scheffner, I.; Haller, H.; Gwinner, W.; Thum, T. Urinary MiR-210 as a Mediator of Acute T-Cell Mediated Rejection in Renal Allograft Recipients. *Am. J. Transpl.* **2011**, *11*, 2221–2227. [[CrossRef](#)]
152. Betts, G.; Shankar, S.; Sherston, S.; Friend, P.; Wood, K.J. Examination of Serum MiRNA Levels in Kidney Transplant Recipients with Acute Rejection. *Transplantation* **2014**, *97*, e28–e30. [[CrossRef](#)]
153. Grigoryev, Y.A.; Kurian, S.M.; Hart, T.; Nakorchevsky, A.A.; Chen, C.; Campbell, D.; Head, S.R.; Yates, J.R.; Salomon, D.R. MicroRNA Regulation of Molecular Networks Mapped by Global MicroRNA, mRNA, and Protein Expression in Activated T Lymphocytes. *J. Immunol.* **2011**, *187*, 2233–2243. [[CrossRef](#)] [[PubMed](#)]
154. Sarwal, M.M.; Ettenger, R.B.; Dharmidharka, V.; Benfield, M.; Mathias, R.; Portale, A.; McDonald, R.; Harmon, W.; Kershaw, D.; Vehaskari, V.M.; et al. Complete Steroid Avoidance Is Effective and Safe in Children with Renal Transplants: A Multicenter Randomized Trial with Three-Year Follow-Up. *Am. J. Transpl.* **2012**, *12*, 2719–2729. [[CrossRef](#)] [[PubMed](#)]
155. Li, L.; Khatri, P.; Sigdel, T.K.; Tran, T.; Ying, L.; Vitalone, M.; Chen, A.; Hsieh, S.; Dai, H.; Zhang, M.; et al. A Five-Gene Peripheral Blood Diagnostic Test for Acute Rejection in Renal Transplantation. *Am. J. Transpl.* **2012**, *12*, 2710–2718. [[CrossRef](#)]
156. Allison, S.J. Biomarkers in Peripheral Blood Detect Acute Rejection. *Nat. Rev. Nephrol.* **2012**, *8*, 681. [[CrossRef](#)] [[PubMed](#)]
157. Roedder, S.; Sigdel, T.; Salomonis, N.; Hsieh, S.; Dai, H.; Bestard, O.; Metes, D.; Zeevi, A.; Gritsch, A.; Cheeseman, J.; et al. The KSORT Assay to Detect Renal Transplant Patients at High Risk for Acute Rejection: Results of the Multicenter AART Study. *PLoS Med.* **2014**, *11*, e1001759. [[CrossRef](#)]
158. Shen-Orr, S.S.; Tibshirani, R.; Khatri, P.; Bodian, D.L.; Staedtler, F.; Perry, N.M.; Hastie, T.; Sarwal, M.M.; Davis, M.M.; Butte, A.J. Cell Type-Specific Gene Expression Differences in Complex Tissues. *Nat. Methods* **2010**, *7*, 287–289. [[CrossRef](#)]
159. Li, L.; Khush, K.; Hsieh, S.-C.; Ying, L.; Luikart, H.; Sigdel, T.; Roedder, S.; Yang, A.; Valentine, H.; Sarwal, M.M. Identification of Common Blood Gene Signatures for the Diagnosis of Renal and Cardiac Acute Allograft Rejection. *PLoS ONE* **2013**, *8*, e82153. [[CrossRef](#)]
160. Crespo, E.; Roedder, S.; Sigdel, T.; Hsieh, S.-C.; Luque, S.; Cruzado, J.M.; Tran, T.Q.; Grinyó, J.M.; Sarwal, M.M.; Bestard, O. Molecular and Functional Noninvasive Immune Monitoring in the ESCAPE Study for Prediction of Subclinical Renal Allograft Rejection. *Transplantation* **2017**, *101*, 1400–1409. [[CrossRef](#)]
161. Khatri, P.; Roedder, S.; Kimura, N.; De Vusser, K.; Morgan, A.A.; Gong, Y.; Fischbein, M.P.; Robbins, R.C.; Naesens, M.; Butte, A.J.; et al. A Common Rejection Module (CRM) for Acute Rejection across Multiple Organs Identifies Novel Therapeutics for Organ Transplantation. *J. Exp. Med.* **2013**, *210*, 2205–2221. [[CrossRef](#)]
162. Sigdel, T.K.; Bestard, O.; Tran, T.Q.; Hsieh, S.-C.; Roedder, S.; Damm, I.; Vincenti, F.; Sarwal, M.M. A Computational Gene Expression Score for Predicting Immune Injury in Renal Allografts. *PLoS ONE* **2015**, *10*, e0138133. [[CrossRef](#)]
163. The Urine Common Rejection Module (uCRM) Is a Sentinel Assay for Graft Rejection. ATC Abstracts. Available online: <https://atcmeetingabstracts.com/abstract/the-urine-common-rejection-module-ucrm-is-a-sentinel-assay-for-graft-rejection/> (accessed on 6 May 2023).
164. Novacescu, D.; Feciche, B.O.; Cumpanas, A.A.; Bardan, R.; Rusmir, A.V.; Bitar, Y.A.; Barbos, V.I.; Cut, T.G.; Raica, M.; Latcu, S.C. Contemporary Clinical Definitions, Differential Diagnosis, and Novel Predictive Tools for Renal Cell Carcinoma. *Biomedicines* **2022**, *10*, 2926. [[CrossRef](#)] [[PubMed](#)]
165. Novacescu, D.; Cut, T.G.; Cumpanas, A.A.; Latcu, S.C.; Bardan, R.; Ferician, O.; Secasan, C.-C.; Rusmir, A.; Raica, M. Evaluating Established Roles, Future Perspectives and Methodological Heterogeneity for Wilms’ Tumor 1 (WT1) Antigen Detection in Adult Renal Cell Carcinoma, Using a Novel N-Terminus Targeted Antibody (Clone WT49). *Biomedicines* **2022**, *10*, 912. [[CrossRef](#)] [[PubMed](#)]
166. Novacescu, D.; Cut, T.G.; Cumpanas, A.A.; Bratosin, F.; Ceausu, R.A.; Raica, M. Novel Expression of Thymine Dimers in Renal Cell Carcinoma, Demonstrated through Immunohistochemistry. *Biomedicines* **2022**, *10*, 2673. [[CrossRef](#)] [[PubMed](#)]
167. Radu-Cosnita, A.D.; Nesi, A.; Berzava, P.L.; Cerbu, S.; Cosma, A.; Comsa, S.; Sarb, S.; Ferician, A.M.; Ferician, O.C.; Cimpean, A.M. Anti-Chloride Intracellular Channel Protein 1 (CLIC1) Antibodies Induce Tumour Necrosis and Angiogenesis Inhibition on In Vivo Experimental Models of Human Renal Cancer. *Anticancer Res.* **2022**, *42*, 1313–1325. [[CrossRef](#)] [[PubMed](#)]
168. Ferician, A.M.; Ferician, O.C.; Cumpanas, A.D.; Berzava, P.L.; Nesi, A.; Barmayoun, A.; Cimpean, A.M. Heterogeneity of Platelet Derived Growth Factor Pathway Gene Expression Profile Defines Three Distinct Subgroups of Renal Cell Carcinomas. *Cancer Genom. Proteom.* **2022**, *19*, 477–489. [[CrossRef](#)]
169. Ferician, A.M.; Ferician, O.C.; Nesi, A.; Cosma, A.A.; Caplar, B.D.; Melnic, E.; Cimpean, A.M. The Mutually Mediated Chloride Intracellular Channel Protein 1 (CLIC1) Relationship between Malignant Cells and Tumor Blood Vessel Endothelium Exhibits a Significant Impact on Tumor Angiogenesis, Progression, and Metastasis in Clear Cell Renal Cell Carcinoma (CcRCC). *Cancers* **2022**, *14*, 5981. [[CrossRef](#)]
170. Sigdel, T.K.; Vitalone, M.J.; Tran, T.Q.; Dai, H.; Hsieh, S.-C.; Salvatierra, O.; Sarwal, M.M. A Rapid Noninvasive Assay for the Detection of Renal Transplant Injury. *Transplantation* **2013**, *96*, 97–101. [[CrossRef](#)]

171. Halloran, P.F.; Madill-Thomsen, K.S.; Reeve, J. The Molecular Phenotype of Kidney Transplants: Insights From the MMDx Project. *Transplantation* **2023**. [[CrossRef](#)]
172. Nakorchevsky, A.; Hewel, J.A.; Kurian, S.M.; Mondala, T.S.; Campbell, D.; Head, S.R.; Marsh, C.L.; Yates, J.R.; Salomon, D.R. Molecular Mechanisms of Chronic Kidney Transplant Rejection via Large-Scale Proteogenomic Analysis of Tissue Biopsies. *J. Am. Soc. Nephrol.* **2010**, *21*, 362–373. [[CrossRef](#)]
173. Sigdel, T.K.; Gao, Y.; He, J.; Wang, A.; Nicora, C.D.; Fillmore, T.L.; Shi, T.; Webb-Robertson, B.-J.; Smith, R.D.; Qian, W.-J.; et al. Mining the Human Urine Proteome for Monitoring Renal Transplant Injury. *Kidney Int.* **2016**, *89*, 1244–1252. [[CrossRef](#)]
174. Solez, K.; Colvin, R.B.; Racusen, L.C.; Sis, B.; Halloran, P.F.; Birk, P.E.; Campbell, P.M.; Cascalho, M.; Collins, A.B.; Demetris, A.J.; et al. Banff '05 Meeting Report: Differential Diagnosis of Chronic Allograft Injury and Elimination of Chronic Allograft Nephropathy ('CAN'). *Am. J. Transpl.* **2007**, *7*, 518–526. [[CrossRef](#)] [[PubMed](#)]
175. Colvin, R.B. Chronic Allograft Nephropathy. *N. Engl. J. Med.* **2003**, *349*, 2288–2290. [[CrossRef](#)] [[PubMed](#)]
176. Nankivell, B.J.; Borrows, R.J.; Fung, C.L.-S.; O'Connell, P.J.; Allen, R.D.M.; Chapman, J.R. The Natural History of Chronic Allograft Nephropathy. *N. Engl. J. Med.* **2003**, *349*, 2326–2333. [[CrossRef](#)] [[PubMed](#)]
177. Bosmans, J.-L.; Ysebaert, D.K.; Verpooten, G.A. Chronic Allograft Nephropathy: What Have We Learned from Protocol Biopsies? *Transplantation* **2008**, *85*, S38–S41. [[CrossRef](#)] [[PubMed](#)]
178. Cosio, F.G.; Grande, J.P.; Larson, T.S.; Gloor, J.M.; Velosa, J.A.; Textor, S.C.; Griffin, M.D.; Stegall, M.D. Kidney Allograft Fibrosis and Atrophy Early after Living Donor Transplantation. *Am. J. Transpl.* **2005**, *5*, 1130–1136. [[CrossRef](#)] [[PubMed](#)]
179. Serón, D.; Moreso, F. Protocol Biopsies in Renal Transplantation: Prognostic Value of Structural Monitoring. *Kidney Int.* **2007**, *72*, 690–697. [[CrossRef](#)]
180. Quintana, L.F.; Solé-Gonzalez, A.; Kalko, S.G.; Bañon-Maneus, E.; Solé, M.; Diekmann, F.; Gutierrez-Dalmau, A.; Abian, J.; Campistol, J.M. Urine Proteomics to Detect Biomarkers for Chronic Allograft Dysfunction. *J. Am. Soc. Nephrol.* **2009**, *20*, 428–435. [[CrossRef](#)]
181. Quintana, L.F.; Campistol, J.M.; Alcolea, M.P.; Bañon-Maneus, E.; Sol-González, A.; Cutillas, P.R. Application of Label-Free Quantitative Peptidomics for the Identification of Urinary Biomarkers of Kidney Chronic Allograft Dysfunction. *Mol. Cell Proteom.* **2009**, *8*, 1658–1673. [[CrossRef](#)]
182. Johnston, O.; Cassidy, H.; O'Connell, S.; O'Riordan, A.; Gallagher, W.; Maguire, P.B.; Wynne, K.; Cagney, G.; Ryan, M.P.; Conlon, P.J.; et al. Identification of B2-Microglobulin as a Urinary Biomarker for Chronic Allograft Nephropathy Using Proteomic Methods. *Proteom. Clin. Appl.* **2011**, *5*, 422–431. [[CrossRef](#)]
183. Bañón-Maneus, E.; Diekmann, F.; Carrascal, M.; Quintana, L.F.; Moya-Rull, D.; Bescós, M.; Ramírez-Bajo, M.J.; Rovira, J.; Gutierrez-Dalmau, A.; Solé-González, A.; et al. Two-Dimensional Difference Gel Electrophoresis Urinary Proteomic Profile in the Search of Nonimmune Chronic Allograft Dysfunction Biomarkers. *Transplantation* **2010**, *89*, 548–558. [[CrossRef](#)]
184. Jung, H.-Y.; Lee, C.-H.; Choi, J.-Y.; Cho, J.-H.; Park, S.-H.; Kim, Y.-L.; Moon, P.-G.; Baek, M.-C.; Berm Park, J.; Hoon Kim, Y.; et al. Potential Urinary Extracellular Vesicle Protein Biomarkers of Chronic Active Antibody-Mediated Rejection in Kidney Transplant Recipients. *J. Chromatogr. B* **2020**, *1138*, 121958. [[CrossRef](#)]
185. Kurian, S.M.; Heilman, R.; Mondala, T.S.; Nakorchevsky, A.; Hewel, J.A.; Campbell, D.; Robison, E.H.; Wang, L.; Lin, W.; Gaber, L.; et al. Biomarkers for Early and Late Stage Chronic Allograft Nephropathy by Proteogenomic Profiling of Peripheral Blood. *PLoS ONE* **2009**, *4*, e6212. [[CrossRef](#)]
186. Scian, M.J.; Maluf, D.G.; David, K.G.; Archer, K.J.; Suh, J.L.; Wolen, A.R.; Mba, M.U.; Massey, H.D.; King, A.L.; Gehr, T.; et al. MicroRNA Profiles in Allograft Tissues and Paired Urines Associate with Chronic Allograft Dysfunction with IF/TA. *Am. J. Transpl.* **2011**, *11*, 2110–2122. [[CrossRef](#)]
187. Maluf, D.G.; Dumur, C.I.; Suh, J.L.; Scian, M.J.; King, A.L.; Cathro, H.; Lee, J.K.; Gehrau, R.C.; Brayman, K.L.; Gallon, L.; et al. The Urine MicroRNA Profile May Help Monitor Post-Transplant Renal Graft Function. *Kidney Int.* **2014**, *85*, 439–449. [[CrossRef](#)] [[PubMed](#)]
188. Zununi Vahed, S.; Omidi, Y.; Ardalan, M.; Samadi, N. Dysregulation of Urinary MiR-21 and MiR-200b Associated with Interstitial Fibrosis and Tubular Atrophy (IFTA) in Renal Transplant Recipients. *Clin. Biochem.* **2017**, *50*, 32–39. [[CrossRef](#)] [[PubMed](#)]
189. Soltaninejad, E.; Nicknam, M.H.; Nafar, M.; Sharbafi, M.H.; Keshavarz Shahbaz, S.; Barabadi, M.; Yekaninejad, M.S.; Bahrami, T.; Ahmadpoor, P.; Amirzargar, A. Altered Expression of MicroRNAs Following Chronic Allograft Dysfunction with Interstitial Fibrosis and Tubular Atrophy. *Iran. J. Allergy Asthma Immunol.* **2015**, *14*, 615–623.
190. Iwasaki, K.; Yamamoto, T.; Inanaga, Y.; Hiramitsu, T.; Miwa, Y.; Murotani, K.; Narumi, S.; Watarai, Y.; Katayama, A.; Uchida, K.; et al. MiR-142-5p and MiR-486-5p as Biomarkers for Early Detection of Chronic Antibody-Mediated Rejection in Kidney Transplantation. *Biomarkers* **2017**, *22*, 45–54. [[CrossRef](#)] [[PubMed](#)]
191. Mas, V.; Maluf, D.; Archer, K.; Yanek, K.; Mas, L.; King, A.; Gibney, E.; Massey, D.; Cotterell, A.; Fisher, R.; et al. Establishing the Molecular Pathways Involved in Chronic Allograft Nephropathy for Testing New Noninvasive Diagnostic Markers. *Transplantation* **2007**, *83*, 448. [[CrossRef](#)] [[PubMed](#)]
192. Lee, J.R.; Muthukumar, T.; Dadhania, D.; Ding, R.; Sharma, V.K.; Schwartz, J.E.; Suthanthiran, M. Urinary Cell mRNA Profiles Predictive of Human Kidney Allograft Status. *Immunol. Rev.* **2014**, *258*, 218–240. [[CrossRef](#)] [[PubMed](#)]
193. O'Connell, P.J.; Zhang, W.; Menon, M.C.; Yi, Z.; Schröppel, B.; Gallon, L.; Luan, Y.; Rosales, I.A.; Ge, Y.; Losic, B.; et al. Biopsy Transcriptome Expression Profiling to Identify Kidney Transplants at Risk of Chronic Injury: A Multicentre, Prospective Study. *Lancet* **2016**, *388*, 983–993. [[CrossRef](#)]

194. Li, L.; Greene, I.; Readhead, B.; Menon, M.C.; Kidd, B.A.; Uzilov, A.V.; Wei, C.; Philippe, N.; Schroppel, B.; He, J.C.; et al. Novel Therapeutics Identification for Fibrosis in Renal Allograft Using Integrative Informatics Approach. *Sci. Rep.* **2017**, *7*, 39487. [[CrossRef](#)]
195. Peruzzi, L.; Deaglio, S. Rejection Markers in Kidney Transplantation: Do New Technologies Help Children? *Pediatr. Nephrol.* **2023**. [[CrossRef](#)] [[PubMed](#)]
196. Bergan, S.; Brunet, M.; Hesselink, D.A.; Johnson-Davis, K.L.; Kunicki, P.K.; Lemaitre, F.; Marquet, P.; Molinaro, M.; Noceti, O.; Pattanaik, S.; et al. Personalized Therapy for Mycophenolate: Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. *Ther. Drug Monit.* **2021**, *43*, 150. [[CrossRef](#)] [[PubMed](#)]
197. Kuypers, D.R.J. Inpatient Variability of Tacrolimus Exposure in Solid Organ Transplantation: A Novel Marker for Clinical Outcome. *Clin. Pharmacol. Ther.* **2020**, *107*, 347–358. [[CrossRef](#)]
198. Eid, L.; Tuchman, S.; Moudgil, A. Late Acute Rejection: Incidence, Risk Factors, and Effect on Graft Survival and Function. *Pediatr. Transplant.* **2014**, *18*, 155–162. [[CrossRef](#)]
199. Pollock-BarZiv, S.M.; Finkelstein, Y.; Manlihot, C.; Dipchand, A.I.; Hebert, D.; Ng, V.L.; Solomon, M.; McCrindle, B.W.; Grant, D. Variability in Tacrolimus Blood Levels Increases the Risk of Late Rejection and Graft Loss after Solid Organ Transplantation in Older Children. *Pediatr. Transplant.* **2010**, *14*, 968–975. [[CrossRef](#)]
200. Marquet, P.; Cros, F.; Micallef, L.; Jacqz-Aigrain, E.; Woillard, J.-B.; Monchaud, C.; Saint-Marcoux, F.; Debord, J. Tacrolimus Bayesian Dose Adjustment in Pediatric Renal Transplant Recipients. *Ther. Drug Monit.* **2021**, *43*, 472. [[CrossRef](#)]
201. Davis, S.; Gralla, J.; Klem, P.; Tong, S.; Wedermyer, G.; Freed, B.; Wiseman, A.; Cooper, J.E. Lower Tacrolimus Exposure and Time in Therapeutic Range Increase the Risk of de Novo Donor-Specific Antibodies in the First Year of Kidney Transplantation. *Am. J. Transplant.* **2018**, *18*, 907–915. [[CrossRef](#)] [[PubMed](#)]
202. Kirk, A.D.; Hale, D.A.; Mannon, R.B.; Kleiner, D.E.; Hoffmann, S.C.; Kampen, R.L.; Cendales, L.K.; Tadaki, D.K.; Harlan, D.M.; Swanson, S.J. Results from a Human Renal Allograft Tolerance Trial Evaluating the Humanized CD52-Specific Monoclonal Antibody Alemtuzumab (CAMPATH-1H). *Transplantation* **2003**, *76*, 120–129. [[CrossRef](#)]
203. Kowalski, R.J.; Post, D.R.; Mannon, R.B.; Sebastian, A.; Wright, H.I.; Sigle, G.; Burdick, J.; Elmagd, K.A.; Zeevi, A.; Lopez-Cepero, M.; et al. Assessing Relative Risks of Infection and Rejection: A Meta-Analysis Using an Immune Function Assay. *Transplantation* **2006**, *82*, 663–668. [[CrossRef](#)]
204. Egli, A.; Humar, A.; Kumar, D. State-of-the-Art Monitoring of Cytomegalovirus-Specific Cell-Mediated Immunity after Organ Transplant: A Primer for the Clinician. *Clin. Infect. Dis.* **2012**, *55*, 1678–1689. [[CrossRef](#)] [[PubMed](#)]
205. Ginevri, F.; Azzi, A.; Hirsch, H.H.; Basso, S.; Fontana, I.; Cioni, M.; Bodaghi, S.; Salotti, V.; Rinieri, A.; Botti, G.; et al. Prospective Monitoring of Polyomavirus BK Replication and Impact of Pre-Emptive Intervention in Pediatric Kidney Recipients. *Am. J. Transpl.* **2007**, *7*, 2727–2735. [[CrossRef](#)] [[PubMed](#)]
206. Rittà, M.; Costa, C.; Sinesi, F.; Sidoti, F.; Di Nauta, A.; Mantovani, S.; Piccighello, A.; Simeone, S.; Ricci, D.; Boffini, M.; et al. Evaluation of Epstein-Barr Virus-Specific Immunologic Response in Solid Organ Transplant Recipients with an Enzyme-Linked ImmunoSpot Assay. *Transpl. Proc.* **2013**, *45*, 2754–2757. [[CrossRef](#)] [[PubMed](#)]
207. Abate, D.; Saldan, A.; Mengoli, C.; Fiscon, M.; Silvestre, C.; Fallico, L.; Peracchi, M.; Furian, L.; Cusinato, R.; Bonfante, L.; et al. Comparison of Cytomegalovirus (CMV) Enzyme-Linked Immunosorbent Spot and CMV Quantiferon Gamma Interferon-Releasing Assays in Assessing Risk of CMV Infection in Kidney Transplant Recipients. *J. Clin. Microbiol.* **2013**, *51*, 2501–2507. [[CrossRef](#)]
208. Walker, S.; Fazou, C.; Crough, T.; Holdsworth, R.; Kiely, P.; Veale, M.; Bell, S.; Gailbraith, A.; McNeil, K.; Jones, S.; et al. Ex Vivo Monitoring of Human Cytomegalovirus-Specific CD8+ T-Cell Responses Using QuantiFERON-CMV. *Transpl. Infect. Dis.* **2007**, *9*, 165–170. [[CrossRef](#)] [[PubMed](#)]
209. Mella, A.; Mariano, F.; Dolla, C.; Gallo, E.; Manzione, A.M.; Di Vico, M.C.; Cavallo, R.; De Rosa, F.G.; Costa, C.; Biancone, L. Bacterial and Viral Infection and Sepsis in Kidney Transplanted Patients. *Biomedicines* **2022**, *10*, 701. [[CrossRef](#)]
210. Mafi, S.; Essig, M.; Rerolle, J.-P.; Lagathu, G.; Crochette, R.; Brodard, V.; Schwartz, B.; Gouarin, S.; Bouvier, N.; Engelmann, I.; et al. Torque Teno Virus Viremia and QuantiFERON[®]-CMV Assay in Prediction of Cytomegalovirus Reactivation in R+ Kidney Transplant Recipients. *Front. Med.* **2023**, *10*, 1180769. [[CrossRef](#)]
211. Newell, K.A.; Asare, A.; Kirk, A.D.; Gisler, T.D.; Bourcier, K.; Suthanthiran, M.; Burlingham, W.J.; Marks, W.H.; Sanz, I.; Lechler, R.I.; et al. Identification of a B Cell Signature Associated with Renal Transplant Tolerance in Humans. *J. Clin. Investig.* **2010**, *120*, 1836–1847. [[CrossRef](#)]
212. Sagoo, P.; Perucha, E.; Sawitzki, B.; Tomiuk, S.; Stephens, D.A.; Miqueu, P.; Chapman, S.; Craciun, L.; Sergeant, R.; Brouard, S.; et al. Development of a Cross-Platform Biomarker Signature to Detect Renal Transplant Tolerance in Humans. *J. Clin. Investig.* **2010**, *120*, 1848–1861. [[CrossRef](#)]
213. Brouard, S.; Le Bars, A.; Dufay, A.; Gosselin, M.; Foucher, Y.; Guillet, M.; Cesbron-Gautier, A.; Thervet, E.; Legendre, C.; Dugast, E.; et al. Identification of a Gene Expression Profile Associated with Operational Tolerance among a Selected Group of Stable Kidney Transplant Patients. *Transpl. Int.* **2011**, *24*, 536–547. [[CrossRef](#)]
214. Orlando, G.; Hematti, P.; Stratta, R.J.; Burke, G.W.; Di Cocco, P.; Pisani, F.; Soker, S.; Wood, K. Clinical Operational Tolerance after Renal Transplantation: Current Status and Future Challenges. *Ann. Surg.* **2010**, *252*, 915–928. [[CrossRef](#)]

215. Lozano, J.J.; Pallier, A.; Martinez-Llordella, M.; Danger, R.; López, M.; Giral, M.; Londoño, M.C.; Rimola, A.; Souillou, J.P.; Brouard, S.; et al. Comparison of Transcriptional and Blood Cell-Phenotypic Markers between Operationally Tolerant Liver and Kidney Recipients. *Am. J. Transpl.* **2011**, *11*, 1916–1926. [[CrossRef](#)]
216. Danger, R.; Pallier, A.; Giral, M.; Martínez-Llordella, M.; Lozano, J.J.; Degauque, N.; Sanchez-Fueyo, A.; Souillou, J.-P.; Brouard, S. Upregulation of MiR-142-3p in Peripheral Blood Mononuclear Cells of Operationally Tolerant Patients with a Renal Transplant. *J. Am. Soc. Nephrol.* **2012**, *23*, 597–606. [[CrossRef](#)]
217. Viklicky, O.; Krystufkova, E.; Brabcova, I.; Sekerkova, A.; Wohlfahrt, P.; Hribova, P.; Wohlfahrtova, M.; Sawitzki, B.; Slatinska, J.; Striz, I.; et al. B-Cell-Related Biomarkers of Tolerance Are up-Regulated in Rejection-Free Kidney Transplant Recipients. *Transplantation* **2013**, *95*, 148–154. [[CrossRef](#)]
218. Rebollo-Mesa, I.; Nova-Lamperti, E.; Mobillo, P.; Runglall, M.; Christakoudi, S.; Norris, S.; Smallcombe, N.; Kamra, Y.; Hilton, R.; Indices of Tolerance EU Consortium; et al. Biomarkers of Tolerance in Kidney Transplantation: Are We Predicting Tolerance or Response to Immunosuppressive Treatment? *Am. J. Transpl.* **2016**, *16*, 3443–3457. [[CrossRef](#)]
219. Roedder, S.; Li, L.; Alonso, M.N.; Hsieh, S.-C.; Vu, M.T.; Dai, H.; Sigdel, T.K.; Bostock, I.; Macedo, C.; Metes, D.; et al. A Three-Gene Assay for Monitoring Immune Quiescence in Kidney Transplantation. *J. Am. Soc. Nephrol.* **2015**, *26*, 2042–2053. [[CrossRef](#)]
220. Bohne, F.; Martínez-Llordella, M.; Lozano, J.-J.; Miquel, R.; Benítez, C.; Londoño, M.-C.; Manzia, T.-M.; Angelico, R.; Swinkels, D.W.; Tjalsma, H.; et al. Intra-Graft Expression of Genes Involved in Iron Homeostasis Predicts the Development of Operational Tolerance in Human Liver Transplantation. *J. Clin. Investig.* **2012**, *122*, 368–382. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.