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Biomarkers in Kidney Transplantation: Translating to clinical practice

Biomarcadores em Transplantação Renal: do laboratório à prática clínica

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ABSTRACT

Improving long-term graft survival is a major challenge in kidney transplantation. Ischaemia-reperfusion injury is a critical early allograft insult that enhances the risk of delayed graft function, which is common in deceased-donor transplantation. Delayed graft function complicates the post-transplant management and has a negative impact on both short and long-term outcomes. The development of effective interventions to prevent and attenuate the injury caused by ischaemia-reperfusion is constricted by the limited ability of early detection of kidney damage. In recent years, clinical and translational research has focused on improvements in the diagnosis of acute kidney injury and provided prognostic information that is helpful in the post-transplant care. Numerous biomarkers in kidney transplantation have been evaluated in the past decade, but, so far, evidence to support their use in routine practice is limited. The purpose of this review is to examine the current status of three biomarkers for early diagnosis and prognosis of delayed graft function, namely urinary neutrophil gelatinase-associated lipocalin, oxidative stress and cystatin C. In addition, the concept of a biomarker is addressed, as well as the existing challenges and perspectives for developing a biomarker. This review discusses current literature and reflects the author's own interpretation and experience.

Keywords: Biomarker, Delayed graft function, Kidney transplantation

RESUMO

Melhorar a sobrevivência do transplante renal a longo prazo é um desafio. A lesão provocada pela isquemia e reperfusão constitui uma agressão precoce do enxerto renal e aumenta o risco de atraso de função do enxerto, que é comum no transplante de dador cadáver. A ocorrência de atraso de função do

enxerto condiciona a evolução do pós-transplante e tem um impacto negativo nos resultados imediatos e a longo prazo do transplante renal. O desenvolvimento de intervenções eficazes na prevenção e atenuação da lesão causada pelo processo de isquemia-reperfusão do órgão transplantado tem estado limitado pela ausência de marcadores precoces da lesão e disfunção renal.

Nos últimos anos, a investigação clínica e de translação tem conseguido melhorar a capacidade de diagnóstico da lesão aguda do enxerto renal e fornecido alguma informação de prognóstico, que pode ser útil no seguimento pós-transplante. Nas últimas décadas têm sido investigados inúmeros biomarcadores no transplante renal, mas a sua translação para a prática clínica não tem sido escassa. Esta revisão tem como objetivo a descrição do contexto atual de três biomarcadores para o diagnóstico precoce de atraso na função do enxerto: a lipocalina associada à gelatinase dos neutrófilos (NGAL), o stress oxidativo e a cistatina C. Adicionalmente serão abordados alguns conceitos básicos e as perpetivas de desenvolvimento de um biomarcador, com base numa revisão da literatura e na interpretação e experiencia pessoal de uma investigação em curso nessa área.

Palavras-chave: Atraso de função do enxerto, Biomarcadores, Transplante Renal

INTRODUCTION

Late failure of kidney transplants remains an important clinical problem and one of the leading causes of end-stage renal disease. Despite significant improvements in one-year kidney allograft survival, the rate of chronic graft loss after the first year remains significant and the actual kidney allograft half-life only showed a marginal improvement over the past decade[1]. The reasons for this slight improvement remain unclear. It is possible that some important determinants of long-term graft survival may not have changed sufficiently to improve the overall outcomes of kidney transplantation[2]. Patient death with a functioning allograft and chronic allograft failure are the two major causes of late transplant loss. The causes of chronic allograft failure are multifactorial and influenced by numerous immunological and non-immunological factors[1,2]. Generally, kidney transplants stabilize after recovering from the stress of implantation until declining of graft function due to specific diseases or conditions, such as recurrent renal disease, antibody-mediated rejection or a common process involving interstitial fibrosis and tubular atrophy the entity encompassed by the previous descriptive term "chronic allograft nephropathy", and more recently simply 'fibrosis/atrophy'[2,3].

Improving long-term graft survival is a major challenge in kidney transplantation. A patient submitted to a renal transplant would wonder if his or her transplanted kidney will work well and how long will it last. When will it be possible to identify valuable markers to distinguish patients at increased risk of graft dysfunction or of losing their transplant? Can biomarkers signal early transplant dysfunction, a process that is often undetectable? Can biomarkers help clinicians fine-tune their prognoses?

As in every other domain in medicine, in organ transplantation early diagnosis and timely intervention will improve outcomes. Clinicians need and continually look for tools to aid them on clinical assessment and to enhance their ability to identify the "vulnerable" patient at risk for graft dysfunction. Biomarkers are one such tool. They will allow to better identify high-risk individuals, to diagnose dysfunction promptly and accurately, and to effectively prognosticate outcomes and treat patients with a tailored and more individualized intervention.

WHAT IS A BIOMARKER?

Biomarker is a very broad term that can be used to describe any indicator of a biological state. The term biomarker, or biological marker, was introduced in 1989 as a Medical Subject Heading (MeSH) term and it was defined as a "*measurable and quantifiable*

biological parameters (eg, specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances), which serve as indices for health- and physiology-related assessments." More recently, in 2001, the definition was standardized by the Biomarker Definitions Working Group[4] as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*". In practice, biomarkers can improve understanding about a disease and provide new knowledge of pathological mechanisms, making possible the earlier diagnosis and the delivery of more efficacious and safer therapies. Presently, it is not well established how biomarkers are categorized. Within the field of health care, biological markers are commonly classified based on the sequence of events from exposure to disease[5]: *biomarkers of exposure*, which are used in risk prediction, and *biomarkers of disease*, which are used in screening, diagnosis and prognosis.

To be clinically useful and prevent false-positive screening tests, a biological marker should be highly sensitive and specific in detecting disease or any other outcome. Regardless of the purpose for its use, it should be accurate, reproducible and standardized across different clinical units. Furthermore, it should be easily measured in a standard biological source (as blood or urine), obtained from a person (as blood pressure or electrocardiogram), or image-based (echocardiogram or computerized tomography scan), so that the information would be readily available and easy to interpret by clinicians[5,6]. In summary and according to the Food and Drug Administration, an ideal biomarker should be specific, sensitive, predictive, robust, simple, accurate, and inexpensive.

BIOMARKERS IN KIDNEY TRANSPLANTATION

In organ transplantation, initial graft dysfunction is one of the most important early post-operative problems, mainly due to the unavoidable ischemia-reperfusion injury that occurs in the transplanted organ. In kidney transplantation, ischemic injury of the renal allograft is a critical early insult that augments the risk of acute tubular necrosis and long-term graft loss[7,8]. The development of effective interventions is constricted by the limited ability of early detection of graft dysfunction. Current clinical indicators of kidney injury, like serum creatinine, are inadequate for timely diagnosis and prognosis. Thus, application of biomarkers in the field of kidney transplantation will allow to detect incipient graft dysfunction or rejection, will refine diagnoses and enable more effective post-transplant management, and thereby potentially improve short-term (e.g., delayed graft function, acute rejection) and long-term (e.g., allograft failure) outcomes.

Discovery of biomarkers is expanding at an unprecedented rate. Numerous biomarkers in kidney transplantation have been evaluated in the past decade, but, so far, evidence to support their use in routine practice is limited. In this article, we review the promising role of three biomarkers of delayed graft dysfunction, namely, neutrophil gelatinase-associated lipocalin, oxidative stress, and cystatin C.

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN

Neutrophil gelatinase-associated lipocalin (NGAL) is one the most promising biomarkers of acute kidney injury in a variety of acute clinical settings[9-11]. Neutrophil gelatinase-associated lipocalin, also known as human neutrophil lipocalin or lipocalin-2, is a 25-kDa glycoprotein belonging to the lipocalin family and originally it was found covalently bounded to gelatinase in activated neutrophils. This lipocalin behaves as a bacteriostatic agent in acute infections and it is released to blood from activated neutrophils during inflammatory processes. It was demonstrated that NGAL exists in two separate body pools, the systemic and the renal one. In the systemic pool, NGAL is normally expressed at very low concentrations in several human tissues. After glomerular filtration, circulating NGAL is reabsorbed in the proximal tubules, catabolized and finally released with urine in small quantities. In the renal pool, NGAL is rapidly released from renal tubular cells in response to various insults to the kidney. Thus, in steady situations, NGAL is found in urine only in trace. In contrast, when massive NGAL quantities are expressed and excreted in urine this usually indicates injury and damage of proximal tubular cells, due to

ischaemia-reperfusion injury, hypoxia, nephrotoxins or chronic progressive changes[12,13].

Ischaemia-reperfusion injury is an inevitable consequence of kidney transplantation procedure and can be considered a form of post-transplantation acute kidney injury. For this reason, several studies investigated the utility of NGAL for the diagnostic and prognostic of acute graft dysfunction following kidney transplantation, with promising results[14-19]. Recently, the prognostic value of NGAL on graft function at one year post-transplantation was also examined[18,20].

The larger study on this subject is from Hollmen and colleagues[18]. These researchers demonstrated that urinary NGAL (uNGAL) is an early predictor of delayed graft function (DGF) following renal transplantation, in a prospective cohort study of 176 adult recipients transplanted with deceased-donor kidneys. Urine was collected before transplant, at then at days 1, 3, 7 and 14, and NGAL was measured at each time point. The uNGAL measured in the first morning following transplantation predicted DGF (defined as the need for dialysis during the first week after transplantation), particularly in cases where early graft function was expected on the basis of diuresis and decreasing plasma creatinine concentration. Patients who needed dialysis in the first post-transplant week had a slower decrease in uNGAL compared with recipients without DGF, and levels of uNGAL at day-1 predicted DGF (area under the curve (AUC) of 0.75). In 15 of 112 cases with urine output higher than 1L at day-1, uNGAL was a predictor of DGF, as well in 19 of 86 cases with a day-1 decrease in creatinine over than 1 mg/dl (AUC 0.74). Other authors showed also prominent results. Parikh *et al.* in a prospective study that included 53 consecutive patients undergoing living or deceased-donor transplantation (children and adults), measured NGAL in urine samples collected within the first 24 hours after transplantation and reported an AUC-ROC of 0.9 in predicting DGF[14]. Hall and coworkers evaluated uNGAL within 6h after transplantation in a 91-patient cohort of adults with a kidney graft from a deceased-donor, and predicted subsequent DGF with an AUC-ROC of 0.81[16].

Across a range of clinical studies, both urine and plasma NGAL has been shown to be a useful discriminatory marker of renal injury and an early predictor of DGF, with a performance greater than serum creatinine, the most commonly used surrogate measurement of glomerular filtration rate. In our experience, and similarly to other authors[14,18], we have chosen to measure NGAL in urine (uNGAL), instead of blood, since uNGAL represents tubule damage in the kidney rather than filtration from blood[13,21]. Although plasma NGAL is freely filtered by the glomerulus, it is largely reabsorbed in proximal tubules by efficient megalin-dependent endocytosis[11]. Thus, any urinary excretion of NGAL is likely only when there is concomitant proximal renal tubular injury that precludes NGAL reabsorption and/or increases *de novo* NGAL synthesis. Accordingly, an increased level of NGAL in urine usually indicates injury and damage of tubular cells and seems to be more specific compared to serum NGAL, which can be produced by other organs and released into the circulation following a transplant surgery[21]. The non-invasive nature of sample collection and the reduced number of interfering proteins were also other advantages taken in account when we choose to measure this biomarker in urine[10].

However, despite the undoubtedly value of urinary markers of kidney injury, their use in transplant recipients can be also a drawback because of possible transient graft anuria, which may preclude the availability of urine and consequently the lack of sample to measure NGAL. The persistent urine production by the native kidneys and the usual fluctuations of hydration status in these patients can also induce potential changes in urinary biomarker concentration, which can be another inconvenience to measure NGAL in urine. The genesis and sources of plasma and urinary NGAL require further clarification. However, despite the uncertainty of whether NGAL level performs better in urine or plasma/serum, both plasma/serum and urine NGAL levels appear to perform similarly well and provide a relevant advantage compared with serum creatinine, which is an insensible marker of kidney injury[9].

OXIDATIVE STRESS

Oxidative stress is one of the most important components of the ischaemia-reperfusion process[22-24]. It reflects an imbalance between reactive oxygen species (ROS) and cellular mechanisms for detoxifying

the reactive intermediates or for repairing the resulting damage. Disturbances in the normal state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell.

Oxygen free radicals or, more generally, ROS are products of normal cellular metabolism. It has been estimated that the average person has around 10,000–20,000 free radicals attacking each body cell every day. Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals, which gives a considerable reactivity to the free radical. The well-known radicals derived from oxygen, such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) represent the most important class of radical species generated in living systems[25].

In physiological conditions, ROS are produced specifically to serve essential biological functions, as in defence against infections. In these conditions, the rates of free radical production and elimination are equal, leading to a steady state that is presumably tolerated by the cell. The antioxidant defence mechanisms can be divided into two major groups: endogenous, mainly enzymes, such as superoxide dismutases (SOD), catalases, glutathione reductases (GR) and peroxidases (GPx) and small molecules, mostly exogenous, acting as free radical scavengers (vitamins A, C, and E, carotenoids and polyphenol)[25]. In some pathological conditions, an imbalance between ROS generation and antioxidant capacity leads to enhanced ROS activity and oxidative stress. When these antioxidant mechanisms cannot counterbalance the amount of free radicals generated, cell damage and tissue injury can take place[26].

Reactive oxygen species may cause tissue injury via several mechanisms. As they are potent oxidizing and reducing agents, ROS directly damage cellular membranes and modify several biological molecules, such as lipids, proteins, and nucleic acids. The by-products of these reactions can serve as biomarkers of oxidative stress[22,23]. Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation generates a huge variety of secondary products, including reactive carbonyl compounds, such as malondialdehyde (MDA). This aldehyde is the principal and most studied product of polyunsaturated fatty acid peroxidation, and for this reason MDA is a marker widely used to assess lipid peroxidation[25,26].

Markers of oxidative stress, including elevated levels of MDA and reduced antioxidant activity have been reported in renal patients[27,28]. There is huge amount of literature concerning oxidative stress and renal disease but data about kidney transplantation in the early stages are scarce. The restoration of kidney function after transplantation can lead to improvement of the oxidative stress[29], but some studies report increased systemic biomarkers of oxidative stress in kidney transplant recipients[30,31], specifically in the early phase of transplantation[32,33] and, thereafter, coexisting with chronic allograft tubular atrophy/interstitial fibrosis[30,34].

It has long been suspected that oxidative stress contributes to injury of ischaemic and reperfused tissues. In the setting of kidney transplantation, not only are there ischaemic and reperfusion periods obligated by the preservation and implantation procedures, but placement of the kidney into an immune milieu can also act as an adjuvant for oxidative damage. Reactive oxygen species are generated during both phases of ischaemia-reperfusion. As in other clinical conditions, if the scavenging capacity of kidney is beneath the excessive ROS generated, such oxidative imbalance may trigger a robust inflammatory response within the transplanted organ and lead to cellular destruction, tissue damage and graft dysfunction[33,35]. Thus, severe reperfusion injury is a risk factor for DGF and detection of ROS could be an early warning of graft injury. Waller and coworkers studied blood samples in porcine kidney allografts before and after reperfusion injury and demonstrated that both plasma carbonyl and 8-isoprostane (product of protein and lipid damage by free radicals respectively) could be reliable biomarkers to predict the reperfusion injury[36]. To the author's knowledge, no similar studies were done on this topic in human kidney transplantation.

A wide range of antioxidant enzymes may potentially exert a protective influence by limiting the production of ROS and the damage of oxidative stress following ischaemia-reperfusion injury of kidney graft. Conflicting results are reported in the literature on the activities of antioxidant enzymes in kidney transplant patients. Glutathione compounds and SOD have been reported to increase[37,38] decrease[31] or not

change[39] following renal transplantation. Whitin *et al.*[37] reported a rapid increase in plasma GPx activity after transplantation. The plasma GPx activity was two times higher 3 days after transplantation in adult patients who received a kidney transplant from a related donor; and rapidly increased over the first 2 weeks post-transplant in adult recipients from a deceased-donor and paediatric patients undergoing kidney transplantation from related donors. Zachara *et al.*[38] have shown that plasma GPx activity increased rapidly 3 days after renal transplantation, and doubled two weeks later. Both of these studies suggested that monitoring plasma GPx might be a useful marker for monitoring transplanted kidney function and a valuable tool for post-operative detection of DGF.

Not only in the early post-transplant period but also at longer-term, oxidants and antioxidants can be as biomarkers of graft dysfunction with diagnostic accuracy. Oxidative stress is believed to be a common pathway that leads to both immunological and non-immunological stress in the setting of kidney transplantation and to the development or progression of chronic allograft nephropathy. Increased plasma and intragraft levels of MDA and reduced antioxidant activity were found in kidney allografts with chronic tubular atrophy/interstitial fibrosis, which suggests the possibility of early detection, even when graft dysfunction is undetectable by serum creatinine[30,34,40].

The understanding about oxidative stress significantly advanced in the last decade, but these experimentally derived ideas have yet to be fully integrated into clinical practice. General evidence for involvement of ROS in hypoxia-reoxygenation injury includes detection of lipid peroxidation. Malondialdehyde is an end-product of lipid peroxidation and it is a frequently measured biomarker of oxidative stress. Studies on this topic are limited in kidney transplantation. Therefore, more research is needed to clearly define the role and clinical value of MDA and other oxidative stress markers in kidney transplantation.

CYSTATIN C

Cystatin C (CysC) is a low molecular mass protein that is produced at a constant rate by nearly all-human nucleated cells. This cystatin is freely filtered through the normal glomerular membrane, almost completely reabsorbed and degraded by proximal tubular cells, but it is not secreted by the tubules. Although its clearance cannot be measured because of this catabolism, its plasma or serum concentration has been shown to be independent of muscular mass, inflammatory diseases, sex, age or diet, and these properties make it a good measure of glomerular filtration rate (GFR) compared to the traditional measurement of creatinine[41,42]. As a result of this finding, several prediction equations have been derived from both paediatric and adult patients to estimate GFR from the serum CysC concentration[43,44]. Most of the studies that compared CysC levels or CysC derived equations with gold standard methods found CystC to be superior or, at least, equivalent to serum creatinine[42]. Some studies on selected patient groups, whose muscle mass is either reduced or undergoes rapid changes, also demonstrated CysC as a sensitive marker of GFR and independently of body composition[41].

Renal transplant recipients are a target group for whom precise determination of GFR is crucial. Allograft function following renal transplantation is commonly monitored using serum creatinine. However, plasma creatinine is far from being an ideal marker of GFR, despite its convenience and low cost. Since the first publication in 1998[45], quite a few original clinical papers have addressed the question of the use of CysC in kidney transplantation. A good number of studies identified serum CysC (or CysC-based equations) as a promising, easily measurable marker to estimate GFR with a higher diagnostic value than serum creatinine (or creatinine-based equations) and 24-hour creatinine clearance for evaluation of GFR in the follow-up of adult kidney transplant patients[46-48]. Very recently, Masson *et al.*[48] validated both of CysC-based CKD-EPI equations (2012) in 670 kidney transplant recipients and concluded that both performed better than the serum creatinine-based CKD-EPI equation (2009).

Glucocorticoid medication can be shortcoming in using serum CysC in this population and it is important to take into account when interpreting this serum marker. Glucocorticoid therapy is one of the few circumstances identified that have an impact on the production of CysC in a dose-dependent manner, leading to systematic underestimation of GFR[49]. Very

large doses of glucocorticoids have been described to increase the production of CysC[49], whereas low and medium doses of glucocorticoids do not seem to alter the production of CysC[50]. This, however, does not preclude the use of CysC in detecting impaired renal function in renal transplant patients on glucocorticoids, given that many studies have shown CysC to be still significantly more accurate in detecting impaired renal function in this patient group[49,50].

For kidney recipient follow-up, the ability to detect rapid changes in GFR is clinically more important than accuracy itself. For this reason, and due to the promising findings of CysC in kidney transplantation, the role of this marker in detecting post-transplant renal damage earlier than serum creatinine has been investigated[51-54]. Thervet *et al.*[51] in a prospective study of 30 renal transplant patients found that CysC allowed earlier diagnosis of renal function recovery than serum creatinine, particularly in patients with DGF. In a prospective study that included 30 consecutive patients with end-stage renal disease undergoing renal transplantation, Le Bricon *et al.* evaluated CysC as a marker of allograft function during the early post-operative transplantation period. Serum CysC was more sensitive than serum creatinine for detecting decreases in GFR and predicting DGF. Furthermore, a more prominent rise in plasma CycC values allowed a more rapid diagnosis of acute rejection or treatment nephrotoxicity, with the potential for more timely intervention[52]. Hall *et al.* also confirmed these findings in a cohort of 78 deceased-donor renal recipients and shown that CysC outperformed serum creatinine as a predictor of poor early graft function and the need for dialysis within the first week of kidney transplantation. The authors also proved that CysC was a good prognostic marker of graft function at 3 months[53]. In a recent article published in June 2012, Liu *et al.* evaluated the clinical value of CysC for the diagnosis of an acute rejection episode after renal transplantation in 76 recipients and concluded that CysC can predict acute rejection episode after renal transplantation[55]. Urine CysC was also studied recently by Hall *et al.*[54] in a prospective multicenter study that included 91 deceased-donor kidneys transplants. Serial urine samples were collected for 2 days following transplant and on the first post-operative day urine CysC was a predictor of DGF and of 3-month allograft function.

Cystatin C displays several good characteristics that make it a viable biomarker for early detection of DGF. Of the three markers addressed in this review, serum CysC is probably the biomarker most used and closest to the clinical validation in kidney transplantation.

THE AUTHOR'S EXPERIENCE

The Renal Transplant Unit at the Centro Hospitalar do Porto conducted a prospective, longitudinal study in 40 consecutive end-stage renal disease patients undergoing living or deceased-donor kidney transplantation, from December 2010 to May 2011. This study aimed to identify early markers of graft dysfunction in the peritransplant period and investigate their accuracy in predicting DGF (defined as dialysis requirement within the first week after transplantation). The receiver operating characteristic curve analysis is commonly used to evaluate biomarker utility in clinical diagnosis of disease, especially during biomarker development research. Using this statistical tool, the present study demonstrated uNGAL, MDA and CysC as good diagnostic markers on identifying patients with graft dysfunction in the early post-transplant period and who required dialysis in the first week (*articles submitted or in draft*). When analyzed separately, all three biomarkers predicted who would develop DGF with about the same degree of accuracy, and all of them with a diagnostic performance superior to serum creatinine.

Despite these encouraging results, this is not enough to certify any of these markers as diagnostic and prognostic biomarkers with wide clinical utility. Before each of these (or any other) biomarkers can be deployed in the clinic, they have to be repeatedly tested in hundreds of patients to assure that they serve as effective markers of acute graft dysfunction and prognostic indicators of dialysis-based DGF. It is a long and laborious pathway from identifying to validating a reliable biomarker.

Delayed graft function is a common complication affecting renal grafts immediately after transplantation. Since DGF has so many detrimental effects, accurate and early identification of features of DGF is remarkably important because it would allow more targeted and personalised treatment approaches.

Several studies were done in renal transplantation to identify biomarkers for the diagnosis of DGF. However, there is still no routine application of any of these markers in clinical transplantation. The first step was taken in the long march to translate a biomarker from the laboratory into the clinical practice. Generation of prospective data will now be necessary for validation and demonstration of the clinical utility of these markers in other centres and transplant recipients, across different practices and sets of variables. If validated, these biomarkers will be a major advantage for transplant recipients by allowing their care team to detect acute kidney injury before the risk of graft dysfunction becomes too high and the possibility of intervention less effective.

CONCLUSIONS

In renal transplant patients, early detection of impaired kidney function is critical so that efforts to prevent further deterioration of graft function or rejection can be instituted. Biomarker investigations are now an integral part of clinical research. The overall expectation of a biomarker is to enhance the ability to detect earlier an ongoing biological process and predict which patients will respond better to which interventions. To bring biomarkers to the clinic, it is mandatory to show a useful clinical application that is supported by the validation data. In the field of kidney transplantation, some biomarkers have successively gone the process of discovery and of validation, but fall short in their ability to contribute decisively to patient care. In a time of greater economic constraints and more personalized medicine, biomarkers are certain to have a presence in transplant care and coordinated and collaborative efforts should be made to implement novel biomarkers into the clinical practice.

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