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Biomarkers in Renal Transplantation: Evolution of Strategies

Editorial

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The benefits conferred by renal transplantation (RT), such as the improved quality of life, prolonged survival and cost-effectiveness, are compromised by premature allograft losses from the deleterious effects of ischaemia-reperfusion injury (IRI) leading to delayed graft function (DGF), acute rejection (AR), infections, calcineurin-inhibitor toxicity, chronic allograft dysfunction (CAD), recurrent disease and cardiovascular deaths with functioning graft [1]. Current management of RT is largely reliant on monitoring the late manifestation of graft injury, such as serum creatinine level. Protocol surveillance biopsy is an invasive method of monitoring graft status, which has several limitations [2]. Non-invasive approach of RT management by measurement of biomarkers has been studied to assess their efficacy in prevention, prediction, early diagnosis and treatment of above conditions in both experimental and clinical settings. Biomarkers are measurable indicators of the presence or severity of pathological conditions affecting renal allografts. They indicate a change in expression or state of a protein that correlates with the risk, progression of a disease, or with the susceptibility of the disease to a given treatment, which can be detected and measured in blood, urine, perfusate and tissues [3, 4].

Evaluation of biomarkers in RT began in 1980 by measurement of urinary enzymes such as alanine aminopeptidase, microprotein and beta-glucuronidase in reversible and irreversible rejection episodes [5]. Since then, over 15000 biomarkers have been examined with respect to solid organ transplantation based on functional immune assay and non-invasive test based on blood gene expression [6]. Development of laboratory techniques such as real-time polymerase chain reaction, microarray profiling, mass spectrometry, in-situ hybridisation, gene and protein expression analysis, immunohistochemistry, enzyme-linked immunosorbent spot (ELISPOT) assay, flow cytometry and immune cell functional assays have been employed in wide range of RT scenarios [7, 8].

Despite development of newer immunosuppressive agents, tissue typing, sensitive cross-match techniques and regular monitoring of antibodies, both cell-mediated (CM) and antibody-mediated rejection (AMR) remain prevalent following living donor (LD) and deceased donor (DD) RT, which impact long-term allograft survival. Sarwal et al., have examined the predictive capacity of transcriptional kidney Solid Organ Response Test (kSORT) and interferon-ELISPOT assay in the Evaluation of sub-clinical Acute Rejection (sc-AR) PrEdiction (ESCAPE) Study in 75 consecutive RT recipients, who received 6-months protocol biopsies. The kSORT assay showed high accuracy predicting sc-AR (specificity, 98%; positive predictive value 93%) whereas the ELISPOT showed high precision ruling out sc-T-CMAR (specificity = 70%, negative predictive value = 92.5%), but could not predict sc-AMR, unlike kSORT [9].

In a cohort of RT recipients from Europe, genotype of single nucleotide polymorphisms (SNPs) was done and genetic variants in donor/recipient was associated with risk and severity of AR and allograft survival. Acute rejection was associated with presence of loci encompassing PTRO, coding for a receptor-type tyrosine kinase essential for B cell receptor signalling and ciliary gene CCDC67 [10]. Analysis of transcriptome of pre-transplantation biopsy specimens, showed significant difference in C3 gene expression between LD and DD, which was related to the length of cold ischaemia time, which correlated well with 2-year graft function [11].

Ischaemia-reperfusion injury occurs following restoration of blood flow and oxygenation of implanted kidney due to releases of free radicals which can lead to DGF and primary non-function (PNF). The adverse effects of DGF on long-term graft survival is well established, which occurs more frequently in donation after circulatory death (DCD) RTs compared to donation after brain death or LD RTs. Neutrophil gelatinase-associated lipocalin (NGAL) is released by activated neutrophils, which accumulates in the proximal convoluted tubules (PCT) after acute tubular injury. The urinary level of NGAL and expression of NGAL in the PCT were significantly increased in the episodes AR reflecting acute tubular injury [12]. In a study including 49 LD RT recipients, the serum NGAL correlated with graft function recovery and long-

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term graft function [13]. Urinary NGAL was evaluated in 124 RT recipients, which showed 10% increased risk of DGF and 15% risk of CAD progression in association with NGAL [14].

Hypothermic machine perfusion (HMP) is used in DCD RT for preservation purpose. In a prospective study, perfusate biomarkers such as NGAL, kidney injury molecule-1 (KIM-1), interleukin-18 (ILR-18) and liver-type fatty acid-binding protein (L-FABP) were found to correlate with reduced e-GFR at 6 months, but not with the incidence of DGF. However, most patients with "undesirable" biomarkers levels experienced acceptable 6-month allograft function, suggesting these biomarker characteristics should not be used in isolation for discard decisions [15].

Smith et al., performed proteomic analysis of serum of 54 RT recipients and observed correlation between the serum aminocyclase-1 (ACY-1) levels at day 1 and 3 post-transplant and DGF, slow graft function. immediate graft function and long-term graft function. ACY-1 is expressed predominantly in the PCT in pig and human kidneys and DGF results from acute tubular injury causing significant increase of ACY-1 level [16]. The level of lactic dehydrogenase and ILR-18 were found to be elevated in the perfusate of the HMP kidneys, which correlated with the incidence of DGF and PNF, but there was no significant association with 1-year graft survival. Glutathione S-transferase and aspartate transaminase were found to be significantly associated with DGF [17, 18].

Proteomics and metabolomics biomarkers studies in RT have been explored to generate diagnostic fingerprints. Chemokines CXCl-9 and CXCL-10 have been discovered in proteomics studies, which may help in guiding and individualising immunosuppressive regimens and predict acute and chronic T-CMAR and AMR [19]. Critical genes (CXCR4, CCL5 and ITGB2) were identified in 112 specimens when examined by microarray profiling technique, which are useful in assessing organ quality and predict kidney graft function [20].

Detection of Fas-ligand, granzyme B (GZMB) and perforin (PRF) in the blood and urine was significantly elevated in AR following RT. However, a recent meta-analysis concluded neither GZMB nor PRF, if evaluated alone, could be a convincing non-invasive diagnostic biomarker of AR in clinical practice. Combined GZMB and PRF post-RT may be a better choice in AR evaluation to direct allograft biopsy and earlier therapeutic intervention [21].

Micro-ribonucleic acid (miRNA) is a noncoding small molecule, discovered in 1993, plays an important role in the regulation of immune and adaptive immune response, which can be measured in blood, urine, urine cell pellets and tissues [22]. The miRNA has been extensively studied in RT recipients who developed acute kidney injury, AMR, CAMR and CAD and their detection before appearance of histological changes has significant implication in clinical transplantation [23, 24]. De novo donor-specific antibodies (DSA) do not always contribute to CAMR in RT. Investigation of miRNAs by microarray profiling revealed significant underexpression of miR-142-5p in patients with DSA. After DSA production, miR-486-5p and its target PTEN/foxO3 mRNA were significantly over-expressed ($p < 0.01$) and under-expressed ($p < 0.01$), respectively, in patients with biopsy-proven CAMR, compared with non-CAMR. Thus miRNA expression patterns may serve as non-invasive diagnostic biomarkers to evaluate immune response and RT status [25].

Urine miRNAs were compared between RT recipients diagnosed with CAD and those with normal function, which showed differential expression of miRNAs in recipients of CAD, which was identified as potential biomarker for monitoring graft function and anticipating progression to CAD [26]. In another study comprising of 47 RT recipients, two aberrant urinary miR-21 and miR0-200b expression levels were accompanied by renal allograft dysfunction [24].

Shrestha et al., observed significant elevation of cross-linking enzyme tissue transglutaminase and urinary epsilon(gamma-glutamyl)-lysine levels in the Fisher-to-Lewis rat model of chronic allograft nephropathy, which correlated with degree of tubulointerstitial fibrosis [27]. After RT, donor-derived cell-free DNA (ddcfDNA) can be detected in the recipient's blood and urine. Introduction of digital droplet PCR and massive parallel sequencing have been the major breakthrough in the investigation technique. Increased levels of ddcfDNA during AR even weeks to months before histological features of AR points to a possible role of ddcfDNA as an early non-invasive rejection biomarker [28].

The ultimate goal of biomarker studies is to find non-invasive biomarkers of transplant pathologies by using recipient's urine and serum, those indicate changes at the molecular level before the development of phenotype, that would predict allograft outcome, response to therapy and possibly reveal novel targets for therapeutic interventions [29]. Future strategies should be targeted to develop biomarkers in relation to the non-HLA antibodies, C4D-negative AMR, the role of the innate immune system in AR and complement-system-associated molecules.

Due to multifactorial aetiology of RT pathologies, biomarker studies need to be standardised and validated prospectively in large cohort of patients to eliminate the effects of confounding variables. The US "Clinical Trials in Organ Transplantation" (CTOT), the Canadian "Biomarkers in Transplantation" (BIT) project and the European study of "Reprogramming the Immune System for Establishment of Tolerance" (RISET) have been established for evaluations of biomarkers in transplantation in multicentre studies. The successful transfer of biomarkers to clinic will lead to personalised transplantation medicine, including improved donor-recipient matching, individual immunosuppressive regimens and individual risk assessment for DGF, AR, CAD and graft tolerance. These improvements will translate into improved graft and patient survivals and reduced cost to the health care providers [30].

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