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AIM AND SCOPE

World Journal of Nephrology (*World J Nephrol*, *WJN*, online ISSN 2220-6124, DOI: 10.5527) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJN covers topics concerning kidney development, renal regeneration, kidney tumors, therapy of renal disease, hemodialysis, peritoneal dialysis, kidney transplantation, diagnostic imaging, evidence-based medicine, epidemiology and nursing. Priority publication will be given to articles concerning diagnosis and treatment of nephrology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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REVIEW

Kidney regeneration: Where we are and future perspectives

Joao Paulo Zambon, Renata S Magalhaes, Inkap Ko, Christina L Ross, Giuseppe Orlando, Andrea Peloso, Anthony Atala, James J Yoo

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Abstract

In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, approximately 40% of recipients will die or lose graft within 10 years. The limitations of current therapies for renal failure have led researchers to explore the development of modalities that could improve, restore, or replace the renal function. The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding cell sources and mechanisms to develop a bioengineering kidney. Due to kidneys peculiar anatomy, extracellular matrix based scaffolds are rational starting point for their regeneration. The perfusion of detergents through the kidney vasculature is an efficient method for delivering decellularizing agents to cells and for removing of cellular material from the tissue. Many efforts have focused on the search of a reliable cell source to provide enrichment for achieving stable renal cell systems. For an efficient bioengineered kidney, these cells must be attached to the organ and then maturated into the bioractors, which simulates the human body environment. A functional bioengineered kidney is still a big challenge for scientists. In the last ten years we have got many improvements on the field of solid organ regeneration; however, we are still far away from the main target. Currently, regenerative centers worldwide have been striving to find feasible strategies to develop bioengineered kidneys. Cell-scaffold technology gives hope to end-stage renal disease patients who struggle with morbidity and mortality due to extended periods on dialysis or immunosupression. The potential of bioengineered organ is to provide a reliable source of organs, which can be refunctionalized and transplanted.

Key words: Kidney regeneration; Stem cells; Decellularization; Extra cellular matrix; Regenerative medicine

Core tip: In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, 20% of recipients will experience an episode of acute rejection within 5 years of transplantation, and approximately 40% of recipients will die or lose graft function within 10 years. The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding possible cell sources and mechanisms to develop a bioengineering kidney.

Zambon JP, Magalhaes RS, Ko I, Ross CL, Orlando G, Peloso A, Atala A, Yoo JJ. Kidney regeneration: Where we are and future perspectives. *World J Nephrol* 2014; 3(3): 24-30 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/24.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.24

INTRODUCTION

In the United States, approximately 1 million patients live with end-stage renal disease (ESRD), with over 100000 new diagnoses every year. Although hemodialysis has in-

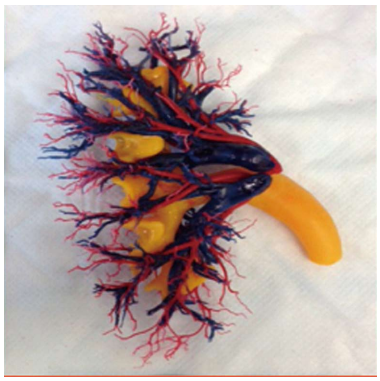

Figure 1 Vascular and collecting system casting of a kidney. Red: Arteries; Blue: Veins; Yellow: Ureter and collecting system. Permission of Wake Forest Institute for Regenerative Medicine.

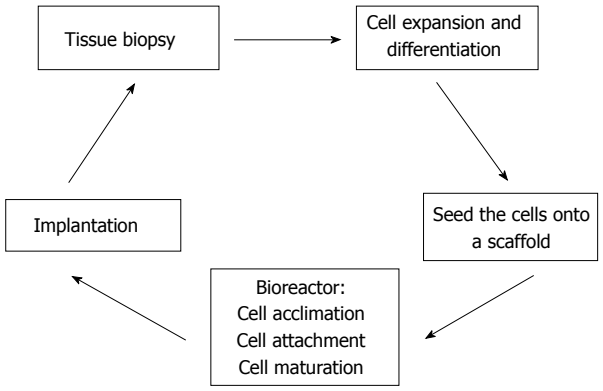

Figure 2 Regeneration of a tissue/organ.

creased the survival of patients with ESRD, kidney transplantation remains the only potential curative treatment. In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, 20% of recipients will experience an episode of acute rejection within 5 years of transplantation, and approximately 40% of recipients will die or lose graft function within 10 years[1-4].

The limitations of current therapies for renal failure have led researchers to explore the development of alternative modalities that could improve, restore, or replace either partial or total renal function. Tissue engineering and regenerative medicine represents one of the newest innovations in modern-day science. It represents a broad spectrum of methodologies and techniques aiming to repair, augment, and regenerate damaged organs and tissues. The basis of tissue engineering is that cells can be expanded *in vitro,* placed on a tissue scaffold made of suitable biomaterial, and then implanted into the host[5-7].

Within the field of organ bioengineering, the methodology of seeding cells on supporting scaffolding material has shown great promise for generating viable organs. A simple structure such as vessels, bladders, upper airways, and urethras have been implanted into patients with acceptable results in the short and midterms. The structural simplicity of these organs enables them to meet the oxygen and nutrient requirements *via* diffusion from adjacent host tissues while angiogenesis has time to occur. Unfortunately, complex organs such as the kidney cannot be viably incorporated without the reconnection of new structure to the host vasculature, a task that has presented insurmountable challenges experimentally let alone clinically[8-12].

The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding possible cell sources and mechanisms to develop a bioengineering kidney.

STRUCTURAL AND FUNCTIONAL CONSIDERATIONS OF HUMAN KIDNEYS

The kidneys are the primary organs for maintaining fluid, electrolyte, and acid-base balance. They produce hormones such as rennin, erythropoietin, and convert a precursor of vitamin D, 1,25-dihydroxyvitamin D, to active metabolite. Each kidney has more than thirty different cell types, approximately 2 million glomeruli, numerous arterioles, capillaries, and tubules that interconnect in a three dimensional pattern to filter the blood and excrete waste through the collecting system[13]. Figure 1 represents an example of a kidney's vascular cast.

WHOLE KIDNEY REGENERATION

Ideally, a bioengineered kidney must be biocompatible, non-immunogenic and support cell growth. The basic idea for solid organ regeneration is to harvest a tissue biopsy from a donor and expand these cells in culture. Subsequently, they are seeded into a scaffold, and placed into a bioreactor in order to promote cell acclimation, attachment, and maturation. Once this is achieved, the cell-seeded scaffold can be implanted on the host[14,15]. Figure 2 represents a potential methodology for solid organ regeneration.

KIDNEY DECELLULARIZATION AND EXTRACELLULAR MATRIX PRESERVATION-BIOLOGICAL SCAFFOLD PRODUCTION

Extracellular matrix (ECM) is the naturally occurring scaffold material secreted and manufactured by the resident cells of each tissue and organ. The complex 3D organization of the ECM and its components are dictated by the tissue from which ECM is derived. The structural and functional molecules of the ECM are in a state of dynamic equilibrium within the surrounding tissues, and also provide the means by which cells communicate with each other and the external environment. The ECM contains growth factors and others bioinductive factors, which facilitates the remodeling process, cell attachment and tissue integration[16,17].

Due to their peculiar anatomy and physiology, ECM based scaffolds are a rational starting point for kidney re-

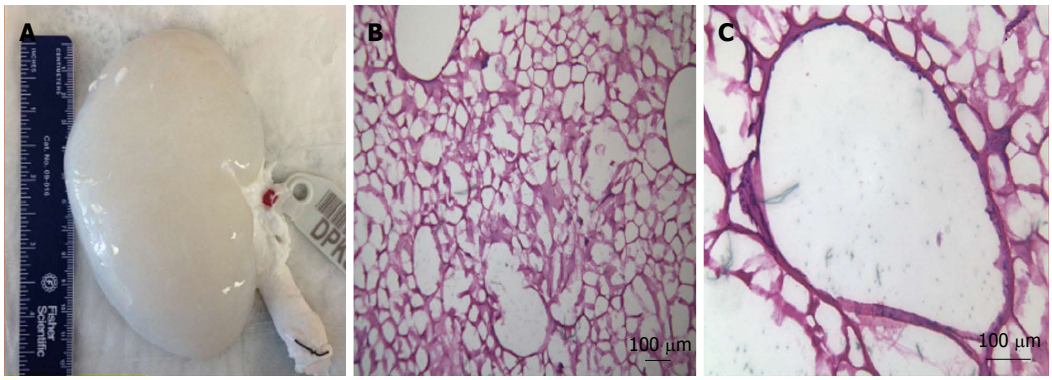

Figure 3 A decellularized pig kidney scaffold and its extra cellular matrix after decellularization. A: Decellularized pig kidney scaffold; B: Hematoxylin and eosin staining of the decellularized pig kidney scaffold shows a decellularized extracellular matrix (× 200); C: Hematoxylin and eosin staining of the decellularized pig kidney scaffold shows a decellularized extracellular matrix (× 400). Permission of Wake Forest Institute for Regenerative Medicine.

generation. For this purpose, several protocols have been described for whole kidney decellularization. The perfusion of detergents and enzymes (*e.g.*, DNase) through the kidney vasculature is an efficient method for delivering decellularizing agents to cells and for removing of cellular material from the tissue. However, in spite of being effective decellularization agents, the effects of these agents on kidney microvasculature have not been established, and further studies are necessary to elucidate them[18-20]. Figure 3 represents a decellularized pig kidney scaffold and its extra cellular matrix after decellularuzation.

CELL EXPANSION AND DIFFERENTIATION

The kidney has approximately 30 different specialized cell types. For an efficient scaffold, all cells must be characterized for repopulation, which represents a challenging task. Many efforts have focused on the search of a reliable cell source and optimal growth conditions to provide adequate enrichment for achieving stable renal cell expansion systems[4].

Regarding vascular anatomy, the main renal artery splits into segmental, interlobar, interlobular, and arcuate arteries. The venous system is formed by a complex net of veins, which drains to the main renal vein. Each kidney has approximately 2 million glomeruli, which are responsible for renal filtration process and an extensive net of capillaries[13]. Figure 4 represents a pig kidney's glomerulus.

ADULT KIDNEY STEM CELLS

The regenerative capacity of a tissue is determined in part by whether it contains endogenous stem cells. These stem cell populations are housed in a niche, which regulates stem cell survival, self-renewal, and differentiation. In a normal environment, stem cells remain quiescent in the niche for long periods until they are activated by the requirement of new cells to maintain the tissue or because of the tissue damage. With regards to renal adult stem cells, a subset has been found in the Bowman's capsule, glomeruli, pericytes, proximal tubules, and renal papilla. These cells expressed stem cell markers such as CD24, CD133, CD146 and Pax-2[21-25].

Regenerative mechanisms after acute renal failure have not been well established, but appear that tubular cells, growth factors and cytokines are involved in this process as well demonstrated by Humphreys *et al*[26]. They reported *via* a genetic maping technique, that tubular epithelial cells were the predominant source of regeneration after kidney ischemic injury, and distal tubular cells were more involved with growth factor production such as epidermal, IGF-1, and hepatocyte growth factor[26,27].

Harari-Steinberg *et al*[28] identified in human kidneys nephron progenitor cells (hNPCs), which were capable of generation of kidney structures and functional repair of chronic renal disease. These cells expressed NCAM1$^+$ and had a high clonogenic potential. Moreover, when grafted in aggregates into a chorioallantoic membrane of the chick embryo they generate renal structures. Ultimately, hNPCs were injected directly into the kidney's parenchyma of mice with chronic kidney disease. They reported that treatment with hNPCs halted disease progression and increased creatinine clearance throughout the 12-wk study period[28].

Buzhor *et al*[29] also demonstrated that human adult kidney epithelial cells (hKEpCs) positive for NCAM1$^+$ overexpressed nephron progenitor markers, acquired a mesenchymal fate and produce epithelial renal tissue on single-cell grafting in chick chorioallantoic membrane and mouse.

Rinkevich *et al*[30] demonstrated an *in vivo* clonal analysis of progenitor cells found in mammalian kidneys. They used a long-term *in vivo* genetic lineage tracing and clonal analysis of individual cells isolated from kidneys and demonstrated that tissue and lineage-restricted precursors cells from tubules and non tubules structures such as glomerulus are directly involved in the kidney recovery after injury. As a future direction, the isolation and characterization of kidney precursor cells offer a

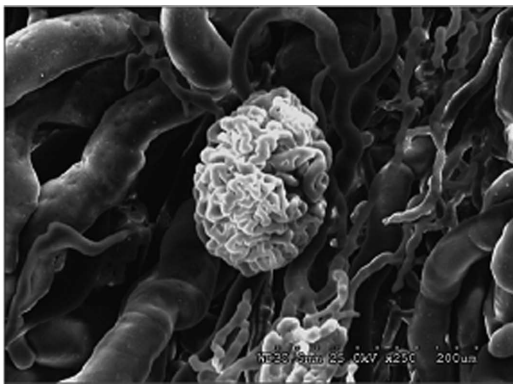

Figure 4 Normal kidney glomerulous visualized through scanning electron microscopy (magnification × 250). Permission of Wake Forest Institute for Regenerative Medicine.

therapeutic target to increase or restore the regenerative capacity of the mamallian kidney[30].

BONE MARROW DERIVED STEM CELLS

The bone marrow contains two major populations of stem cells, hematopoietic stem cells (HSCs), and mesenchymal stromal cells (MSC), which provide stromal support for HSCs. These stem cell therapies derived from bone marrow have been used to repair a variety of organs in experimental models. A possible explanation for apparent plasticity of these cells is a mechanism of transdifferentiation, dedifferentiation or cell fusion[31].

Despite studies have shown promising results with bone marrow derived stem cells, the biological relevance and clinical importance have not been well demonstrated for kidney regeneration. Initially, bone-marrow stem cells have been considered as a source of "replacement" cells that could be used for the treatment of different diseases. However, studies in experimental transplantation models or direct injection of these cells into tissue have shown that the contribution of bone-marrow cells to nonhematopoietic cell fates is uncommon. Therefore, the potential of these cells for whole organ regeneration is far from being considered as a treatment option[32,33].

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSC) are stromal cells that can be found and isolated from different tissues. Because of their multilineage differentiation potential and their ability to migrate to the site of injury, they have been studied in the last 10 years as a therapeutic agent in kidney injury. It has been demonstrated that MSC are involved in immune response through the activation of T and B-lymphocytes. In addition, they stimulate interleukins, β-TGF, hepatocyte, fibroblast, and vascular endothelial growth factors[34,35].

Adipocyte derived stem cells (ADSC) are a type of mesenchymal stem cell that in the last decade have been explored as an attractive source of cells with regenerative properties. These cells are abundant, easily harvested with low morbidity, and seem to stimulate angiogenesis. Regarding kidney regeneration, de Almeida *et al*[36] demonstrated in an experimental model of acute renal failure that the injection of ADSC in mice reduced renal fibrosis at six weeks; however, they did not find the injected cells in the kidney. Chen *et al*[37] also demonstrated in rats that an intra-renal injection of ADSC attenuated the deterioration of renal function at 14 d, improved angiogenesis, and preserved the architecture integrity[36,37].

Despite being an attractive source of stem cells for kidney regeneration, the exact mechanism of action of MSCs has not been established. Most of the experimental studies have been performed with rodents and have short follow up. Further studies with a longer follow up and using large animal models should be performed before translating to clinical trials.

HUMAN AMNIOTIC FLUID AND PLACENTA DERIVED STEM CELLS

Amniotic fluid, due to its contact with the fetus, has been considered an interesting source for undifferentiated or partially differentiated cells. The molecular composition of amniotic fluid and the presence of nutritive substances play a key role in the proliferation and differentiation of different cell types.

Human amniotic stem cells (HASC) express surface markers and transcription factors distinctive of embryonic stem cells (ESCs). These include octamer-binding transcription factor 4 (OCT-4) and stage specific embryonic antigen (SSEA-4)[38]. HASCs have high replicative self-renewal potential and multilineage differentiation capacity. Perin *et al*[39,40] showed that HASC integrated into metanephric structures after being injected into embryonic kidneys, which improved repair/recovery of kidneys with acute tubular necrosis[39-41].

In the field of cell therapy and regenerative medicine, many studies have been done to establish reliable animal models for different types of disease targeting the feasibility and benefits of human amniotic stem cell therapy.

EMBRYONIC STEM CELLS

ESC are pluripotent cells derived from blastocysts. These cells propagate readily and remain undifferentiated when cultured with leukemia inhibitory factor (LIF). When LIF is withdrawn, ESCs form aggregates called embryoid bodies that generate a variety of specialized cell types. However, the extraction of these cells involves the destruction of embryos, therefore their use is associated with controversial ethical dilemmas[42].

In spite of the self-renewing potential and the capability of differentiation into tissues derived from the three germ layers, ESCs are associated with uncontrolled growth and teratoma formation. Kidney regeneration studies have demonstrated that renal progenitor cells derived from ESCs differentiated into glomerular-like

structures and integrated into renal proximal tubules when implanted *in vivo*[42,43].

GENERATION OF KIDNEY TISSUE FROM EMBRYONIC RUDIMENTS

This approach for generating kidney tissue can be achieved by the use of undifferentiated or partially developed kidney precursor cells derived from early embryos and fetal tissue. Dekel *et al*[44] transplanted in immunodeficient mice human or pig kidney precursors, which were obtained from 7 to 8 wk human fetus or 3.5 to 4 wk pig gestation. The rudimentar kidneys survive, grow and complete nephrogenesis, forming a functional organ able to produce urine. The successful organogenesis was achieved only when early progenitors with mesenchymal cells and ureteric bud branches were transplanted. Nevertheless, as well as embryonic cells, this approach also involves the destruction of embryos and is associated with controversial ethical dilemmas[44].

SOMATIC NUCLEAR CELL TRANSFER

The first renal tissue created *via* therapeutic cloning techniques has been described by Lanza *et al*[45] in a bovine model. In this study, a skin fibroblast nucleus was microinjected into an enucleated oocyte that was transplanted *in vitero* for 12 wk. Cloned renal cells were then seeded into a biodegradable scaffold and transplanted *in vivo* to follow the growth of the construct. The authors reported that the kidney-like organ was capable of secreting urinary fluid, confirming that the implant contained cells capable of filtration, reabsorption and secretion. However, like ESCs, this technique also is associated with controversial ethical dilemmas[45,46].

INDUCED PLURIPOTENT STEM CELLS

Induced pluripotent stem cells (iPSC) were first described by Takahashi and Yamanaka in 2006 when they reprogrammed human fibroblasts to become pluripotent stem cells by the addition of four different genes: *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. Despite being a good source of cells, not all adult stem cells can be reprogrammed using the same method, which means that each cell type may have critical factors. Unlike ESCs, iPS cells have no ethical issues and no immune rejection. On the other hand, these cells are reprogrammed through the addition of oncogenes, which increase the risk of uncontrolled growth[47-49].

The surrogate application of iPSC as representative of kidney disease is increasingly becoming reality given recent advances involving the production of iPSC from both mesangial and epithelial cells derived from urine[45]. In addition to that, iPSCs have been generated from proximal tubular cells and podocytes[46]. Despite promising results, some issues should be highlighted before clinical application. First of all, there are no established differentiation protocols for moving from pluripotent state to functional kidney cell. Second, there are no optimal culture conditions for targeting cells. Third, a stepwise differentiation depends on specific factors, which must be identified[50,51].

BIOREACTOR CULTURE-CELL ACCLIMATION, ATTACHMENT AND MATURATION

For whole organ regeneration, cells need to be attached to the organ and then maturated. The bioreactor simulates the human body environment; however, each organ depends on different conditions such as perfusion rate, temperature, CO_2 concentration, growth factors, and nutrients, *etc*. Due to kidney complexity, the recellularization of a functional kidney is still a big challenge for scientists. In 2013, Song *et al*[52] published the first experimental orthotopic transplantation of a bioengineered kidney in rats. They repopulated acellular rat kidneys with endothelial and epithelial cells through the renal artery and ureter respectively. Engrafted epithelial cells were found to reestablish polarity and organize in tubular structures expressing Na/K-ATPase and aquaporin similar to native proximal tubular epithelium. Also, epithelial cells formed structures resembling native distal tubular epithelium and lined the renal pelvis similar to native transitional epithelium. Transmission and scanning electron microscopy of regenerated kidneys showed perfused glomerular capillaries with engrafted podocytes and formation of foot processes. However, as they suggested, before translating this technology, it will require optimization of cell seeding protocols, upscaling of biomimetic organ culture, as well as isolation, differentiation, and expansion of the required cell types[52].

FUTURE DIRECTIONS

In the field of regenerative medicine and whole organ regeneration many efforts have been carried out to translate this technology for clinical practice. The ultimate goal is to provide a feasible and reliable therapy for different types of diseases. In this context, although there are many improvements on kidney regeneration, there are still many hurdles to be overcome, and as our knowledge improves, more complex questions remain unclear.

A functional bioengineered kidney is still a big challenge for scientists. In the last ten years we have got many improvements on the field of solid organ regeneration; however, we are still faraway from the main target. Currently, regenerative centers worldwide have been striving to find feasible strategies to develop bioengineered kidneys. Cell-scaffold technology gives hope to end-stage renal disease patients who struggle with morbidity and mortality due to extended periods on dialysis or immunosupression. The potential of bioengineered organ is to provide a reliable source of organs, which

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REVIEW

Chronic kidney disease prediction is an inexact science: The concept of "progressors" and "nonprogressors"

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Abstract

In 2002, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) instituted new guidelines that established a novel chronic kidney disease (CKD) staging paradigm. This set of guidelines, since updated, is now very widely accepted around the world. Nevertheless, the authoritative United States Preventative Task Force had in August 2012 acknowledged that we know surprisingly little about whether screening adults with no signs or symptoms of CKD improve health outcomes and that we deserve better information on CKD. More recently, the American Society of Nephrology and the American College of Physicians, two very well respected United States professional physician organizations were strongly at odds coming out with exactly opposite recommendations regarding the need or otherwise for "CKD screening" among the asymptomatic population. In this review, we revisit the various angles and perspectives of these conflicting arguments, raise unanswered questions regarding the validity and veracity of the NKF KDOQI CKD staging model, and raise even more questions about the soundness of its evidence-base. We show clinical evidence, from a Mayo Clinic Health System Renal Unit in Northwestern Wisconsin, United States, of the pitfalls of the current CKD staging model, show the inexactitude and unpredictable vagaries of current CKD prediction models and call for a more cautious and guarded application of CKD staging paradigms in clinical practice. The impacts of acute kidney injury on CKD initiation and CKD propagation and progression, the effects of such phenomenon as the syndrome of late onset renal failure from angiotensin blockade and the syndrome of rapid onset end stage renal disease on CKD initiation, CKD propagation and CKD progression to end stage renal disease all demand further study and analysis. Yet more research on CKD staging, CKD prognostication and CKD predictions are warranted. Finally and most importantly, cognizant of the very serious limitations and drawbacks of the NKF K/DOQI CKD staging model, the need to individualize CKD care, both in terms of patient care and prognostication, cannot be overemphasized.

Key words: Acute kidney injury; Chronic kidney disease; Chronic kidney disease staging; Estimated glomerular filtration rate; End stage renal disease; National Kidney Foundation Kidney Disease Outcomes Quality Initiative; Renal replacement therapy; Serum creatinine; Syndrome of late onset renal failure from angiotensin blockade; Syndrome of rapid onset end stage renal disease

Core tip: In 2002, the National Kidney Foundation established a novel chronic kidney disease (CKD) staging paradigm. In 2012, the authoritative United States Preventative Task Force questioned the validity of asymptomatic CKD screening. The American Society of Nephrology and the American College of Physicians have opposite recommendations regarding this controversy.

We examined the evidence-base and limitations of CKD staging. Furthermore, we show clinical evidence of pitfalls of the current CKD staging model and the failings of current CKD prediction models. We called for more research into CKD to end stage renal disease translations including the impact of acute kidney injury on this continuum. CKD care and prognostication must be individualized.

Onuigbo MAC, Agbasi N. Chronic kidney disease prediction is an inexact science: The concept of "progressors" and "nonprogressors". *World J Nephrol* 2014; 3(3): 31-49 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/31.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.31

INTRODUCTION

In 2002, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) instituted new guidelines that established a novel chronic kidney disease (CKD) staging paradigm[1,2]. In this model, by use of pre-specified estimated glomerular filtration rates (eGFR) ranges, CKD was characterized into five stages: Ⅰ, Ⅱ Ⅲ Ⅳ and Ⅴ[1,2]. More recent updates of the NKF KDOQI guidelines have incorporated the degree of associated proteinuria to plausibly enhance the utility, applicability, validity and clinical relevance of this CKD staging paradigm[3]. Furthermore, it must be acknowledged that the entire paradigm of the NKF K/DOQI 2002 CKD staging and the design of various CKD risk stratification scoring models and published CKD progression prediction formulas are all firmly predicated on the validity of this albeit unproven and only assumed archetype of a predictable linear and time-dependent progression of CKD through stages Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ, and finally to inexorably end in end stage renal disease (ESRD) and the need for renal replacement therapy[1-3]. Thus, note worthily, this NKF KDOQI CKD staging archetype assumes that CKD patients follow this linear, predictable, smoothly progressive and time-dependent curve to advance through the increasing CKD stages before inexorably reaching ESRD and the need for renal replacement therapy (RRT). Indeed, current nephrology literature on ESRD outcomes in CKD patients, is unquestionably primarily and almost entirely predicated on this presumed theory of a smooth, continuous, predictable and time-dependent linear progressive loss of eGFR[4-8]. This presumption is so strongly held that several reports over the decades have described CKD progression to ESRD in terms of annual rates of GFR decline or as mean annualized eGFR slopes in mL/min per 1.73 m^2 every year[4,6,7,9-18]. A detailed investigative analysis of these reports has been recently published in a recent review[19]. Undeniably, this generally accepted consensus classic view of CKD-ESRD progression presumes that functional nephron loss in chronic progressive kidney disease, and therefore as measured by serum creatinine, is orderly and mathematically definable[9].

However, it must be acknowledged that such a premise of predictable linear time-dependent progressive step-wise decline in kidney function, with mathematically linear falling eGFR over time, and with eGFR methodically marching through these incremental projected CKD stages Ⅰ through Ⅴ and then inexorably ending in symptomatic ESRD and the need for renal replacement therapy is unproven, untested and potentially flawed[20-32]. Contrary to the classic premise of a smooth predictable linear decline of renal function from CKD to ESRD described in the preceding section of this chapter, more and more data have over the years suggested that the natural pattern of progression from CKD to ESRD followed a more staccato and unpredictable course[20-32]. Moreover, and again contrary to the NKF schema of a predictable linear CKD staging referred to above, the results of new analysis of CKD databases suggest a high degree of variability of CKD staging among CKD patients, and this CKD staging variability is often unpredictable, and with significant intra-patient variability, such that our current knowledge of this very important patient group can, at best, be described as limited and incomplete[19,33-36].

A recent publication in the *Kidney International* journal put CKD smack back into the realms of public discourse when it was reported that 59% of Americans had a lifetime risk of developing CKD and that Americans had a 1 in 3 chance of CKD risk[37]. Whereas, it has been known for decades that eGFR declines in parallel with age[37,38], to what extent this is true and linearly predictable at the individual (older) CKD level remains unclear[19,33-36]. To further buttress the latter sentiment and concerns regarding the validity of current CKD staging paradigms, in August 2012, the authoritative United States Preventative Task Force had in a very comprehensive analysis acknowledged that we know surprisingly little about whether screening adults with no signs or symptoms of CKD improve health outcomes and that we deserve better information on CKD[39].

HOW MUCH DO PHYSICIANS AND OTHER HEALTHCARE PROVIDERS EMBRACE THE NKF K/DOQI 2002 CKD GUIDELINES?

Without a doubt, the introduction of the K/DOQI 2002 CKD guidelines has heralded a growing appreciation of the impact of CKD in health outcomes[3,40]. Undoubtedly, there has followed an exponential increase in nephrology specialty referrals and nephrology specialty office visits for purported "CKD follow up" since the introduction of the 2002 NKF KDOQI CKD clinical practice guidelines[41-49]. However, given the above limitations and constraints of the K/DOQI CKD clinical practice guidelines model, one interesting question is to what extent physicians and providers have embraced its recommenda-

tions and structure[50]. An online survey, developed by the NKF KDOQI Education Committee, was sent to 16323 healthcare providers of which 951 completed the survey[50]. Whereas 78% of all providers reported using the NKF KDOQI guidelines in their practice, the most common barrier perceived by physicians was lack of evidence supporting the KDOQI guidelines[50]. Notably, compared to physician extenders and allied health professionals, not surprising, we must admit, a larger proportion of nephrologists cited lack of evidence and too much influence from industry as barriers to the implementation of the KDOQI guidelines[50]. Surely and undoubtedly, and this fact was recognized very much earlier on, the validity of the clinical practice guidelines of the NKF K/DOQI 2002 report is limited in some areas because of the paucity of evidence-based data[40].

UTILITY OF CKD PREDICTION MODELS IN THE NEPHROLOGY LITERATURE

Tangri *et al*[51] in a recent 2011 publication devised and validated different new CKD prediction models using demographic, clinical, and laboratory data from two independent Canadian cohorts of patients with CKD stages Ⅲ to Ⅴ (estimated GFR, 10-59 mL/min per 1.73 m^2) who were referred to nephrologists between April 1, 2001, and December 31, 2008[51]. The most accurate model included age, sex, estimated GFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin (C statistic, 0.917; 95%CI: 0.901-0.933 in the development cohort and 0.841; 95%CI: 0.825-0.857 in the validation cohort)[51]. In the validation cohort, this model was more accurate than a simpler model that included age, sex, estimated GFR, and albuminuria (integrated discrimination improvement, 3.2%; 95%CI: 2.4%-4.2%; calibration (Nam and D'Agostino 2 statistic, 19 *vs* 32); and reclassification for CKD stage 3 (NRI, 8.0%; 95%CI: 2.1%-13.9%) and for CKD stage 4 (NRI, 4.1%; 95%CI: -0.5%-8.8%)[51]. The conclusion was that a model using routinely obtained laboratory tests can accurately predict progression to kidney failure in patients with CKD stages Ⅲ to Ⅴ[51].

In a subsequent 2013 review of CKD prediction models, 13 studies describing 23 models were analyzed[52]. Eight studies (11 models) involved kidney failure, five studies (6 models) involved all-cause mortality, and three studies (6 models) involved cardiovascular events[51-64]. Measures of eGFR or serum creatinine level were included in 10 studies (17 models), and measures of proteinuria were included in 9 studies (15 models)[51-64]. Only 2 studies (4 models) met the criteria for clinical usefulness, of which 1 study (3 models) presented reclassification indices with clinically useful risk categories[52]. A 2013 VA study of 1866 participants aged 65 and older with CKD with an eGFR less than 30 mL/min per 1.73 m^2 body surface area (BSA), concluded that a new CKD prediction model using commonly available clinical measures (age, congestive heart failure, systolic blood pressure, eGFR, potassium, and albumin) showed excellent ability to predict the onset of ESRD within the next year in elderly adults[65]. Peeters et al in an analysis of 595 Canadian CKD patients in stages Ⅲ-Ⅴ CKD, developed an eight-variable model [including age, sex, eGFR, albuminuria, calcium, phosphate, bicarbonate, albumin] and the conclusion was that this more complex model also accurately predicted the progression to kidney failure[66]. Despite these foregoing claims and assertions regarding the utility of CKD prediction models in patient care and in individual patient CKD prognostication, from direct clinical experience, and after a critical unbiased review of currently available CKD progression prediction models, and related CKD literature, we have concluded that all currently published CKD progression prediction models remain untested, not validated, and of limited use for individual CKD patient prognostication, at any given pre-specified point in time[19-36].

LIFETIME CKD RISK MODELING AMONG THE US POPULATION–HOW ACCURATE AND RELIABLE CAN SUCH POPULATION WIDE ESTIMATES BE?

Grams *et al*[37], in a Markov Monte Carlo model simulation study of current United States black and white populations had concluded that from birth, the overall lifetime risks of CKD stages 3a+, stage 3b+, stage 4+, and ESRD were 59.1%, 33.6%, 11.5%, and 3.6%, respectively[37]. According to this very publicized report, the risk of CKD increased with age, with approximately one-half the CKD stage 3a cases developing after 70 years of age[37]. However, to what extent this is true and linearly predictable at the individual (older) CKD level remains unclear[67]. The clinical relevance, the validity and real life implications of these estimates remain to be more appropriately elucidated[29-32]. Moreover, as is evident from the analysis in the next paragraph, such population-based disease risk estimates can often be shown to be very significantly off-mark, more so when validated against actual reported ESRD incidence values as available in the United States Renal Data System (USRDS).

OVERESTIMATION OF ESRD INCIDENCE RATES AMONG US CKD COHORTS-REPORT OF A COMPARATIVE ANALYSIS

A 2007 United States of America Centers for Disease Control report revealed that 16.5% of the United States. population 20 years of age and older had CKD as defined by eGFR < 60 mL/min per 1.73 m^2 BSA[68]. This CKD prevalence estimate, at the time of this report, translated that > 20 million adult Americans had CKD[68]. Besides, three United States studies published between 2004 and 2009, among different CKD patient population cohorts,

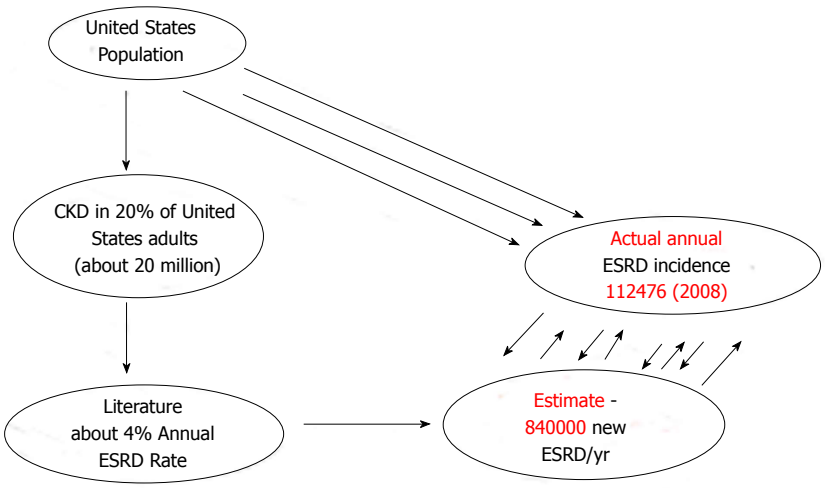

Figure 1 Gross overestimation of annual incident end stage renal disease patients for 2008 from projected estimates based on annualized end stage renal disease rates from three cited concurrent United States chronic kidney disease cohort studies. CKD: Chronic kidney disease; ESRD: End stage renal disease.

had demonstrated annualized ESRD rates among CKD patients of 4%, 8.2% and 4.5%, respectively[29,69,70]. A meta-analysis of these three studies produced a weighted average annualized ESRD incidence rate of 4.2%[30-32]. This weighted average annualized ESRD incidence rate of 4.2% among the entire United States CKD population from 2007 would produce an estimated 840000 new ESRD cases/year for 2008[30-32]. This represented a gross over-estimation when compared to the actual reported USRDS incident ESRD for 2008 of 112476[30-32,71] (Figure 1). Thus, the estimated ESRD incidence for 2008 of 840000 was a whopping 650% gross overestimate over the actual recorded number of new ESRD patients in the United States for 2008 of 112476[30-32,71].

EVIDENCE OF CKD STAGE VARIABILITY AND UNPREDICTABILITY AMONG STUDIED CKD COHORTS: THE PROBABLE IMPACT OF THE EFFECTS OF THE SYNDROME OF LATE ONSET RENAL FAILURE FROM ANGIOTENSIN BLOCKADE

In a 2011 retrospective analysis, Sikaneta et al, analyzed the longitudinal changes during a 1.1-year observation period of eGFR and CKD stages among 1262 patients, mean age 71.25 years, drawn from two large Canadian renal clinics[35]. This study demonstrated CKD stage variability (defined by changes in CKD stages) and reported that CKD stage changed in 40% of the cohort (including 7.4% in whom CKD stage improved) whereas CKD stage remained static in 762 (60.4%) patients, the majority of this CKD cohort[35]. Furthermore, although CKD stage had remained static in 762 (60.4%) patients, that is to say, they did not experience a change in CKD stage during the initial observation period, 204 (40%) of 512 patients from this subgroup who were available for follow-up 2.3 years later still ended up on dialysis, suggesting subsequent acute unanticipated yet irreversible ESRD, a picture that is consistent with the newly described syndrome of rapid onset end stage renal disease which syndrome is expatiated in greater detail in a following section of this review[72]. This Canadian study demonstrated the clear unpredictability of CKD staging and prognostication at the individual patient level[35,36]. Moreover, the observation that 7.4% of this Canadian CKD cohort actually demonstrated improved CKD stage is still more dramatic[35,36]. In an editorial correspondence, we had speculated that some of these CKD patients who revealed improved CKD stages during follow up may indeed represent some CKD patients who had experienced acute worsening kidney function while on angiotensin blockade and who following withdrawal of angiotensin inhibition had exhibited improved kidney function[36]. This would only further strengthen the existence of the previously unrecognized syndrome of late onset renal failure from angiotensin blockade, a syndrome that we first described in 2005[73-77]. In another Canadian study, Levin et al studied 4231 CKD Ⅳ patients characterized by an index eGFR of less than 30 mL/min per 1.73 m^2, at least 3 subsequent eGFR values available for analysis, and no less than 4 months of follow-up between January 2000 and January 2004[33]. In this CKD cohort, mean age of 67 years, median follow-up of 31 mo, during the first 2 years of follow-up, 24% started dialysis therapy, 1% received a transplant, 7% died, and 1% was lost to follow-up[33]. The conclusion from this study was that the clinical course of patients with CKD stage 4 was unpredictably variable[33].

A JUNE 2011 MAYO CLINIC LABORATORY DATABASE TWO-YEAR SNAP SHOT ANALYSIS OF CKD STAGE CHANGES IN CKD IV PATIENTS

In June 2011, we pulled by IT reporting, all stage Ⅳ CKD patients with eGFR in the 15.0-29.9 mL/min per 1.73 m^2 BSA range in a Mayo Clinic Laboratory Database reported between April 19, 2009 and April 19, 2011[19,30,32,75]. All patients who had received renal replace-

ment therapy for acute kidney injury (AKI) or ESRD were excluded from the analysis. We included for analysis, all patients with at least three recorded eGFR values, and with a minimum of 6 mo between the first and the last reported eGFR estimations[19,30,32,78]. After excluding 62 ESRD patients, and all who received renal replacement therapy for AKI, 241 patients qualified for this analysis - 102 males and 139 females. In over 95% of the patients, eGFR remained stable and did not vary by as much as 5 eGFR points (< 25% from baseline) over the two-year study period[19,30,32,78]. The conclusion following this snapshot cross sectional analysis was that eGFR in the majority of CKD stage Ⅳ patients remained stable after two years of follow up[19,30,32,78].

EVIDENCE IN THE CKD LITERATURE OF THE EXISTENCE OF CKD" PROGRESSORS" AND CKD "NONPROGEESSORS"

Our experience studying the natural history of CKD at the Mayo Clinic Health System Renal Unit in Northwestern Wisconsin, United States, suggests that some CKD patients are able to maintain stable, albeit diminished eGFR levels over several years, the so-called "nonprogressors" or "asymptomatic" CKD patients, whereas, other CKD patients, for often unclear reasons, have an apparent enhanced propensity to progressively lose eGFR over time, the so-called "progressors" or "symptomatic" CKD[31,79].

In a 2012 retrospective report from South Korea, of 347 CKD Ⅲ patients, enrolled between January 1997 and December 1999, who were followed up through June 2010, a period of 10 years, 167 patients (48.1%) did not progress, 60 (17.3%) progressed to stage 4 and 120 (34.6%) progressed to stage 5, with 91 (26.2%) starting dialysis[80].

Other investigators, as reviewed below, have also documented such disparate behavior of individual patient CKD stages over time, with the additional introduction of the terms, "improvers" and "nonimprovers", to describe this dichotomy of observed CKD nonprogression *vs* progression, respectively, with follow up[81-85]. In a 2-year follow-up of the MDRD study, GFR remained stable in 19% of patients and improved in 11%[81]. In the African American Study of Kidney Disease and Hypertension (AASK) trial, however, over a longer follow up period of 8.8 years and with Bayesian models, eGFR improved among only 3.3%, with a mean slope of +1.06 mL/min per 1.73 m^2 per year[82]. In another study of individual GFR progression trajectories over 12 years of follow-up in participants in AASK trial, Li *et al*[83] demonstrated that many patients with CKD have a nonlinear GFR trajectory or a prolonged period of nonprogression. In this study, 352 (41.6%) patients showed a > 0.9 probability of having either a nonlinear trajectory or a prolonged nonprogression period; in 559 (66.1%), the probability was > 0.5. Baseline eGFR > 40 mL/min per 1.73 m^2 and urine protein-creatinine ratio < 0.22 g/g were associated with a higher likelihood of a non progression period. 74 patients (8.7%) had both a substantial period of stable or increasing eGFR and a substantial period of rapid eGFR decrease[83]. In another population with mild CKD receiving primary care through a large integrated health care system between 2004 and 2009, eGFR rose over time among 41.3%[84]. In yet another retrospective study of patients before nephrology referral, eGFR did not progress among 16% of those with stages 3-5 of CKD[85].

CKD "IMPROVERS" AND "NONIMPROVERS" IN A 2013 FRENCH REPORT

Recently, French investigators examined 406 patients in the NephroTest cohort with measured glomerular filtration rates (mGFR) measured by 51Cr-EDTA clearance at least 3 times during at least 2 years of follow-up[86]. Individual examination of mGFR trajectories by 4 independent nephrologists classified patients as "improvers", defined as those showing a sustained mGFR increase, or "nonimprovers"[86]. Twelve patients with erratic trajectories were excluded. Measured GFR improved over time in 62 patients (15.3%). Their median mGFR slope was + 1.88 (IQR, 1.38, 3.55) mL/min per year; it was 22.23 (23.9, 20.91) for the 332 "nonimprovers". "Improvers" had various nephropathies, but not diabetic glomerulopathy or polycystic kidney disease[86]. They did not differ from "nonimprovers" for age, sex, cardiovascular history, or CKD stage, but their urinary albumin excretion rate was lower. GFR improvement is possible in CKD patients at any CKD stage through stage 4-5[86]. It is noteworthy that this GFR improvement is associated with a decrease in the number of metabolic complications over time. In conclusion, this French report showed that renal function can improve over time in a significant proportion of CKD patients, even at a severe stage of CKD[86]. Indeed, the 15.3% prevalence of GFR improvement observed in this cohort is consistent with the few reports previously published.

These observations warrant further study of CKD progression or nonprogression among stable population-based asymptomatic CKD patients to improve our knowledge of the natural history of CKD and to help optimize CKD care around the world - our soon to be introduced new IT Software Program, The CKD Express© IT Software Program, which would help accelerate the harnessing of such new information about the natural history of CKD will be discussed in a later section of this review[49,87,88].

GRAPHICAL CASE SUMMARIES OF SELECTED INDIVIDUAL PATIENT-LEVEL

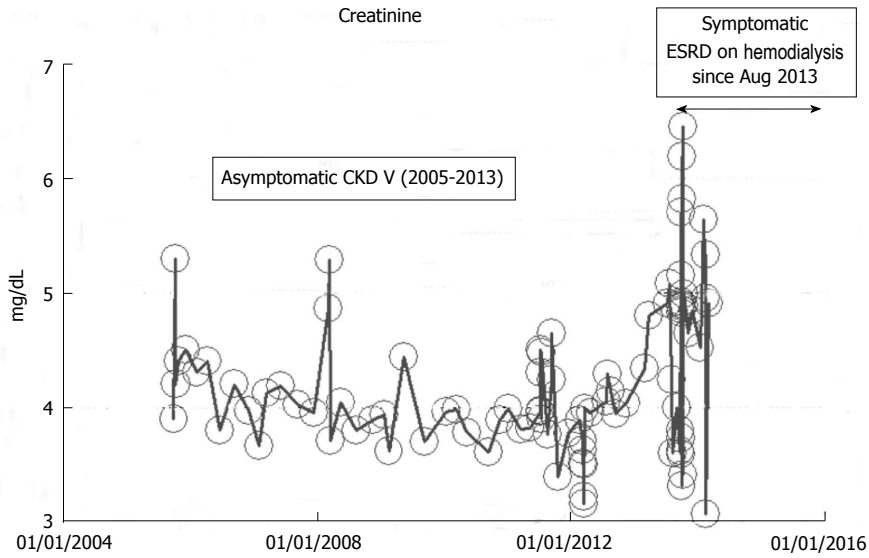

Figure 2 A now 82-year-old white male on maintenance hemodialysis August 2013-March 2014, after 8 years of asymptomatic chronic kidney disease V, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. CKD: Chronic kidney disease; ESRD: End stage renal disease.

SERUM CREATININE TRAJECTORIES OVER SEVERAL YEARS TO DEMONSTRATE CKD STAGING UNPREDICTABILITY AS SEEN AT THE MAYO CLINIC HEALTH SYSTEM RENAL UNIT, NORTHWESTERN WISCONSIN, UNITED STATES, 2002-2014: THE CONCEPT OF "SYMPTOMATIC" *VS* "ASYMPTOMATIC" CKD

From our experience of studying the natural history of CKD at the Mayo Clinic Health System Renal Unit, Northwestern Wisconsin, United States, during the period, 2002-2014, and from a dispassionate review of the nephrology literature, it would appear that some CKD patients are able to maintain stable, albeit diminished eGFR levels over years, the so-called "non-progressors" or "asymptomatic" CKD patients, whereas, other CKD patients, for often unclear reasons, have the enhanced propensity to progressively lose eGFR over time, the so-called "progressors" or "symptomatic" CKD[32,78-86]. We will show in this section of this review, graphical representations, some selected CKD patients that we have encountered in the last 12 years in our Mayo Clinic Health System Renal Unit, Northwestern Wisconsin, United States, that demonstrate these patterns of behavior: (1) as at March 2014, a now 82-year-old white male, ex-smoker, with a history of hypertension and CKD has been on maintenance hemodialysis for symptomatic ESRD since August 2013. However, for 8 years, between 2005 and 2013, despite a stable CKD stage V with serum creatinine of 4.0-5.0 mg/dL (eGFR 8-14 mL/min per 1.73 m^2 BSA), he had remained asymptomatic. He had an AVF created in June 2005 which was subsequently revised for non-maturation in January 2006. For unclear reasons, he then became symptomatic in July 2013 with nausea, vomiting and anorexia, and has been on maintenance hemodialysis since August 2013 (Figure 2); (2) as at February 2014, a now 78 year-old white male, with a history of hypertension, type Ⅱ diabetes mellitus, obesity, recurrent UTI and CKD V, has over the last 7 years, between 2006 and 2013, despite a stable CKD stage V with serum creatinine of 4.5-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 BSA), had remained asymptomatic. He continues on alternate monthly course of prophylactic short course of oral Levofloxacin for recurrent UTI prophylaxis. (Figures 3 and 4); and (3) as at early 2014, a now 78-year-old white male, diagnosed with Wegener's Granulomatosis with AKI on CKD in 2005. Serum creatinine had increased from 2.0 mg/dL to 3.5-4.0 mg/dL. The Wegener's Granulomatosis was treated with standard chemotherapy (Prednisone), and he has been in remission since 2006, albeit with a new baseline serum creatinine of 3.5-4.0 mg/dL (eGFR 16-22 mL/min per 1.73 m^2 BSA). He has remained otherwise an asymptomatic CKD IV patient between 2006 and early 2014 (Figures 5 and 6).

UNPREDICTABILITY OF THE IMPACT OF AKI EVENTS ON CKD PROGRESSION-A BRIEF REVIEW OF SELECTED CASES FROM THE MAYO CLINIC HEALTH SYSTEM RENAL UNIT, EAU CLAIRE, NORTHWESTERN WISCONSIN, UNITED STATES

In a just published book chapter, we had extensively reviewed the multifaceted nature of renal outcomes following AKI in CKD patients[89-91]. As a result of the var-

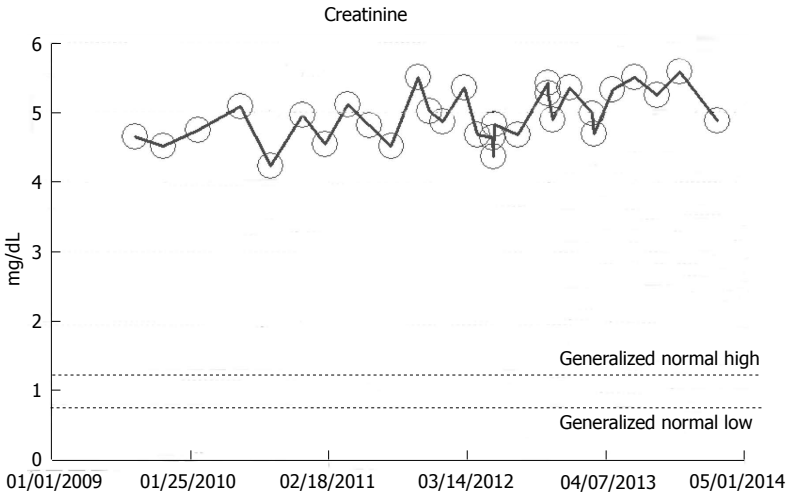

Figure 3 A now 78-year-old white male on with asymptomatic stable chronic kidney disease V for 8 years, 2006-2014, serum creatinine of 4.5-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 Body Surface Area), as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

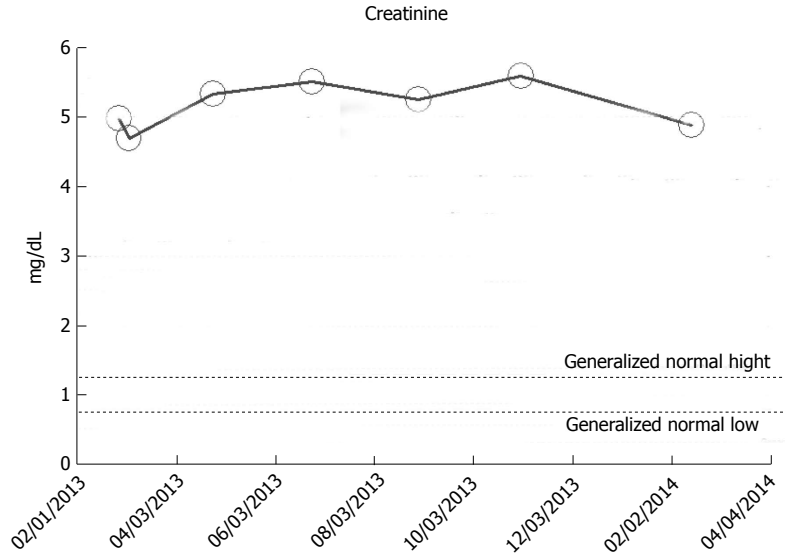

Figure 4 A now 78-year-old white male on with asymptomatic stable chronic kidney disease V for 8 year, 2006-2014, serum creatinine of 5.0-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 Body Surface Area), in the last one year, February 2013-March 2013, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

ied nature of these renal outcomes, we dubbed this "the rainbow syndrome of too many colors"[91]. We would briefly describe three patients with AKI on CKD and three observed different outcomes to demonstrate the array of possible renal outcomes following AKI on CKD - complete recovery of renal function back to baseline, partial recovery following AKI, and a third outcome where despite multiple repeated AKI events over several years, a 92-year-old Caucasian female was still able to maintain her baseline serum creatinine (Figure 7). In the following section, we shall describe the previously unrecognized syndrome of acute yet irreversible ESRD following unpredictably on an AKI event in a CKD patient, the syndrome of rapid onset end stage renal disease or (SORO-ESRD)[19,30,72,89].

A-48 year-old obese hypertensive Caucasian male patient, with otherwise stable stage Ⅱ CKD, baseline serum creatinine of 1.2 mg/dL (eGFR > 60 mL/min per 1.73 m^2 BSA), in January 2013, developed AKI on CKD following fever of unknown origin (FUO) complicating methicillin-resistant Staphylococcus Aureus (MRSA) bacteremia. He at the time of admission in January 2013 was on hydrochlorothiazide 25 mg daily, Lotrel (5 mg of amlodipine + 10 mg of benazepril) daily. Lotrel was promptly discontinued on admission and Amlodipine only was continued. MRSA septicemia was treated with parenteral antibiotics, guided by antibiogram[91]. A kidney biopsy revealed acute interstitial nephritis. Peak serum serum creatinine was 3.3 mg/dL. He exhibited full recovery of kidney function as at March 2013 (Figure 8).

An 83-year-old white woman with hypertension and otherwise stable CKD Ⅲ had undergone a right hemicolectomy procedure together with 150 cm small intestinal resection with end-to-end ileo-colic anastomosis for colon cancer in October 2011. This was further complicated by dehydration and diarrhea and the need for a second laparoscopic procedure in November 2011. She experienced AKI in October 2011 following the initial surgical procedure and another AKI episode in November 2011 from hypovolemic dehydration with peak creatinine values as shown in Figure 9. She subsequently partially recovered kidney function and since December 2011, she has maintained a serum creatinine of 1.8-2.2 mg/dL through March 2014. She otherwise remains asymptomatic, and continues to feel great at her current age of 85 years. Her baseline serum creatinine was 1.2 mg/dL in

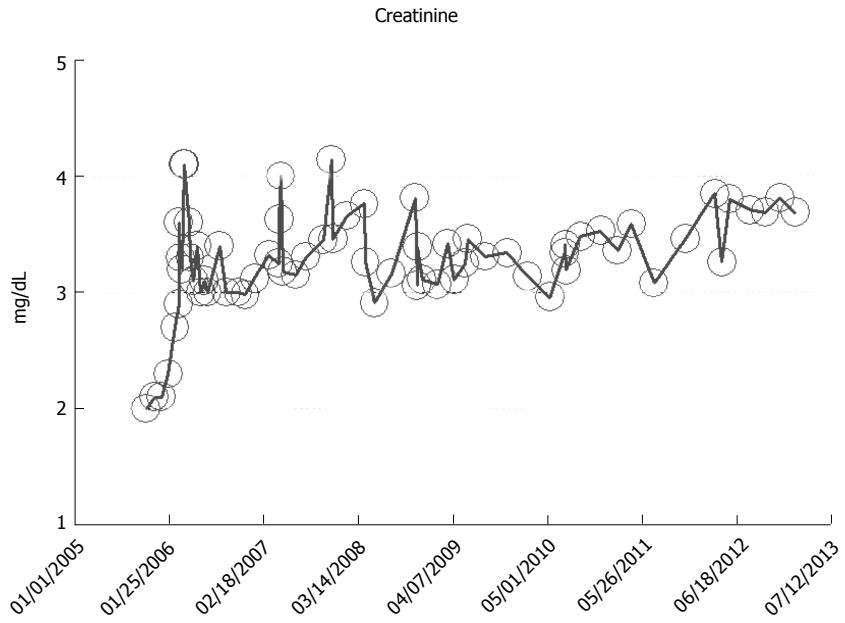

Figure 5 A now 78-year-old white male with treated Wegener's Granulomatoisis in remission since 2006, with new stable chronic kidney disease IV 2008-2014, following initial acute kidney injury on chronic kidney disease in 2005, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program.

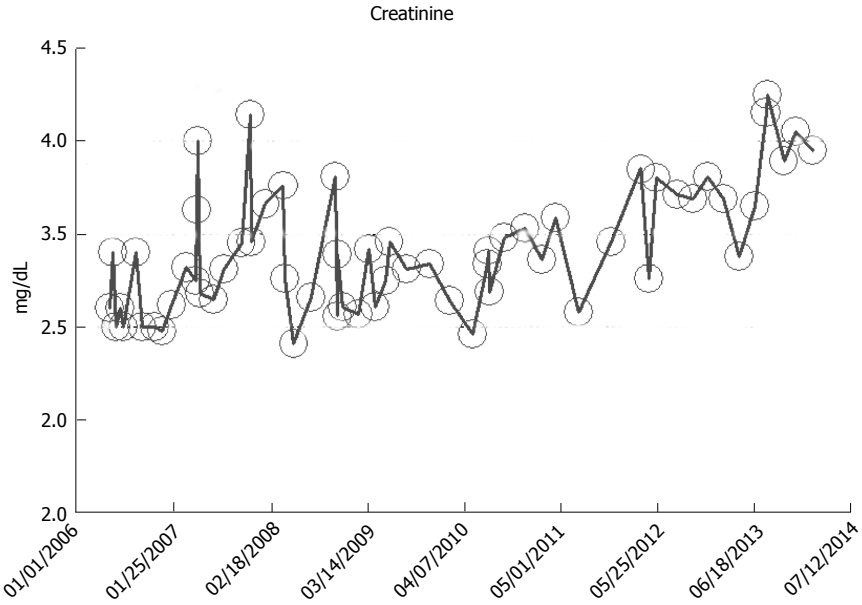

Figure 6 The same patient as in Figure 5 has remained otherwise an asymptomatic chronic kidney disease IV patient between 2006 and early 2014, serum creatinine of 3.5-4.0 mg/dL (eGFR 16-22 mL/min per 1.73 m^2 Body Surface Area), as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

January 2006, 1.3-1.4 mg/dL in 2010-2011 (CKD stage Ⅲ, eGFR approximately 36-38 mL/min per 1.73 m^2 BSA), AKI intervened in October-November 2011, and she has had a new higher baseline serum creatinine of 2.0-2.6 mg/dl (eGFR 20-25 mL/min per 1.73 m^2 BSA), CKD stage IV, in the last three years, between December 2011 and March 2014 (Figure 9).

A 92-year-old white woman in March 2014, with a history of multiple AKI events between 2005 and 2014, over nearly 10 years, but is still able to generally maintain an otherwise asymptomatic CKD Ⅳ status with serum creatinine of 1.7-2.0 mg/dL (CKD stage Ⅳ, eGFR 20-29 mL/min per 1.73 m^2 BSA), through March 2014, the same baseline serum creatinine she had 10 years ago in 2005 (Figure 7). The repeat episodes of AKI events are shown in Figure 7 and were caused by the following causes: (1) Pyelonephritis, treated with Bactrim, June 2006, peak creatinine was 3.0 mg/dL; (2) Left lower lobe pneumonia with diarrhea, dehydration and hypotension, July 2007, peak creatinine was 3.43 mg/dL; (3) Pyelonephritis, September 2009, peak creatinine was 2.37 mg/dL; and (4) Acute gastroenteritis and dehydration, March 2012, peak creatinine was 2.75 mg/dL.

THE SYNDROME OF RAPID ONSET END STAGE RENAL DISEASE

The commonly held consensus of the propagation of CKD to ESRD is that of a predictable, linear, progressive, relentless, time-dependent and knowable decline in renal function, with predictably increasing serum creatinine or falling eGFR, leading inexorably to ESRD and the need for renal replacement therapy[1-4,6,7,9-18]. This universally accepted paradigm of CKD-ESRD progression will be referred to in this review as the "classic" pattern

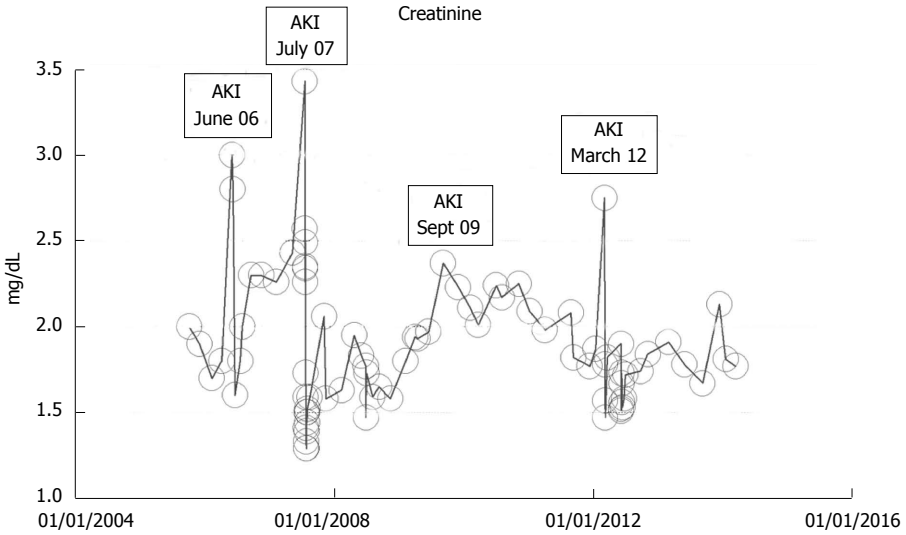

Figure 7 Serum creatinine trajectory in a now 92-year-old Caucasian female patient who despite multiple repeated AKI episodes between 2006 and 2012, has otherwise maintained a baseline serum creatinine of 1.7-2.0 mg/dL through March 2014, as monitored and tracked through a simulation of The chronic kidney disease Express IT Software Program. AKI: Acute kidney injury.

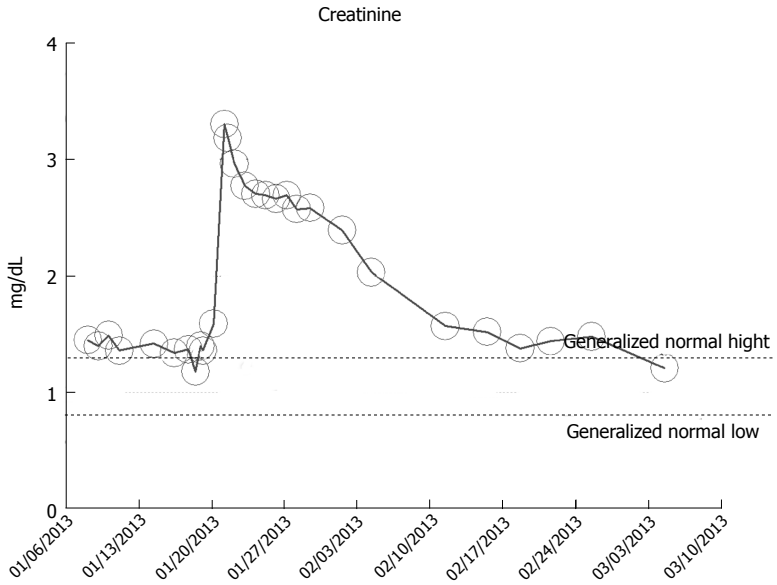

Figure 8 Serum creatinine trajectory in an 48-year-old obese hypertensive male patient who developed acute kidney injury from biopsy-proved acute interstitial nephritis complication methicillin-resistant Staphylococcus Aureus septicemia, with full renal recovery after one month, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. AKI: Acute kidney injury.

of CKD to ESRD progression[19,92].

What's more, whereas 25.2% of ESRD Medicare patients all experienced antecedent AKI, for many decades now, this relationship between antecedent AKI and irreversible ESRD had been fortuitously blamed on the so-called "residual confounding"[93]. However, in 2010, we put to rest any doubts as to the direct causality between antecedent AKI and the precipitation of acute yet irreversible ESRD, when we first described the previously unrecognized syndrome of rapid onset end stage renal disease or SORO-ESRD in the journal, *Renal Failure*[72]. We have defined the syndrome of rapid-onset ESRD as the unpredictable, unanticipated and accelerated progression from a-priori stable CKD to irreversible ESRD, requiring permanent RRT, following a new episode of AKI precipitated by antecedent new medical/surgical events, with the interval between AKI and the need for RRT represented by a period of often less than less than two weeks, measured only in days following surgically induced AKI[19,30,72,92,94,95]. We have also described this syndrome of acute yet irreversible ESRD in renal transplant recipients[19,92,94,95]. We would briefly describe in this section, CKD patients who demonstrate features of the "classic" CKD to ESRD translation (Figures 9 and 11), and patients who on the other hand exhibit the features of the syndrome of rapid onset end stage renal disease or SORO-ESRD (Figure 12).

We were the first to aptly circumscribe this syndrome of rapid onset ESRD as a distinct clinical entity[19,30,72,92,94,95]. Our findings have received significant collaborating support following the publication of several more recent reports demonstrating the not uncommon occurrence of SORO-ESRD, in incident adult ESRD populations in both the US and Canada[96-99]. In fact, a recent 2012 review in the journal *Kidney International* concluded that AKI can cause ESRD directly, and increase

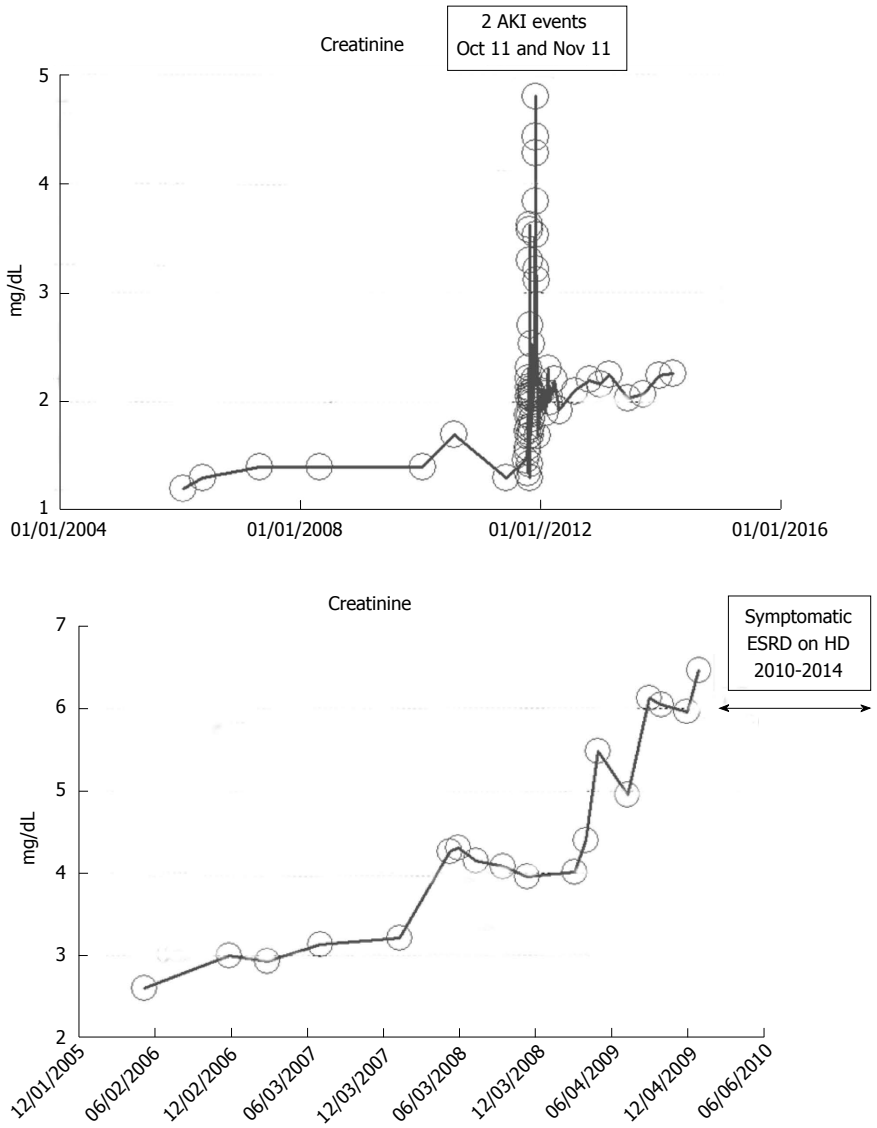

Figure 9 Serum creatinine trajectory in an 85-year-old obese hypertensive female patient who developed acute kidney injury following a complicated right hemicolectomy with small intestinal resection and ileo-colic anastomosis in October 2011, with partial but stable renal recovery (chronic kidney disease IV) after one month, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program.

Figure 10 Serum creatinine trajectory in a 82-year-old white male patient with features of the predictable linear chronic kidney disease to end stage renal disease progression from 2006 through 2010 when he started hemodialysis for symptomatic uremia, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. ESRD: End stage renal disease; HD: Hemodialysis.

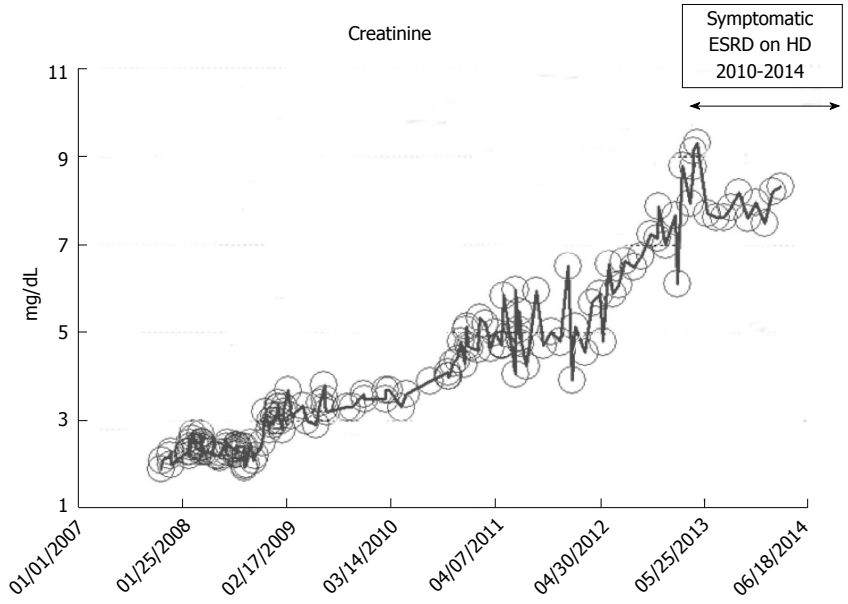

Figure 11 Serum creatinine trajectory in a 52-year-old white male patient with features of the predictable linear chronic kidney disease to end stage renal disease progression from 2007 through 2010 when he started hemodialysis for symptomatic uremia, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. ESRD: End stage renal disease; HD: Hemodialysis.

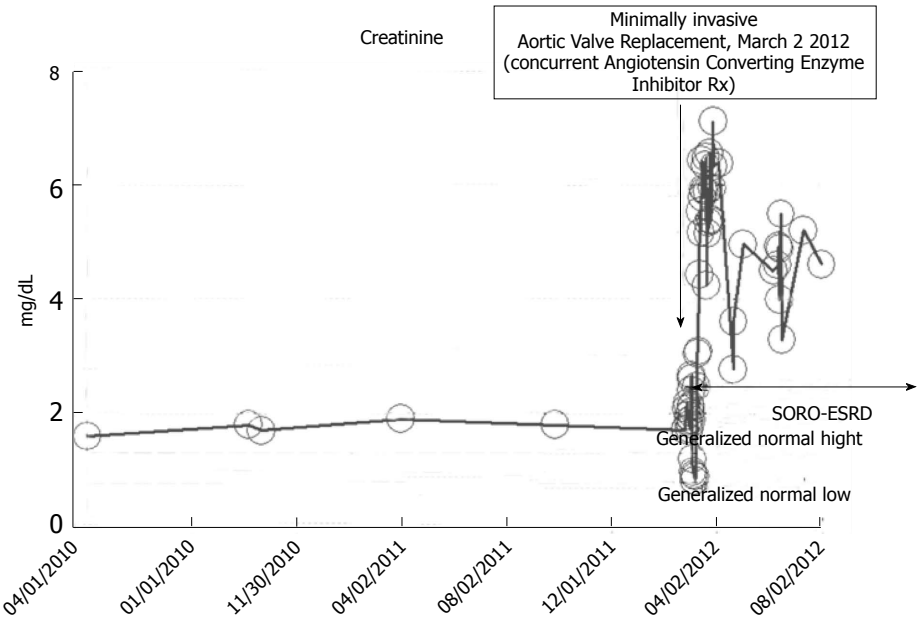

Figure 12 Serum creatinine trajectory, 2010-2012, with post-operative acute kidney injury precipitating acute yet irreversible end stage renal disease in March 2012 while on concurrent ACE inhibition, following minimally invasive aortic valve replacement in a then 73-year-old thin white woman. SORO-ESRD: Syndrome of rapid onset end-stage renal disease.

the risk of developing incident CKD and worsening of underlying CKD and even went further to posit that the distinction between AKI and CKD may be artificial[100].

"Classic" CKD to ESRD pattern

An 82-year-old while male with a history of hypertension, low ejection fraction ischemic cardiomyopathy, type II diabetes mellitus, coronary artery disease, obesity and hypothyroidism developed progressively worsening renal failure with predictable linear increase in serum creatinine from May 2006 when serum ceatinine was 2.6 mg/dL through to January 2010 when serum creatinine exceeded 6.0 mg/dL and he developed features of uremia and started renal replacement therapy in the form of in-center outpatient hemodialysis in January 2010 (Figure 10). He died in early 2014, while still on maintenance hemodialysis, from failure to thrive. No laboratory data were available for this patient before May 2006.

"Classic" CKD to ESRD pattern

A 52-year-old while male with a history of hypertension, low ejection fraction ischemic cardiomyopathy, and hypothyroidism developed progressively worsening renal failure with predictable linear increase in serum creatinine from November 2007 when serum ceatinine was 1.9 mg/dL through to December 2010 when serum creatinine exceeded 7.0 mg/dL and he developed features of uremia and started renal replacement therapy in the form of in-center outpatient hemodialysis (Figure 11). He continues on maintenance hemodialysis as at March 2014, the date of this present review.

"SORO-ESRD" pattern in native kidneys

A 73-year-old obese male patient with a baseline serum creatinine of approximately 1.7 mg/dL, 2010-2012, with a past medical history for hypertension, type Ⅱ diabetes mellitus, on concurrent ACE inhibition with Lisinopril 40 mg daily, was admitted to the coronary care unit (CCU) in February 2012 with acutely decompensating heart failure. Acute coronary syndrome was ruled out and the patient subsequently underwent minimally invasive aortic valve replacement for symptomatic aortic stenosis with a 25 mm St. Judes Epic stented tissue valve March 2 2012. He rapidly developed post-operative AKI on CKD and required hemodialysis on the 1st post-operative day (Figure 12). He has since remained on outpatient in-center maintenance hemodialysis for ESRD, from March 2012 through to March 2014, the date of present reporting.

"SORO-ESRD" pattern in a renal transplant recipient

A 53-year-old Caucasian type 1 diabetic woman with simultaneous pancreas-kidney transplantation in 2000 for ESRD and type I diabetes mellitus, maintained on chronic transplant immunosuppression with tacrolimus, cellcept, and prednisone had maintained her allograft CKD stage Ⅲ status throughout most of 2010 with a baseline serum creatinine of 1.6 mg/dL. In January 2011, she suffered from acute transplant pyelonephritis precipitating AKI, further complicated by dehydration from acute gastroenteritis[94]. The patient's serum creatinine rose quickly within days to 5.16 mg/dL (Figure 13). She required hemodialysis for symptomatic renal failure. A transplant kidney biopsy revealed acute tubular necrosis with chronic transplant glomerulopathy but no rejection. She was maintained on in-center outpatient hemodialysis for oliguric irreversible ESRD until January 2012, twelve months later, when she received a new renal allograft re-transplant, a living-related kidney allograft from her then 32-year-old son at Mayo Clinic, Rochester. Additional clinical details of this renal transplant recipient are de-

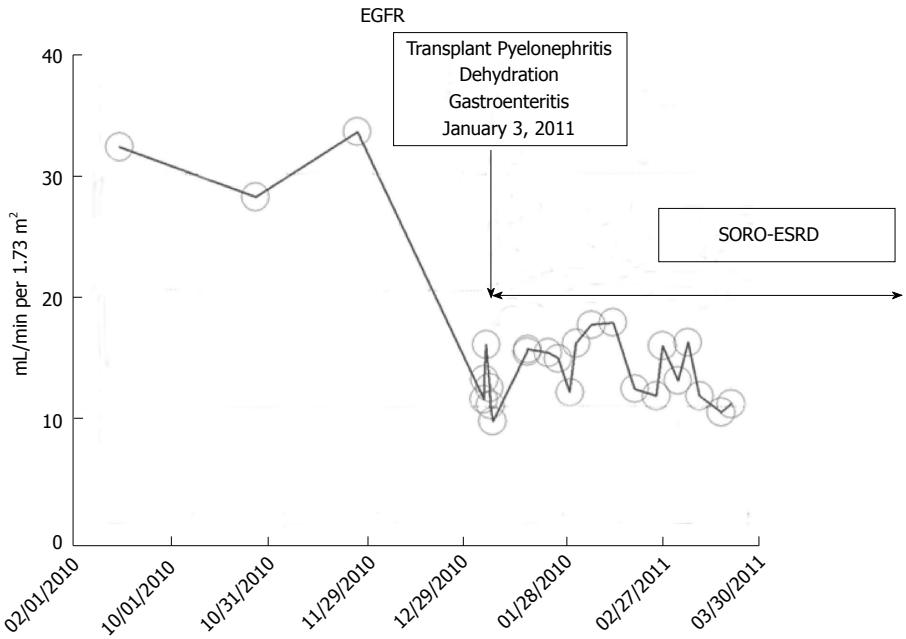

Figure 13 Estimated glomerular filtration rates trajectory in a renal transplant recipient following acute kidney injury and Syndrome of Rapid Onset End-Stage Renal Disease in January 2011, was on maintenance in-center hemodialysis for 12 mo and received a second living related kidney transplant from 32-year-old son in January 2012 at Mayo Clinic, Rochester. SORO-ESRD: Syndrome of rapid onset end-stage renal disease. EGFR: Estimated glomerular filtration rates.

scribed in our recent publication[94].

CKD SCREENING REVISITED-CKD SCREENING CONTROVERSY BETWEEN ACP AND ASN: TO SCREEN OR NOT TO SCREEN?

To further emphasize on the inexactness of CKD prediction and prognostication, the August 2012 United States Preventive Services Task Force (USPSTF) report on CKD screening concluded that we know surprisingly little about whether screening adults with no signs or symptoms of CKD will improve health outcomes and that clinicians and patients deserve better information on CKD[39]. This unsettled state of affairs regarding the appropriateness or otherwise of CKD screening within the asymptomatic population came to the fore and became part of the public discourse when two very respected professional United States medical associations, the American Society of Nephrology (ASN) and the American College of Physicians (ACP) both simultaneously came down on opposite sides of the argument for and against CKD screening among asymptomatic populations, respectively[101,102]. The ASN position was that "while acknowledging the need for further and larger scale clinical research into CKD and how the disease progresses in its early stages, ASN believes current evidence strongly supports the value of early detection of, and screening for, CKD"[102]. Given the present level of evidence-base, and more so from our experience at the Mayo Clinic Health System Renal Unit, in Northwestern Wisconsin, United States, we would side with the USPSTF in cautioning against CKD screening among the otherwise asymptomatic population. Such a wide-out CKD screening model will most likely result in more unnecessary care, escalating costs of CKD care to Medicare, increased referrals to nephrologists, and all of this without any evidence of improved CKD outcomes[32,46,49,103,104]. Additionally, we could not agree more with the USPSTF on the urgent need for more research into CKD progression and prognostication.

MULTIPLE PUTATIVE MECHANISMS OF CKD INITIATION AND PROPAGATION

Clearly, from accruing evidence in the literature, there is a continuing elongating list of putative mechanisms that have been variously incriminated in the initiation and propagation of CKD progression[105-128]. We have analyzed and summarized these mechanisms in an extensively referenced recent review[105]. A critical review of current literature clearly demonstrates that culprit pathogenetic molecule(s) or mechanistic factor(s) responsible for the initiation and propagation of diabetic and/or non-diabetic nephropathy and subsequent progression of CKD to ESRD and the need for permanent renal replacement therapy, remain unverified, unconfirmed, uncertain, and possibly unknown[105-128]. Undeniably, several independent and often conflicting lines of evidence in the literature, from both human and experimental studies, propose a variety of presumed pathogenetic culprit mechanisms and factors[105-128]. Putative roles are ascribed in the literature for oxidative stress, inflammation, underlying genetic predispositions including variations of the non-muscle myosin heavy chain 9 gene (*MYH9*) on chromosome 22 and variants at chromosome 6q24-27 among African-Americans, advanced glycosylation end products and the interaction of these end products on the multili-

gand receptor of the immunoglobulin superfamily receptor for advanced glycation end products, intrarenal angiotensin II and/or renin production, lipid toxicity, podocyte injury and apoptosis, cytokine/chemokine/growth factor release causing renal injury, asymmetric dimethylarginine, and uric acid[105-128]. We posit that our current understanding of these different plausible pathways remain infantile at best, and that more studies are warranted[105]. We surmise that our better understanding of these mechanisms and processes that influence CKD initiation and CKD propagation, and our ability to decipher the relative contributions of these pathways and/or factors in CKD-ESRD translation, together with the impact of AKI on this continuum of CKD pathogenesis would implicitly help improve our overall ability to prognosticate and care for our CKD patients, in general[31,32,79,105].

THE CKD EXPRESS©-A NEWLY INTRODUCED IT SOFTWARE TO ENHANCE AND OPTIMIZE CKD CARE

From the foregoing review, it is very clear that there remains a significant knowledge gap of our understanding of the natural history of CKD, in general. As a result of these inherent deficiencies in the CKD knowledge base, in an MBA "New Product" class at the University of Wisconsin Consortium in 2011-2012, the first author together with a group of fellow MBA students devised a new IT Software Program called The CKD Express©, still currently in United States patent application[32,88,89,129]. This innovative IT Software Program with its unique artificial intelligence (AI), decision support tools (DSS) and other enhancements that include algorithmic components would enable a trained Nurse Practitioner, under the supervision of a nephrologist, to remotely track and remotely manage CKD patients by tracking serum creatinine and simultaneous eGFR trajectories of individual CKD patients indefinitely over time, through established IT system networks linked with the various associated EMR systems[32,87,88,129].This way, CKD patients will carry out pre-specified blood tests including the basic metabolic profile at pre-determined time intervals as determined by the IT Software. Only targeted and identified CKD patients would then need to either be referred to see a nephrologist, or repeat a blood test, urgently be seen in an Urgent Care Center or even the Emergency department as determined by the inbuilt algorithms in The CKD Express©[32,87,88,129]. This would ensure the delivery of convenient, affordable, effective and efficient CKD care around the world[32,49,87,88,129].

CONCLUSION

CKD staging is a useful guide, but its evidence-base is shaky, at best. However, CKD care must be individualized. Clearly, despite decades of painstaking research into the dynamics, processes and mechanisms of translation from CKD to ESRD and the need for renal replacement therapy, the medical community in general, and nephrologists in particular, arguably still remain at a considerable loss in understanding the nuances of such translations. We suggest a complete reappraisal of current nephrology practices and a new push to begin to develop new models of CKD care that correctly recognize the diversity of CKD as representative of a wide spectrum of disease states[31,32]. Most appropriately, Bansal and Hsu, in a 2008 analysis of the long-term outcomes of patients with CKD had strongly echoed the observation that the apparently conflicting and disparate ESRD and/or mortality rates reported in various CKD population cohorts in the literature only emphasized the heterogeneity of different CKD populations[130]. In a recent analysis, in comparison with patients with other underlying causes of CKD, patients with APKD and IgA nephropathy had a statistically significant slower progression rate of CKD to ESRD[131]. Nephrologists must therefore not rely on CKD staging alone to direct management of or risk stratification of patients with CKD[130]. Nephrologists must always consider the etiology and rate of progression of kidney disease, patient age and a wide array of renal cardiovascular disease risk factors[130,131]. Nephrologists must recognize that CKD prediction is at best an inexact science. CKD prediction models are very limited in scope and applicability. Inter-patient and intra-patient CKD stages variability over time is immense. There are "progressors" and "nonprogressors", "improvers" and "nonimprovers", and sometimes the same CKD patient often then exhibits contrasting CKD progression or non-progression patterns at different time periods. The impact of AKI on CKD is multi-faceted, the so-called "rainbow syndrome"[19,30,72,92,94,95]. Moreover, the role of AKI in CKD initiation is even far less well understood[132].

The overarching need to always individualize CKD care cannot be overemphasized as CKD represents a whole wide spectrum of distinctly different clinical disease entities, with each individual patient often subject to a multitude of aggravating factors, some of which often remain unrecognized[19,30,31,72,92,94,95,132,133]. This is in fact how we practice medicine - one patient, and only one patient at a time[19,31]. A soon to be published longitudinal retrospective United Kingdom analysis of approximately 600000 patients strongly emphasized this paramount need for individualized CKD care[134]. This study identified a 5.9% incidence of CKD in the United Kingdom in 2010, and with follow up, CKD stages were static in 50% of patients, progressed in 10%-15% of patients and actually improved in 25%-30% of patients[134].

Finally, we hope that the acceptance and subsequent introduction by Medicare of our newly devised IT Software, The CKD Express© into general CKD care in the United States, will generate enough prospective patient-level serum creatinine trajectories' data over the next few years to begin to help bridge the yawning gaps in our current knowledge of the natural history of CKD[32,87,88,129,135-137]. Every effort to reduce, if not eliminate AKI (Renoprevention) must be emphasized in both general medicine care and in nephrology specialty care,

respectively[32,87,88,129,135,136]. Such reengineering protocols will reduce AKI incidence, potentially save hundreds of millions of healthcare dollars and invariably lead to less ESRD incidence[32,87,88,129,138,139]. A more forceful and pragmatic application of renoprevention strategies in the CCU that aggressively enunciate and articulatethe pre-emptive withholding (from all CKD patients) of nephrotoxics including renin angiotensin aldosterone system blockers, the adoption of measures to aggressively prevent or treat perioperative hypotension, the avoidance of nephrotoxic exposure such as iodinated contrast and nephrotoxic antibiotics, whenever possible, would collectively lead to less AKI, and therefore potentially less SORO-ESRD[32,87,88,129,138-140]. Such paradigms of care would translate into better patient outcomes, hugely significant renal salvage, and indeed massive dollar savings[32,138,139]. Such paradigm shifts would constitute major rethinking in current nephrology practice, a form of nephrology practice reengineering[32,138-140].

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This work is dedicated to the memory of our very dear friends, Mr. Ikechukwu Ojoko (Idejuogwugwu), and Chigbo Eduzor MD (CC), who passed away back home in Nigeria, several years ago, after reported brief illnesses. Idejuogwugwu and CC, you are truly missed. This work is also dedicated to the memories of the 153 Nigerians who died in a fiery plane crash in Lagos, Nigeria, on June 3, 2012. May their souls rest in perfect peace. Finally, we dedicate this work to the majority millions of Nigerians who continue to suffer in poverty, destitution, deprivation amidst plenty, to Nigerians who remain continuously exposed daily to indefensible insecurity in the face of paralyzed, corrupt and weak governments and derelict governance, and we pray for better days for that country. It remains our hope and prayers that someday, we would be celebrating an effective, efficient, affordable, accessible and potent health care delivery system in Nigeria that would veritably and equitably serve all Nigerians, both rich and poor, both privileged and underprivileged. Lastly, we look forward to enhanced CKD care, together with accessible affordable ESRD care to all needy Nigerians.

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REVIEW

Obesity in kidney disease: A heavyweight opponent

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Abstract

Obesity is an important worldwide challenge that must be faced in most developed and developing countries because of unhealthy nutritional habits. The consequences of obesity and being overweight are observed in different organs, but the kidney is one of the most affected. Excess adipose tissue causes hemodynamic alterations in the kidney that can result in renal disease. However, obesity is also commonly associated with other comorbidities such as chronic inflammation, hypertension and diabetes. This association of several aggravating factors is still a matter of concern in clinical and basic research because the pathophysiologic mechanisms surrounding chronic kidney disease development in obese patients remain unclear. This review will discuss the consequences of obesity in the context of renal injury.

Key words: Overweight; Obesity; Kidney disease; Renin-angiotensin system; Diabetes

Core tip: Obesity is unquestionably one of the biggest health challenges the modern world will face this century. It has vast effects on systemic function including cardiovascular disease, metabolic dysfunction and chronic inflammation. All of these factors have a great impact on kidney function, and current data indicate a significant correlation between obesity and kidney disease because of irregular immune activation, altered renal hemodynamics and metabolic mediator signaling. This review focuses on the most recent findings that have begun to elucidate the relationship between obesity and its effect on the kidneys.

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INTRODUCTION

Obesity is unquestionably one of the biggest health challenges the world population faces this century. Statistics indicate that more than 1.4 billion adults over 20 (35%) are overweight, with 11% falling into the obesity category according to World Health Organization statistics. Although the state of being obese and overweight has usually been associated with developed countries and high income, globalization and widespread unhealthy nutritional habits have caused these phenomena to reach epidemic proportions. As a consequence to the rise in obesity rates, comorbidities linked to this disease such as diabetes, car-

Table 1 Recent major multicenter studies regarding the impact of obesity and overweight on the incidence of kidney disease, renal function prognosis and patient survival

Cohort	Number of patients	Country	Result	Ref.
Dialysis patients	1957	Netherlands	Higher mortality with very high or low BMI (< 65 yr)	[157]
Kidney transplant	1810	Netherlands	Higher mortality and kidney graft failure	[158]
Native population	1924	Sweden	Higher Chronic Renal Failure	[13]
National Health and Nutrition Examination Survey Ⅲ	5659	United States	Higher micro-albuminuria with metabolic syndrome	[159]
Hipertension and obesity	4585	Spain	Higher risk of renal insufficiency	[160]
Native population	2585	United States	Higher risk of kidney disease	[12]
Native population	5403	Japan	Higher risk of proteinuria	[161]
Kidney transplant	51927	United States	Lower patient and graft survival. Higher chronic graft failure and delayed graft function	[162]

diovascular disease, and cancer have also increased[1].

Adipose tissue has a great impact on metabolic homeostasis and immunological function. The conjunction of the main obesity-related risk factors defines a clinical condition termed Metabolic Syndrome. This syndrome aggregates a variety of pathologies, including dyslipidemia, thrombosis, low-grade systemic inflammation, elevated blood pressure, hyperglycemia and insulin resistance. Adipose tissue possesses an important influence over the immune response profile *via* direct and indirect mechanisms through the secretion of nonesterified fatty acids, cytokines and endocrine mediators defined as adipokines. Together, these factors contribute to a systemic change in the way the body works, adapts and responds to challenges.

Although many studies have associated obesity with higher morbidity rates and obesity-related diseases[2], some groups argue the contrary. Overweight and obese patients reportedly display higher survival, while patients with low body mass are at a higher risk of general mortality and cardiovascular and many non-cardiovascular disease incidence, a phenomenon referred to as the "obesity paradox"[3,4]. These findings also highlight the complex relationship that obesity has with different pathologies and demonstrates that a closer look is needed to understand the particular effects of being obese and overweight on the organism.

OBESITY AND THE KIDNEY

Obesity affects the function of many organs. The heart is one of the main organs affected by metabolic syndrome, and obesity significantly increases the chances of cardiac dysfunction because of chronic hemodynamic burden, which causes dyspnea, edema, ongoing systemic inflammation, metabolic alterations and other related comorbidities[5]. Other organs such as the liver are also affected by this pathology, with lipid accumulation causing non-alcoholic fatty liver disease[6]. Lung function is also compromised by adipose tissue around the abdomen, rib cage and visceral cavity[7].

The kidney is also responsive to obesity. Several multicenter studies have identified a direct correlation between obesity and renal complications (Table 1). Obesity has a multifactorial mechanism and is considered an independent factor in chronic kidney disease (CKD) development and progression to end-stage renal disease (ESRD)[8]. Studies demonstrate that obesity-induced hypertension and diabetes are strong determinants of CKD. Analyses relating obesity and kidney transplantation revealed that in 1987, 11.6% of adults awaiting a kidney transplant were obese, and in 2001, obesity among adults rose to 25.1%[9]. Concomitantly, body mass index (BMI) among patients initiating dialysis increased from 25.7 kg/m^2 to 27.5 kg/m^2 from 1995 to 2002[10]; and when compared with normal weight persons (BMI, 18.5-24.9 kg/m^2), there is a directly proportional relationship between increased BMI and increased CKD and ESRD risk[11,12]. A study conducted by Ejerblad *et al*[13] examined the association between degrees of obesity and CKD. After making adjustments for many covariates, the investigators found a 2.8-fold increased risk of nephrosclerosis and a 7-fold increased risk of diabetic nephropathy among adults who had a BMI of 35 kg/m^2 or higher compared with a lifetime highest BMI lower than 25 kg/m^2. In adults with no diabetes or hypertension, a lifetime highest BMI of 35 kg/m^2 or higher was associated with a 2-fold increased risk of CKD. Conversely, obese patients had better recovery and benefitted from reduced body weight by diminishing proteinuria[14]. Obesity was recently demonstrated to accelerate IgA nephropathy progression[15]. In this scenario, obesity could be one of the few preventable risk factors for CKD development because it also mediates diabetes and hypertension, which are related to kidney disease progression[14,16,17].

The occurrence of obesity during early life is linked to low glomerular filtration rates (GFRs), while being overweight during adulthood doubles the chances of chronic kidney disease[18]. Many researchers have described the direct impacts obesity has on the kidneys, which include hyperfiltration, elevated glomerular tension, and podocyte stress[19]. Some researchers have also correlated obesity-related inflammation and adipokine deregulation to kidney function. The present review will focus on the impact of obesity on kidney function and discuss its influence on the progression of kidney disease.

Obesity-induced inflammation

Adipose tissue is known for its roles in lipid storage, ther-

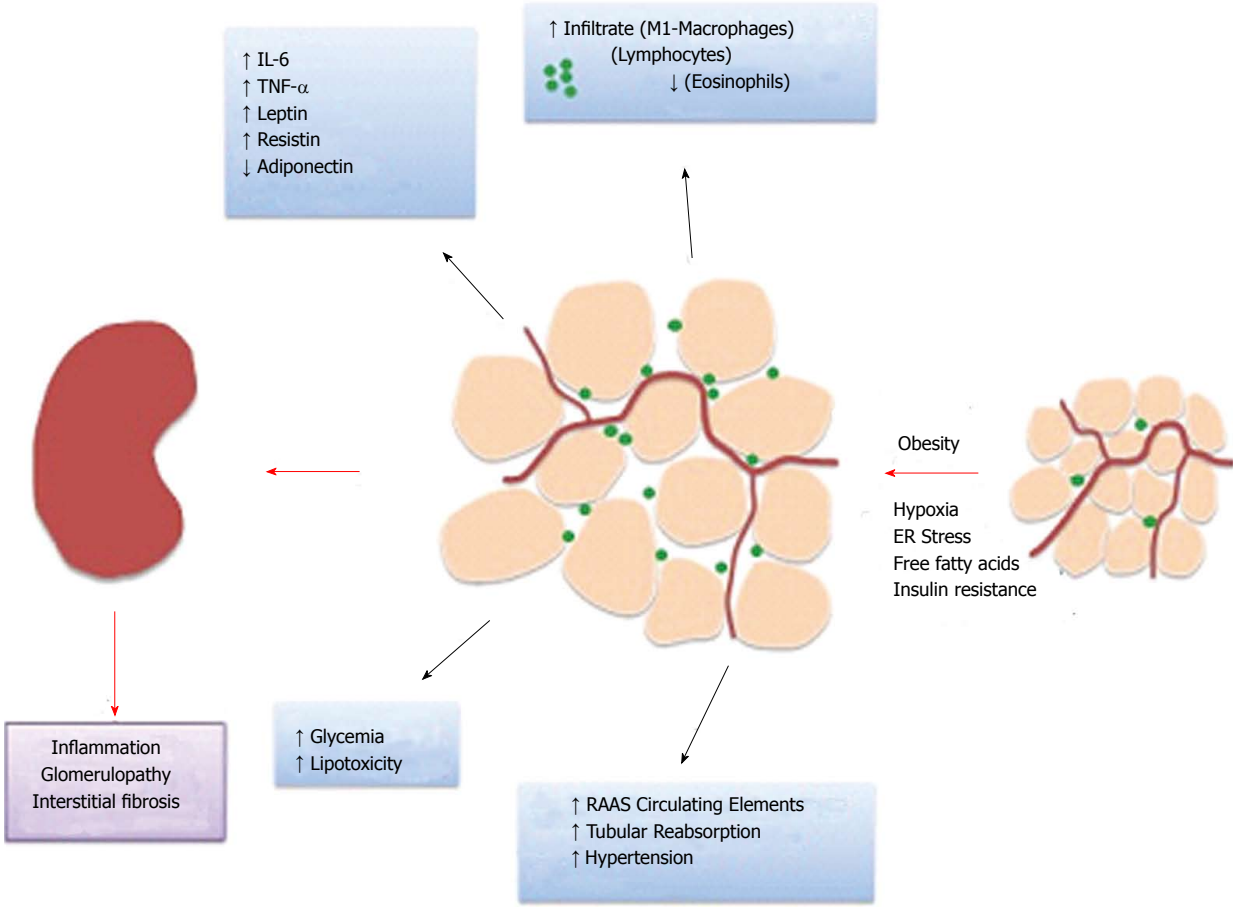

Figure 1 Main factors involved in obesity-induced inflammation, metabolic stress and hemodynamic disorder that participate in kidney function impairment. RAAS: Renin-angiotensin-aldosterone system; ER: Endoplasmic reticulum; TNF-α: Tumor necrosis factor alpha.

mogenesis and metabolic regulation. However, in recent years, focus has been given to its endocrine properties such as cytokine and adipokine secretion (Figure 1).

As previously described, obesity and diabetes are conditions that present a state of low-grade inflammation. Significant evidence supports the concept of adipose tissue as an immunomodulatory organ. Adipose tissue harbors a considerable amount of immune cells such as macrophages, lymphocytes and eosinophils. In obesity, the frequency of infiltrated cells rises, and they acquire a pro-inflammatory profile[20]. Excess free fatty acids that are present in obesity activate diverse inflammatory pathways involving endoplasmic reticulum stress[21], toll-like receptor[22,23], inflammasome and nuclear factor-κB (NF-κB) signaling activation[24,25]. In parallel, adipose tissue becomes hypoxic with adipocyte hypertrophy, which induces a change from aerobic to anaerobic glycolysis and lactate production.

With obesity, adipocyte hypertrophy and hypoxia induce cell death and resident immune cell activation, which in turn promotes inflammatory cell recruitment[26]. Macrophages constitute the principal population of resident and recruited cells in adipose tissue, which have a role in maintaining tissue homeostasis by assisting with the clearance of dead cells and debris. Because of lipid accumulation and adipocyte cell death, non-inflammatory tissue-resident M2 type macrophages and recruited monocytes undergo proliferation and macrophage M1 polarization[27-29]. These cells in turn secrete higher levels of inflammatory cytokines such as TNF-α, IL-6 and MCP-1 and lower levels of anti-inflammatory mediators such as arginase 1[28-30]. IL-4-expressing eosinophil counts also decrease with obesity, which contributes to inflammation[31]. Furthermore, $CD8^+$ and $CD4^+$ Th1 lymphocyte counts also increase while Treg numbers reduce with obesity. In accordance, B cell pro-inflammatory immunoglobulin G2c (IgG2c) production also participates in cell activation[32-35].

Proinflammatory cytokines are also produced by the renal parenchyma in response to hyperglycemia as well as vasoactive peptides such as angiotensin II and endothelin[36]. These molecules activate signaling second messengers such as protein kinase C, MAP kinase and NF-κB, which alter the gene expression of several cytokines and growth factors.

Increased TNF-α levels reduce the expression of nephrin and podocin, causing podocytopathy[37]. Similarly, IL-6 promotes adhesion molecule expression, which increases oxidative stress[38], and IL-6 receptor blockade can inhibit the progression of proteinuria, renal lipid deposition and mesangial cell proliferation[39]. An additional important growth factor for renal injury is transforming growth factor (TGF)-β, which induces podocyte apoptosis, extracellular matrix synthesis and mesangial cell pro-

liferation, thus exacerbating the development of the glomerular lesions associated with diabetes and obesity[40].

While many studies demonstrate the effect of metabolism on the immune system, studies have demonstrated that the reverse also happens; immune cell activation in adipose tissue is a determinant of obesity-linked metabolic changes such as insulin resistance[41]. For example, in response to inflammatory mediators, adipose tissue also down regulates glucose transporter GLUT4 expression, which increases insulin resistance.

Obesity and the adipokine imbalance

In addition to cytokines, adipose tissue is also responsible for the production of many endocrine mediators termed adipokines, which regulate inflammation, food consumption and link immune and metabolic functions. Amongst these are leptin, adiponectin, visfatin, resistin, intelectin and others. These factors are mostly secreted by adipocytes and have imbalanced expression in obesity. Many studies have documented the importance of these cytokines in the regulation of metabolism and inflammation and suggest a role for these cytokines in obesity-related metabolic and inflammatory distortion. Although there is still much to elucidate regarding the role of adipokines in kidney disease, recent studies now have begun to clarify the influence of these mediators in kidney pathology.

Leptin and kidney disease

Leptin was the first adipokine to be characterized and is the best described in the literature. It is secreted by different adipose compartments and induces signaling through Ob-a to Ob-f subtype receptor activation, and the Ob-Rb receptor is the most important. Its main actions are on the nervous system and kidneys. Leptin acts on the nervous system by stimulating neuropeptides that promote satiety and energy consumption. It has been suggested that one develops leptin resistance in obesity because of the absence of many of its effects despite elevated adipokine levels. Hyperleptinemia has also been associated with many cardiovascular and immunologic dysfunctions[42].

Many reports have linked obesity and leptin to hypertension. Studies indicate that this adipokine activates the sympathetic nervous system and may suppress parasympathetic nerve activity, which alters baroreflex control[43,44]. Leptin also increases renal sympathetic nerve activity, as demonstrated by studies on ObR deletion in the central nervous system[45]. Because the sympathetic nervous system contributes to CKD, leptin hypertensive actions may promote kidney disease.

Leptin also holds important pro-inflammatory activity. Its structure resembles other cytokines such as IL-2 and can stimulate many immune cells. Studies demonstrate that leptin induces the production of inflammatory cytokines as IL-6 and TNF-α by monocytes and additionally induces chemokine ligands, reactive oxygen species production and macrophage and monocyte proliferation[42]. Leptin also polarizes $CD4^+$ lymphocytes toward a Th2 profile, which increases the production of inflammatory cytokines such as IL-2 and IFN-γ[42]. Therefore, excess leptin, which is characteristic during obesity, is an important mediator of obesity-related immune and metabolic dysfunction.

Recent studies have also suggested that leptin imposes an important action in the kidneys, as this mediator localizes mainly to the organ after injection[46]. CKD patients demonstrate high leptin levels, as do ESRD patients, and hemodialysis fails to lower these values[47,48]. The kidneys also express the Ob-Ra and Ob-Rb leptin receptor isoforms[49]. In vitro, leptin induces glomerular endothelial cell proliferation, which is augmented in the presence of angiotensin Ⅱ and increases TGF-β1 production. Furthermore, leptin infusion into rats *in vivo* also induced proteinuria, glomerular endothelial cell proliferation and TGF-β1 production and increased collagen type Ⅳ expression[50]. This adipokine also induced type I collagen in mesangial cells, confirming data that link obesity, glomerulosclerosis and glomerulomegaly, which is defined as obesity-related glomerulopathy[51,52].

Adiponectin and kidney disease

Adiponectin is another adipokine with immunomodulatory and metabolic actions. It is present in plasma at a considerable concentration[53], and its receptors R1, R2 and T cadherin are expressed by a wide range of tissues. Adiponectin is negatively correlated with hypertension[54]. It exerts its metabolic actions by increasing glucose uptake and fatty acid oxidation and inhibiting gluconeogenesis. In addition to improving insulin sensitivity, it also possesses potent anti-inflammatory properties[42].

Unlike leptin, low serum adiponectin levels are found in obese patients, and its production is reduced by hypoxia, inflammatory mediators such as IL-6 and oxidative stress[55-57]. Hypoadiponectinemia has been linked to diverse complications in obesity. Mice lacking adiponectin display increased susceptibility to high-fat diet-induced insulin resistance[58]. Moreover, adiponectin overexpression in high-fat diet-fed animals caused less fat accumulation and reduced adipose tissue macrophage infiltration, and it prevented premature death[59].

Recent studies have begun to elucidate the role of adiponectin in kidney injury. Current data demonstrate that adiponectin is secreted not only by adipocytes but also renal tubular cells[60]. Research indicates that plasma adiponectin is inversely correlated with albuminuria in obese patients[61]. Adiponectin-null mice also develop albuminuria and podocyte damage as well as glomerular oxidative stress[62]. These mice also display more expressive albuminuria, fibrosis and macrophage infiltration after 5/6 nephrectomy[63]. Moreover, mice overexpressing adiponectin recover more rapidly and exhibit less interstitial fibrosis after podocyte-specific damage[64]. Metabolic syndrome has also been associated with low adiponectin levels and worse prognosis after kidney transplantation[65]. These data are controversial, however, as some studies describe a direct link between adiponectin levels

and mortality in advanced CKI and kidney transplant patients[66,67]. While recent work suggests that adiponectin causes less intense ischemia-reperfusion kidney injury[68], the contrary was observed when exogenous adiponectin was administered[69]. Furthermore, kidney function also influences adiponectin levels because the kidneys are responsible for its elimination, and kidney transplantation significantly reduces the adiponectin concentration[70].

Resistin and visfatin

Resistin is a recently discovered adipokine with inflammatory properties. Some works suggest that this mediator increases insulin resistance, while others fail to find this correlation[71,72]. Although in mice, it is expressed mainly by adipocytes, in humans it is produced principally by macrophages and monocytes. Although there are still few data on its impact on renal function, some research indicates that serum resistin levels are strongly associated with decreased GFRs and inflammatory biomarkers in CKD[71].

Adipose tissue and the kidneys also synthesize visfatin, and this is upregulated in type-2 diabetic rats, inducing fibrosis and inflammatory pathway activation[73]. In CKD patients, higher visfatin levels also are correlated with decreased GFR and endothelial dysfunction[74,75]. Furthermore, another study with human plasma determined that this mediator was also linked to creatinine levels, inflammation and endothelial damage in kidney recipients, which is negatively related to plasma albumin levels[19].

OBESITY AND RAAS IN KIDNEY DISEASE

The pathophysiologic mechanism surrounding CKD development in obese patients remains unclear, but many events must be linked to ESRD such as altered renal hemodynamics, insulin resistance, hyperlipidemia, inflammation and oxidative stress (Figure 1). Hemodynamic alterations such as higher renal plasma flow, GFR and filtration fraction were linked to obesity when compared with the levels in non-obese patients[76,77]. The effect of BMI on renal hemodynamics was also proven by another work in which GFR and effective renal plasma flow (ERPF) were evaluated with a high-sodium diet. According to this study, ERPF and the GFR were statistically increased when individuals were exposed to a high-sodium diet and compared to another group that was exposed to a low-sodium diet without a change in filtration fraction (FF). However, increased sodium intake-induced changes in the GFR and FF were significantly greater in people with a BMI $\geqslant$ 25 kg/m^2[78]. The hemodynamic effects of overweight on kidney function and albuminuria are enhanced with hypertension, which itself is a clinical complication of obesity. Chagnac *et al*[79] demonstrated that glomerular hyperfiltration could have a relevant role in development of hypertension in obese patients by increasing postglomerular oncotic pressure and proximal tubular sodium reabsorption.

As an individual gains weight, renal mass as well as the glomerular diameter increases[80]. Podocytes are highly specialized cells that support the glomerular basement membrane (GBM) and play an important role in the glomerular filtration barrier *via* their foot processes. With glomerular hypertrophy, podocytes must cover a larger area by expanding these processes. If this podocyte enlargement is not proportional to glomerular hypertrophy, this adaptation could cause podocyte detachment and consequently a loss selectivity of serum protein selectivity[81,82]. Considering that podocytes are cells with limited capacity for cell division and replacement, proteinuria may be detected as is commonly observed in obese patients. Supporting this hypothesis, individuals who reduced their body mass also had significant reductions in proteinuria[14,83].

Extensive studies demonstrate that a lack of podocytes covering the GBM results in the formation of denuded areas, which trigger matrix deposition resulting in glomerulosclerosis in experimental models as well as in human biopsies[84-87]. As kidney injury persists, kidney fibrosis becomes an inevitable outcome in which epithelial-mesenchymal and endothelial-mesenchymal transition events generate matrix-producing fibroblasts in the interstitial space that contribute to renal fibrosis. Accumulation of matrix elements caused by the fibrotic process progressively alters normal kidney architecture by contraction and increased stiffness, resulting in disrupted blood flow supply and nephron function[88,89].

Once a number of podocytes are injured, a vicious cycle starts in which other podocytes also become damaged, accelerating podocyte deterioration and glomerulosclerosis[90]. The extensive loss of glomeruli imposes excessive stress on the remaining glomeruli because of hemodynamic alterations and glomerular hypertrophy, which can subsequently cause further sclerosis of the remaining glomeruli[91]. This could explain the progressive spreading of glomerular damage in later disease stages in which patients develop chronic renal failure[90]. The approach of using new agents to avoid podocyte lesions in different models of acute and chronic kidney disease resulted in less matrix deposition and consequent glomerulosclerosis[92,93].

In obesity, the renin-angiotensin-aldosterone system (RAAS) is commonly activated and is one of the strongest links to renal injury. All of the major components necessary to generate angiotensin Ⅱ (Ang Ⅱ) are found in the kidney[94]. The RAAS is a well-known mechanism to regulate blood pressure, fluids and electrolyte balance[95], and its activation impairs normal pressure natriuresis, increases renal tubular sodium reabsorption, and causes volume expansion. Physical compression of kidneys by visceral adipose tissue in obesity exacerbates these responses and increases blood pressure, leading to hypertension in obese subjects.

RAAS effects are obtained when angiotensinogen (AGT), the precursor of bioactive angiotensin peptides, is cleaved by both renin and angiotensin converting enzyme (ACE) to generate Ang Ⅱ. Ang Ⅱ, which is the active peptide and is the main effector of RAAS, pos-

sesses a dual role in physiology. Ang Ⅱ helps maintain long-term blood pressure and blood volume in the body; conversely, it has also been considered a multifunctional cytokine that plays a role in cell proliferation, hypertrophy, superoxide production, inflammation and extracellular matrix deposition[96]. Ang Ⅱ plays an endocrine role, and its participation in the development of obesity was evidenced by several works in which AGT, ang Ⅱ and ang Ⅱ receptor-deficient mice were protected against high-fat diet-induced obesity[97-99].

There are several pathophysiological conditions, including hypertensive models, in which Ang Ⅱ, in response to increased arterial blood pressure, increases efferent glomerular arteriole resistance and induces TGF-β production[100]. It also impairs the auto-regulation of afferent arterioles by avoiding vasoconstriction[101]. Taken together, Ang Ⅱ directly and indirectly enhances capillary filtration pressure and promotes proteinuria, which is one of the most important factors involved in renal disease progression. Moreover, Ang Ⅱ is also involved in nephrin dephosphorylation during podocyte apoptosis[102], which is a protein that is part of the slit diaphragm and binds to the adjacent nephrins of other podocytes. Ang II decreases the synthesis of negatively charged proteoglycans that are present on the glomerular basement membrane, which impairs the filtration of high molecular weight proteins by electrostatic repulsion[103].

Human adipose tissue expresses all of the RAAS components, including angiotensin, ACE, renin and the AT1 and AT2 receptors. Consequently, the AGT produced by adipose tissue contributes significantly to circulating AGT levels. In humans and mice, a strong relationship has been observed between increased AGT gene expression and obesity[104], supporting a role for adipose AGT in hypertensive obese patients. Weight reduction reduced blood pressure through systemic RAAS suppression and decreased AGT, renin and aldosterone levels in adipose tissue and plasma[105]. Mice with adipose tissue-restricted AGT expression were normotensive, whereas when adipose AGT was overexpressed, the mice became hypertensive[106]. Ang Ⅱ is also involved in adipocyte metabolism by influencing leptin and adiponectin release. Once leptin levels are increased, Ang Ⅱ promotes a number of cellular processes that attenuate leptin signaling and contribute to leptin resistance, which is common in obesity[107]. Conversely, adiponectin was upregulated when RAS was blocked by an ACE inhibitor or Ang II receptor blocker, suggesting Ang Ⅱ participation in the inhibition of adiponectin release[108].

Not only AGT but also aldosterone levels are increased in obese patients. Aldosterone is a mineralocorticoid hormone that is produced in the adrenal glands in response to Ang Ⅱ and a high extracellular potassium concentration, which increases blood pressure *via* sodium retention in the collecting duct. Aldosterone is correlated with increased blood pressure[109] and can also be produced by adipocytes through pathways that are dependent on the Ang Ⅱ-ATI receptor axis and calcineurin signaling[110] as well as pathways that are independent of Ang Ⅱ, in which adipocytes secrete factors that may stimulate the adrenal gland and increase circulating aldosterone levels, resulting in mineralocorticoid receptor activation and increasing blood pressure and hypertension[111]. Aldosterone binds to cytosolic mineralocorticoid receptors and promotes cell signaling pathways, endothelial dysfunction, inflammation and fibrosis independently and in concert with Ang Ⅱ[112]. Moreover, Ang Ⅱ activates the mineralocorticoid receptor in the absence of aldosterone and promotes kidney injury[113,114]. Blocking the mineralocorticoid receptors with antagonists attenuates obesity-induced hypertension and glomerular hyperfiltration[115].

Many clinical trials have been performed to mitigate the effects caused by RAAS. Multiple pharmacological strategies are used to treat CKD patients to diminish proteinuria and blood pressure. These strategies comprehend the use of RAAS-blocking agents alone or combined with ACE inhibitors, angiotensin-receptor blockers, direct renin inhibitors and mineralocorticoid-receptor antagonists[116]. The combination of a pharmacological therapy with reduced sodium intake was a better choice to diminish blood pressure and proteinuria than combined therapies[117]. Attempts to antagonize aldosterone receptors demonstrated promising results to diminish glomerulosclerosis[118].

In summary, the obesity-RAAS-hypertension axis is closely related to renal disease, as the increased release of adipose tissue derived-RAAS elements into the circulation can alter hemodynamic homeostasis. Increased Ang Ⅱ, AGT and aldosterone levels promote increased tubular reabsorption, leading to arterial hypertension and renal vasodilation. These events contribute to glomerular hypertension, which is an important factor in glomerulosclerosis and CKD progression.

OBESITY AND DIABETES IN RENAL DISEASE

Obesity is an important risk factor for hypertension and type 2 diabetes development, which are the leading causes of end-stage renal disease. The relationship between obesity, diabetes and kidney disease is very close because obesity and diabetes alter renal function, leading to renal disease. These renal alterations in both cases include anatomical, physiological and pathological changes (Figure 1).

Physiological and hemodynamic alterations are largely responsible for the subsequent anatomical and histopathological modifications. Among the major hemodynamic changes in obese and/or diabetic patients are increased GFR and intraglomerular capillary pressure[119,120]. Such alterations lead to diabetic nephropathy, increases in kidney weight and size, increased glomerular size, podocyte hypertrophy and mesangial matrix expansion[121].

Diabetes-related renal injuries can be grouped into

Table 2 Summary of the most important changes in the kidney during diabetes

Stages	Features
1 and 2	Hyperfiltration and renal hypertrophy
3	Microalbuminuria and hypertension as clinical features. As histological features: arteriolar hyalinosis, glomerular basement membrane thickening and mesangial matrix expansion
4 (Diabetic Nephropathy)	Proteinuria, nephrotic syndrome and decreased GFR
5	End-stage renal disease

Adapted from Amann *et al*[150]. GFR: Glomerular filtration rate.

five stages that comprise the remodeling that occurs throughout diabetic nephropathy. These stages are summarized in Table 2.

Although obesity and diabetes *per se* are responsible for renal injury, some other factors usually present in these conditions significantly aggravate renal damage such as blood pressure, hyperlipidemia, hyperglycemia, genetic factors[122] and inflammation. Some of these conditions are described in the following sections.

Hypertension in diabetes-related kidney disease

Hypertension and diabetes are two important risk factors in the development of kidney diseases, and when they are present simultaneously, they aggravate renal injury.

Hypertension-induced kidney damage in obesity and diabetes follows a very similar sequence of events including increased renal tubular sodium reabsorption as well as RAAS and sympathetic nervous system activation[123-125]. Such an increase in blood pressure along with increased glomerular capillary pressure and GFR are main contributors to the initial renal damage in obesity and diabetic nephropathy[126].

Given the importance of hypertension in worsening renal injury, especially in diabetic nephropathy, many studies have been performed to demonstrate the importance of controlling blood pressure when treating diabetic nephropathy, and the recommended blood pressure is less than 130/80 mmHg[127]. Several clinical trials have also been developed and have demonstrated renal protection when low blood pressure is achieved[128].

Renin-angiotensin system blockade is an important treatment for controlling blood pressure and decreasing proteinuric kidney disease progression[129-131]. Angiotensin Ⅱ-receptor blocker (ARB) therapy helps prevent the progression from normoalbuminuric (Albumin-Creatinine Ratio < 30 mg/g creatinine) to albuminuric stages (ACR 30-100 mg/g creatinine)[132]. Another important strategy is combined therapy with ARB and ACE inhibitors, which demonstrates a greater decrease in proteinuria than monotherapy[133].

Hyperlipidemia in obesity and diabetes

Dyslipidemia is an important component of metabolic syndrome and is often directly related to obesity and diabetes. Patients with diabetic nephropathy usually have several changes in their lipid profile[134], and the presence of increased blood lipid levels is a risk factor for albuminuria[135]. Several studies have demonstrated a correlation between triglyceride and cholesterol levels with renal function markers. Ravid and colleagues[136] observed a significant and positive correlation between total cholesterol and albuminuria in type 2 diabetic patients in a five-year cohort. Similarly, Klein *et al*[137] noted that type 1 diabetic patients with elevated total cholesterol and low HDL levels also had higher incidence of renal failure.

Although these studies demonstrate significant correlations between dyslipidemia and impaired renal function in diabetic subjects, little is known about the mechanisms by which the increased lipid profile causes kidney damage. Studies have demonstrated lipid deposits in the glomeruli and in the mesangium of obese individuals, suggesting that these lipids may cause kidney damage and lipotoxicity[138]. This glomerular lipotoxicity would be because of renal sterol-regulatory element-binding protein (SREBP-1 and 2) expression, whereas lipotoxicity causes tubulointerstitial fibrosis and inflammation in the proximal tubule epithelial cells[139]. Furthermore, alterations in the coagulation-fibrinolytic system, increased atherosclerosis and endothelial cell damage can also cause or aggravate diabetic nephropathy[140].

Hence, the importance of lipid control in the maintenance of kidney function in diabetic patients has been postulated[141].

Hyperglycemia in diabetes-related renal injury

Vascular alterations in diabetes are largely due to increased blood glucose levels. Hyperglycemia promotes microvascular injury by several mechanisms. The most important mechanisms are as follows: increased intracellular advanced glycated end product (AGE) formation; interaction between AGEs and their receptors, with consequent disruption of cell signaling and function; constant protein kinase C activation[142]; and increased hexosamine pathway activity[143]. Renal endothelial and mesangial cells are susceptible to such hyperglycemia-induced changes[144]. Thus, the hyperglycemia-induced alterations that occur in the kidney are similar to those described above but generate characteristic damage to renal cells. Because of AGE-driven structural changes in extracellular matrix proteins, metalloproteinases lose their ability to degrade the matrix efficiently, which causes basement membrane thickening[145]. In the mesangium, AGE-induced changes include increased pericyte apoptosis and increased vascular endothelial growth factor expression, and these changes in turn cause glomerular hyperfiltration[146].

Because hyperglycemia causes severe damage to the kidneys and other organs, several studies were developed to demonstrate the importance of glycemic control to prevent diabetic nephropathy. One of these clinical trials, the Diabetes Control and Complications Trial, compared conventional and intensive insulin therapy in type 1 diabetic patients. Over approximately 6.5 years, decreased risks for microalbuminuria and overt nephropathy were

observed with intensive glycemic control[147,148]. The Action in Diabetes and Vascular disease: Preterax and Diamicron-MR Controlled Evaluation clinical trial, which was based on type 2 diabetic patients, also observed a reduction in albuminuria and nephropathy progression with insulin therapy intensification in late disease[149].

TREATMENTS AND PHARMACOLOGICAL INTERVENTION

Most treatments and approaches to reduce kidney injury in obese patients focus on managing associated risk factors such as hypertension, diabetes and hyperlipidemia using strategies as nutritional counseling, pharmacological interference and in some cases, surgery.

Dietary treatment consists on the change in nutritional habits and lifestyle. Eating smaller portions, increasing water consumption, minimizing salt ingestion and practicing physical activities are essential for weight reduction. Such practices can prevent and treat obesity which in turn reduces the risk of CKD. However this is a measure that brings long-term results. Treatment of patients with severe obesity focuses on reduction of proteinuria levels. Currently, several studies point out to the combined therapy of RAS inhibitors (ACE inhibitors and Ang Ⅱ receptor antagonists); low calories and low salt diets as presumably the best therapeutic options for obese patients with high levels of proteinuria[117].

Weight loss is also an important factor in this treatment regimen. Surgical intervention to treat obesity is a strategic option that can diminish levels of proteinuria in obese patients by mainly reducing hyperfiltration, attenuating obesity-mediated dyslipidemia and insulin resistance, reducing blood pressure and altering adipokine levels such as leptin and adiponectin which have direct a effect on podocytes, therefore improving kidney function[14,151,152]. Even modest weight loss has been associated with a substantial reduction in blood pressure and risk of diabetes[153]. The benefits of bariatric surgery are attributed to sympathetic nervous system suppression, decreasing therefore overall renal sympathetic activity and reduction on sodium reabsorption[154].

Once patients begin to lose weight, longer-term maintenance is difficult and even with continued treatment, patients may regain their normal condition. To prevent this, there is a need for adjunctive therapies for patients who are not able to lose weight or sustain weight loss solely with lifestyle changes[155]. In this scenario, the introduction of pharmacological treatment by the use of, for instance, noradrenergic agents, gastrointestinal lipase inhibitors and serotonin receptor agonists become an alternative and efficient strategy towards weight loss[156].

CONCLUSION

Obesity has great influence on end-stage renal disease, and it can be either the cause of renal alterations and kidney injury or an aggravating factor when other conditions such as hypertension and diabetes are established. All of these factors represent severe insults to the kidney, resulting in high costs to health systems to manage dialysis patients as well as those with post- cardiovascular events. Therefore, studies that relate these factors are important for developing new strategies to treat obese patients with renal disease to reduce patient mortality and improve quality of life.

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REVIEW

Renin-angiotensin system in the kidney: What is new?

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Abstract

The renin-angiotensin system (RAS) has been known for more than a century as a cascade that regulates body fluid balance and blood pressure. Angiotensin Ⅱ (Ang Ⅱ) has many functions in different tissues; however it is on the kidney that this peptide exerts its main functions. New enzymes, alternative routes for Ang Ⅱ formation or even active Ang Ⅱ-derived peptides have now been described acting on Ang Ⅱ AT_1 or AT_2 receptors, or in receptors which have recently been cloned, such as Mas and AT_4. Another interesting observation was that old members of the RAS, such as angiotensin converting enzyme (ACE), renin and prorenin, well known by its enzymatic activity, can also activate intracellular signaling pathways, acting as an outside-in signal transduction molecule or on the renin/(Pro)renin receptor. Moreover, the endocrine RAS, now is also known to have paracrine, autocrine and intracrine action on different tissues, expressing necessary components for local Ang Ⅱ formation. This *in situ* formation, especially in the kidney, increases Ang Ⅱ levels to regulate blood pressure and renal functions. These discoveries, such as the ACE2/Ang-(1-7)/Mas axis and its antangonistic effect rather than classical deleterious Ang Ⅱ effects, improves the development of new drugs for treating hypertension and cardiovascular diseases.

Key words: Renin-angiotensin system; Angiotensin Ⅱ; Kidney; Hypertension treatment; Receptor

Core tip: Activation of the angiotensin converting enzyme (ACE)/ Angiotensin Ⅱ (Ang Ⅱ)/AT_1 axis leads to vasoconstriction, anti-diuresis, anti-natriuresis, release of aldosterone and anti-diuretic hormone, which can result in hypertension, renal and cardiovascular diseases. Inhibition of renin and ACE or blocking AT_1 receptor is the most used therapies for heart failure and hypertension. Nevertheless, the discovery of local Ang Ⅱ synthesis, new Ang Ⅱ metabolites, receptors and axis of this system, makes possible the development of new drugs and strategies for renal and cardiovascular diseases treatment, such as activation of ACE2/Ang-(1-7)/Mas axis, which presents opposite effects of AT_1 activation by Ang Ⅱ.

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RAS IS NOT ONLY AN ENDOCRINE SYSTEM

The first observation that the arterial blood pressure could be regulated was in 1898 after the discovery of a soluble protein extracted from the kidney that increased

Table 1 Comparison of the components from classic and recent renin-angiotensin system

	Classic RAS	Recent RAS
Hormone process	Endocrine	Paracrine Autocrine Intracrine
Bioactive peptide	Ang Ⅱ	Ang Ⅱ Ang III Ang IV Ang-(1-7) Ang-(3-4) Ang A Alamandine
Receptor	AT_1 AT_2	AT_{1a} AT_{1b} AT_2 Mas Mrg AT4 PRR ACE

Ang: Angiotensin; ACE: Angiotensin converting enzyme; AT_1: Angiotensin type 1 receptor; AT_2: Angiotensin type 2 receptor; AT_4: Angiotensin type 4 receptor; Mas: Ang-(1-7) Mas receptor; Mrg: Mas-related G-protein-coupled receptor; PRR: Renin/(Pro)renin receptor; RAS: Renin-angiotensin system.

blood pressure in rabbits, called "renin"[1]. Over 30 years later, Goldblatt *et al*[2] associated the decrease of blood pressure in kidneys with hypertension by using a silver clamp to partially constrict dogs renal arteries, resulting in reno-vascular hypertension. Using the same methodology as Goldblatt, Braun-Menendez *et al*[3] in 1940 isolated a vasoconstrictor substance responsible for the reno-vascular hypertension from renal venous blood of the hypertensive kidney dog, calling it "hypertensin". Page *et al*[4] independently described a vasoconstrictor substance by injecting renin into cats, called "angiotonin". The same group also described angiotensinogen, first referred to as a "renin activator"[4]. The name "angiotensin" for the vasoconstrictor substance "hypertensin" by Braun-Menendez and "angiotonin" by Page emerged in 1958 after they both agreed on this hybrid name, since these 2 substances proved to be the same potent vasoactive octapeptide. The World Health Organization and the American Heart Association in 1987 suggested the abbreviation Ang for angiotensin, numbering the amino acids residues of angiotensin I (Ang I) as a reference for all angiotensin-derived peptides[5]. The decapeptide Ang I has no physiological effect, but is hydrolyzed by angiotensin converting enzyme (ACE) generating angiotensin Ⅱ (Ang Ⅱ), which was considered the only peptide in renin-angiotensin system (RAS) with biological actions[6].

More than a century since the discovery of renin by Robert Tigerstedt and Bergman[1], the RAS, remains a fascinating subject for research. Although it is well known the distinct roles of RAS in different tissues, such as brain, adipose tissue, gastrointestinal tract and cardiovascular system[7-10], it is on the kidney that Ang Ⅱ has its main function on regulating body fluid content and blood pressure by alterating Na^+ and water homeostasis, intrarenal hemodynamics and glomerular filtration[11,12]. Ang Ⅱ stimulates anti-diuretic hormone secretion in the pituitary gland with increased water reabsorption in the collecting duct, and also increases aldosterone secretion, a steroid hormone synthesized mainly by the adrenal cortex, and a downstream effector of Ang Ⅱ that induces sodium reabsorption and concomitant potassium and hydrogen ion excretion by the kidney[13].

Many new findings suggest new properties of this system, with new enzymes, different routes for Ang Ⅱ formation, new receptors and active Ang Ⅱ-derived peptides (Table 1). The classical axis ACE/Ang Ⅱ/AT_1 is not the only signaling pathway within RAS, since others such as angiotensin converting enzyme 2 ACE2/Ang-(1-7)/Mas receptor and Angiotensin IV/AT_4 indicate new activities for this cascade[14,15]. Besides the inhibition of renin and ACE, and also angiotensin type 1 receptor (AT_1) receptor blockade, activation of the ACE2/Ang-(1-7)/Mas axis is a possible alternative target for new drugs, since some protective effects on renal and cardiovascular function have been reported[14,16-18]. Ang Ⅱ is not the only active peptide of the RAS, there now being physiological properties associated with many Ang Ⅱ-derived peptides[14,15,19]. Ang Ⅱ can be hydrolyzed by > 13 "angiotensinases", proteolytic enzymes such as aminopeptidases, carboxipeptidases, endopeptidases, ACE2 and neprilysin, generating angiotensin Ⅲ (Ang Ⅲ), angiotensin Ⅳ (Ang Ⅳ), angiotensin-(1-7) [Ang-(1-7)], angiotensin-(3-4) [Ang-(3-4)], angiotensin A (Ang A), and alamandine, which can bind to specific receptors or act on the same receptors as Ang Ⅱ[14,15,19-22]. Although AT_1 and AT_2 receptors are the most studied receptors for Ang Ⅱ, two other receptors - Mas receptor for Ang-(1-7), and AT_4 receptor for Ang Ⅳ - have been cloned[14,15]. Ang Ⅱ-derived peptides could have similar effects to Ang Ⅱ, or counteract its effects on renal function. For instance, like Ang Ⅱ, Ang-(1-7) can increase intracellular Ca^{2+} *via* AT_1 receptor, but has the opposite effect to Ang Ⅱ, since it can induce antiproliferative and protective effect through the Mas receptor[23,24]. New functions for well known members of the RAS have been found. For example, ACE, known for its catalytic action on Ang I, also functions as a signal transduction molecule, initiating a series of intracellular events when stimulated[25,26]. Besides increasing catalytic activity of renin and prorenin, the renin/(Pro)renin receptor (PRR), cloned in 2002[27], can also induce an intracellular signaling pathway generating effects in an angiotensin-independent manner[6,27].

It is now considered that RAS assumes paracrine, autocrine and intracrine mechanisms of action in hormone signaling[6,28]. Many tissues and cells, including kidneys, have all the necessary RAS components to form Ang Ⅱ *in situ*[29-31]. Renal levels of Ang Ⅱ are much higher than in the plasma[32], indicating that the source of Ang Ⅱ within the kidney is not only provided by filtered plasma Ang Ⅱ. The kidney expresses all the major components of the RAS, such as angiotensinogen, renin and

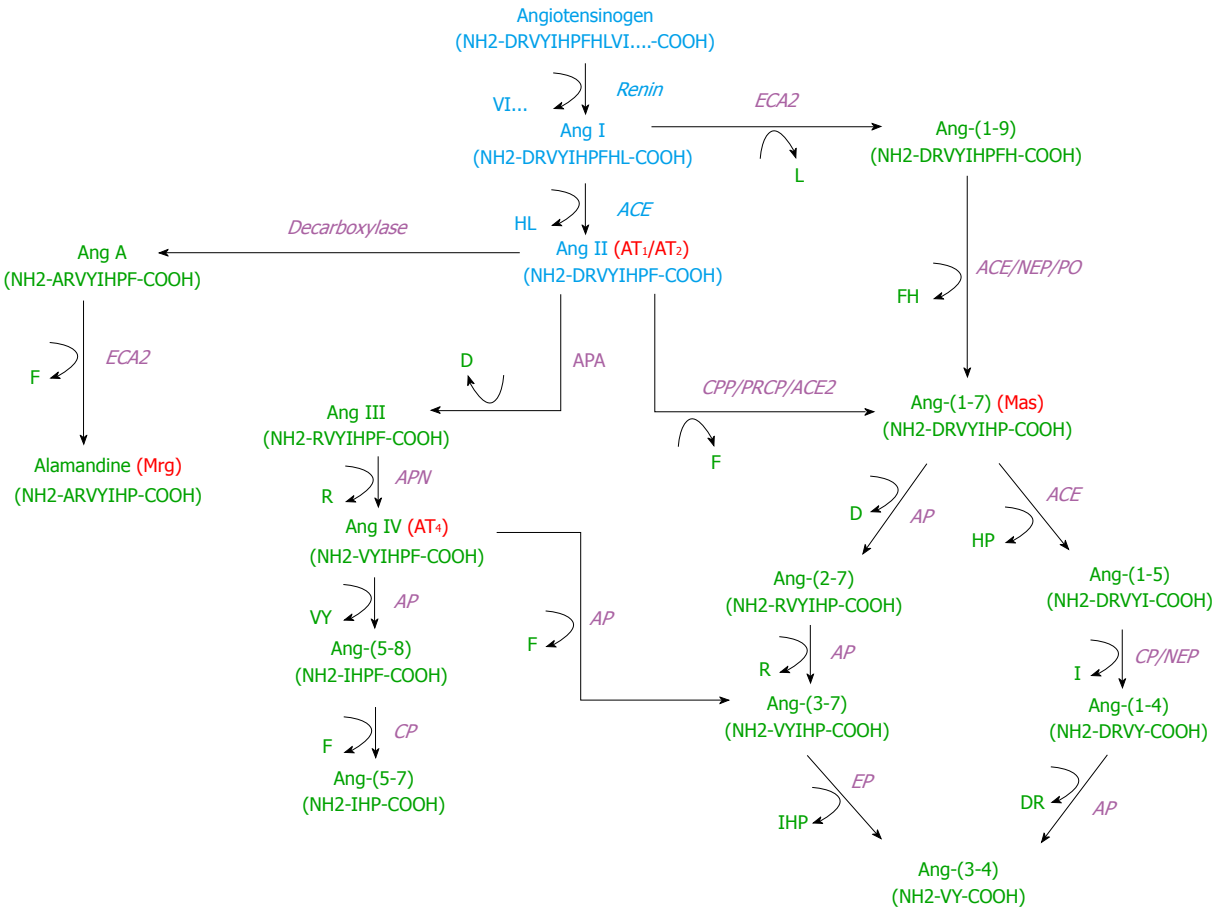

Figure 1 Classic view of renin-angiotensin system cascade (blue) and recent view of renin-angiotensin system cascade (green). AP: Aminopeptidase; APA: Aminopeptidase A; APN: Aminopeptidase N; CP: Carboxypeptidase; EP: Endopeptidase; ACE: Angiotensin converting enzyme; ACE2: Angiotensin converting enzyme 2; CPP: Carboxypeptidase P; PRCP: Prolyl carboxypeptidase; NEP: neprilysin; PO: Prolyl oligopeptidase; Mas: Ang-(1-7) Mas receptor; ACE: Angiotensin converting enzyme. Adaptated from Axelband *et al*[20] with permission.

ACE[29-31]. Locally synthesized Ang Ⅱ can act on cell surface, nuclear and cytoplasmic AT_1 and AT_2 receptors[33-35].

We will describe a novel view of the classic RAS that includes new members, routes, receptors, and new drugs and targets for the treatment of heart failure and hypertension. Due to the high Ang Ⅱ concentration in different compartments of the kidney, and the importance of Ang Ⅱ effects on renal function in physiological and physiopathological conditions, the focus will be on the intrarenal RAS, especially its paracrine and intracrine functions. This new aspect of RAS will improve our present understanding of RAS and the role of its new members, which should benefit the development of new treatments for hypertension and kidney diseases.

NEW MEMBERS OF RAS: ANG Ⅱ-DERIVED PEPTIDES

Classically, renin is secreted by juxtaglomerular cells in response to 3 stimuli: (1) decreased arterial blood pressure, detected by baroreceptors; (2) decreased sodium levels in the macula densa ultrafiltrate; and (3) increased sympathetic nervous system activity. Renin is an enzyme with only one known substrate, angiotensinogen. The reaction catalyzed by renin, generating the decapeptide Ang Ⅰ, is the rate-limiting step in Ang Ⅱ formation. Ang Ⅰ is then converted to Ang Ⅱ by ACE, a monomeric glycoprotein that acts as an exopeptidase to cleave dipeptides from the C-terminus of Ang Ⅰ-(1–10) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) into the octapeptide Ang Ⅱ-(1-8)[36] (Figure 1). The main Ang Ⅱ effects are mediated by the AT_1 receptor, such as vasoconstriction, anti-diuresis, anti-natriuresis, release of aldosterone and anti-diuretic hormone, whereas AT_2 activation counterbalances these effects[19,36,37].

It is widely accepted that small peptides derived from Ang Ⅱ have local physiological effects, especially in the kidney (Figure 1). ACE2 is a transmembrane glycoprotein that shares a 42% of homology with ACE and contains a single active site domain more closely to the N domain of ACE[16,38]. Unlike ACE, ACE2 is a monocarboxypeptidase, generating Ang-(1-7) by the cleavage of a single Phe residue from Ang Ⅱ, and Ang-(1-9), removing the C-terminal Leu residue from Ang Ⅰ [16,38]. Within the renal brush-border vesicles of the rat, Ang-(1-7) is preferentially hydrolyzed by aminopeptidases and neprilysin (NEP) after aminopeptidase blockade, generating Ang-(1-4)[39]. In the basolateral membrane, brush-border vesicles of the pig and purified preparations of

renal NEP Ang I is hydrolyzed primarily to Ang-(1-7) and Ang-(1-4)[40,41]. In sheep proximal tubules, urine and serum, Ang Ⅱ is converted to Ang-(1-7) by both membrane-bound and soluble forms of ACE2[38].

The physiological importance of Ang-(1-7) has become increasingly evident, especially after Santos *et al*[14] found a G protein-coupled receptor for Ang-(1-7), the Mas receptor, using a selective Ang-(1-7) antagonist. The Mas protooncogene was cloned and sequenced in 1986, after being detected by its tumorigenicity in mice[42]. This gene encodes a protein with 7 hydrophobic transmembrane domains, first considered as an "orphan" G protein-coupled receptor[43]. Ang-(1-7) exerts many effects on renal function, such as diuresis and natriureses, and it can be detected in human urine[44]. This peptide is of importance during late gestation in rats, where RAS overactivity is associated with increased kidney and urine levels of Ang-(1-7) and enhanced kidney immunostaining of Ang-(1-7) and ACE2[45].

Diuretic/natriuretic effects of Ang-(1-7) may also be due to the regulation of Na^+ reabsorption within the proximal tubule. *In vivo* and *in vitro* studies showed that Ang-(1-7) is a potent inhibitor of Na^+ reabsorption in this nephron segment, acting on different receptors[46-49]. Ang-(1-7) can bind to distinct receptors and induces different cellular responses depending on the cell type. For instance, in distal tubule cell (MDCK), Ang-(1-7) inhibits ($Na^+ + K^+$)-ATPase activity through the AT_1 receptor to stimulate the PI-PLC/PKC signaling pathway[47], whereas in the proximal tubule, it inhibits Na^+-ATPase *via* the AT_2/G(i/o) protein/cGMP/PKG pathway[48]. Moreover, at different concentrations of Ang-(1-7) (10^{-12}, 10^{-9}, or 10^{-6} mol/L) used in intratubular perfusion in the absence or presence of the Mas receptor antagonist (A779) of rat isolated proximal tubules, it was shown that Ang-(1-7) has a biphasic dose-dependent effect on the Na^+/H^+ exchanger mediated by Mas receptor and gave a moderate increase in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$)[49]. Increased $[Ca^{2+}]_i$ stimulated by Ang-(1-7) also occurred in MDCK cells, but through the AT_1 receptor, which in turn stimulated Ca^{2+} release from endoplasmic reticulum *via* the PLC pathway and Ca^{2+} influx through PLA2-dependent store-operated Ca^{2+} entry[24]. In this way, ACE2/Ang-(1-7)/Mas axis can counteract most of the deleterious effects of ACE/Ang Ⅱ/AT_1. It has been corroborated that acute intravenous infusion of Ang-(1-7) induces diuresis, natriuresis and renal vasodilatation[50].

Like to Ang-(1-7), there is another heptapeptide derived from Ang Ⅱ having the opposite effect to Ang Ⅱ, namely Ang-(2-8), also known as Ang Ⅲ. Ang Ⅱ can be hydrolyzed by aminopeptidase A, generating Ang Ⅲ[51] (Figure 1). Heretofore there has been no evidence of a specific receptor for Ang Ⅲ, and Ang Ⅲ normally binds to AT_1 with greater affinity than to the AT_2 receptor inducing natriuresis on rats[52,53]. Intrarenal Ang Ⅲ induces natriuresis *via* the AT_2 receptor in the proximal tubule by a cGMP-dependent mechanism[51].

Ang Ⅲ can be hydrolyzed by aminopeptidase N generating Ang-(3-8), also called Ang IV, which can be also generated directly from Ang Ⅱ by D-aminopeptidase[20,54]. The receptor for Ang IV, AT_4, was initially detected in the guinea pig hippocampus[15]. Protein purification and peptide sequencing showed that the AT_4 receptor is an insulin-regulated aminopeptidase[54]. AT_4 receptor is also found in the kidney, where this angiotensin-derived fragment can elicit many responses[55]. Aminopeptidases A and N are abundant in the kidney, especially in proximal nephron, and Ang Ⅳ is formed in the glomerulus[56,57]. Ang Ⅳ increases blood flow in the kidney and decreases in Na^+ transport in proximal tubules[55]. Ang Ⅳ induces Ca^{2+} mobilization in human proximal tubule cells[58] through the AT_1 receptor. In AT_4 knockout (-/-) mice, Ang Ⅳ mediated its renal vasoconstrictor effects through AT_{1a} receptors[59].

Ang Ⅱ can also be hydrolyzed to dipeptides that are biological active, and we have found an alternative pathway for Ang-(1-7) formation from Ang Ⅱ by carboxypeptidase N, and posterior generation of Ang-(3-4) with Ang-(1-5) and Ang-(1-4) as intermediate peptides[20] (Figure 1), using isolated basolateral membranes from sheep proximal tubules and different peptidase inhibitors. Ang-(3-4) could counteract inhibition of plasma membrane Ca^{2+}-ATPase promoted by nanomolar concentrations of Ang Ⅱ through conformational changes in the AT_2 receptor and the cAMP/PKA pathway[19,20,37].

Ang (3-4) is remarkably stable in human blood serum and has antihypertensive effects in spontaneously hypertensive rats (SHR)[60,61]. Dias *et al*[62] showed that oral administration of Ang-(3-4) inhibited Na^+-ATPase activity in membranes of SHR and blocked the stimulation of Na^+-ATPase induced by Ang Ⅱ in normotensive rats *via* the AT_2 receptor and the PKA signaling pathway. This effect leads to increased urinary Na^+ concentration, and simultaneous decrease in systolic arterial blood pressure in SHR, but not in normotensive rats[62].

The presence of another angiotensin derived fragment, known as Ang A (Ala-Arg-Val-Tyr-Ile-His-Pro-Phe), occurs in the plasma of healthy humans and in high levels in end-stage patients with renal failure[21,63]. Decarboxylation of Asp^1 of Ang Ⅱ, in the presence of mononuclear leukocytes leads to Ang A generation, which has higher affinity for AT_2 than Ang Ⅱ and the same affinity for the AT_1 receptor[21,63]. As the other Ang Ⅱ-derived peptides, Ang A exerts its effects on the kidney, inducing renal vasoconstrictor responses in normotensive and hypertensive rats, and also in genetically modified mice[64]. Ang A can also be hydrolyzed by ECA2 in rats, mice and humans generating the heptapeptide alamandine (Ala-Arg-Val-Tyr-Ile-His-Pro), a novel peptide of the RAS[22]. Alamandine has long-term antihypertensive effect in SHRs and antifibrotic effects in isoproterenol-treated rats *via* the Mas-related G-protein-coupled receptor, member D (MrgD), and independent of Mas and AT_2 receptor, the known vasodilator receptors of the RAS, since it is blocked by $D\text{-}Pro^7$-angiotensin-(1-7) and PD123319, but not by the Mas antagonist A-779[22]. Most members of Mas-related

G-protein-coupled receptor (Mrg), a novel class of RAS-related receptor, are orphan, with no identified endogenous ligand, but MrgD has been identified as a binding site for alamandine[22].

NEW MEMBERS OF RAS: RECEPTORS

Classically, there are 2 well described Ang Ⅱ receptors, AT_1 and AT_2 receptors. However, newer work on RAS and its effects shows that there are novel members of this system.

Besides the newly described Ang Ⅱ-derived peptides and their corresponding receptors, there are enzyme members of RAS whose actions depend on interaction with receptors. Nguyen *et al*[27] in 2002 cloned the PRR, which contains a specific binding site for renin and its inactive precursor, prorenin; this interaction stimulates their catalytic activity, increasing RAS activation. Prorenin has a "handle" region that binds to the receptor with a 3-4 fold higher affinity than renin and is important in enzymatic activation of prorenin[65].

After binding, renin and prorenin can also act as agonists to its receptor, generating effects in an Ang Ⅱ-independent manner. In the human kidney, PRR is expressed in glomerular mesangial cells, the subendothelium of renal arteries[27], in the distal nephron[66], collecting ducts, and mostly at the apical surface of intercalated cells, where, due to its high expression it stimulates cyclooxygenase-2 (COX-2)-derived prostaglandins to attenuate the anti-natriuretic and vasopressor effects of Ang Ⅱ[67].

However, activation of PRR in kidney is also associated with many pathological conditions. Activation of human PRR and MAPK through an Ang Ⅱ-independent mechanism contributes to the development of nephropathy in prorenin/renin transgenic rats overexpressing the human receptor[68]. PRR is important through the same signaling pathway in diabetic nephropathy by its activation of glomerular ERK. These studies used an AT_{1a} receptor-deficient mice[69] and db/db mice to show that the receptor-bound prorenin leads to the development of nephropathy in type 2 diabetes[70]. In HEK cells, renin and prorenin activate its receptor to promote fibrosis in an Ang II-independent manner[71].

Kohlstedt *et al*[25] in 2004 revealed another unexpected function of the RAS enzymes. Human ACE, usually known by its catalytic action on Ang I in generating Ang Ⅱ, could also function as an outside-in signal transduction molecule. Binding of ACE substrates or inhibitors to this enzyme can stimulate intracellular signaling pathways: ACE inhibitors (perindoprilat and ramiprilat), like the ACE substrate (bradykinin), could also increase COX-2 expression, ACE phosphorylation at Ser1270 and activation of JNK in endothelial cells[25]. The modulation of gene expression in endothelial cell by ACE inhibitors and JNK/c-Jun pathway requires ACE dimerization through the C domain of the enzyme[26]. This indicates that, although ACE is not a cell surface receptor, it is involved in cell functions. Nevertheless, whether ACE works only as a catalytic enzyme or as a signaling molecule in the kidney remains to be elucidated.

BREAKING PARADIGMS

A newly recognized view of RAS assumes that Ang Ⅱ acts beyond cell surface receptors, with endocrine and paracrine action of RAS. Ang Ⅱ also acts through intracellular receptors. Local RAS was first described within the kidney over 20 years ago[29-32], where the levels of Ang Ⅱ are much higher than in plasma[32,72]. Intrarenal Ang Ⅱ levels and local formation in the kidney have been reported by Navar and colleagues[11,32,73-76].

In addition to Ang Ⅱ synthesis in the kidney, there are other well-described mechanisms that play a critical role in high renal Ang Ⅱ levels, and these occur after Ang II endocytosis with the AT_1 receptor[77,78]. Since AT_1 receptors are expressed in different parts of the kidney, such as in the mesangial cells, afferent and efferent arterioles, glomerular podocytes, macula densa and both basolateral and luminal membranes of different nephron segments[79,80], intracellular Ang Ⅱ accumulation by coupled-receptor internalization is one of main sources of renal Ang Ⅱ accumulation.

In Ang Ⅱ-dependent hypertension several groups have shown that Ang Ⅱ can positively amplify it, leading to its high intrarenal levels. Zhuo *et al*[77] showed increased intracellular Ang Ⅱ levels in cortical endosomes, and Ang Ⅱ-infused hypertensive rats mediated by AT_1 receptors. Ang Ⅱ-infused rats through an osmotic minipump also had increased Ang Ⅱ levels in renal interstitial fluid, which is mediated by the AT_1 receptor[81]. Ang Ⅱ endocytosis with AT_1 receptor has been confirmed by the absence of renal Ang Ⅱ accumulation in AT_{1a} receptor-deficient mice ($Agtr1a^{-/-}$)[82,83]. Another possible pathway for increasing the intrarenal Ang Ⅱ level is due to endogenous Ang Ⅱ production, *via* markedly augmentation on angiotensinogen[11,84] and renin expression in collecting ducts[85,86], the secretion of renin and prorenin by these cells into the luminal fluid, leading to its increased urinary levels in Ang Ⅱ-infused hypertensive rats[87]. These results indicating a positive feedback by Ang Ⅱ in the kidney contradict the well-established view that Ang Ⅱ has a negative feedback mechanism in the expression and activity in the RAS, where high Ang Ⅱ levels suppress the release of renin in juxtaglomerular cells and Ang Ⅱ production in the kidney[88], demonstrating the complexity of the system.

Both Ang Ⅱ receptors (AT_1 and AT_2) are expressed in adult kidneys, although AT_2 receptor is less expressed than AT_1 receptor[79]. This intensely local synthesis of high renal levels of Ang Ⅱ, and the wide expression of Ang Ⅱ receptors within the kidney, provides evidence of the pivotal role of Ang Ⅱ in renal physiology, regulating water and solute reabsorption and renal hemodynamic processes that contribute to Na^+ balance and blood pressure regulation. AT_1 receptors in the kidney are responsible for the development of hypertension[89-91]. And AT_1 receptors within the kidney are necessary for cardiac

hypertrophy and hypertension[90,92].

Ang Ⅱ has many effects on different parts of the kidney. As in the systemic circulation, intrarenal Ang Ⅱ also is important in renal hemodynamics. Thereby, long-term treatment with Ang Ⅱ receptor blockers induced unusual proliferative changes in afferent arteriolar smooth muscle cells, narrowing arteriolar lumens and reducing glomerular pressure[93]. Administration of Ang Ⅱ through an osmotic minipump in hypertensive rats leads to marked suppression of Na^+ excretion as well as renal and medullary blood flow[94]. Peritubular capillary Ang Ⅱ infusion enhanced proximal tubular reabsorption and reduced single nephron glomerular filtration rate in rats[95].

Different targets and signaling pathways regulate Na^+ balance within the kidney; rats infused with Ang Ⅱ showed enhanced ENaC expression[96] and activation of the renal Na^+:Cl^- cotransporter[97,98]. *In vitro* studies using isolated basolateral membrane fractions from pig kidney have demonstrated that Ang II stimulates the renal proximal tubule Na^+-ATPase activity *via* PI-PLCβ/PKC pathway[99,100].

It is widely known that intracellular Ca^{2+} mobilization in proximal tubule cells leads to the activation of many Ca^{2+}-dependent intracellular signaling pathways, including those associated with Na^+ reabsorption[101]. Ang Ⅱ microperfusion techniques in rabbit superficial segment of proximal tubules *in vitro* regulated Na^+ reabsorption *via* PKC and intracellular Ca^{2+}[102]; low concentrations of Ang Ⅱ inhibited membrane Ca^{2+}-ATPase *via* AT_1/AT_2 receptors heterodimers and PKC in isolated fractions of basolateral membranes of proximal tubule, increasing cytosolic Ca^{2+} concentration in proximal tubule cells[37,103]. Luminal Ang Ⅱ stimulates AT_1/AT_2 receptors heterodimerization that increases sarco/endoplasmic reticulum Ca^{2+}-ATPase activity and promotes Ca^{2+} mobilization in proximal tubule cells[101].

The intracrine/intracellular system is new paradigm. Cells that express all the necessary components for synthesis can generate Ang Ⅱ internally[28,29]. Ang Ⅱ can be secreted and exert autocrine effects, or remain inside the cell and have its effects[6,35]. An alternative way for the intracellular source of Ang Ⅱ is the internalization of extracellular Ang Ⅱ after binding to the AT_1 surface receptor[82,83]. Not all internalized Ang Ⅱ-AT_1 complex is degraded in lysosomes, thereby increasing its concentration within the cell, and the AT_1 receptor may be relocated to other organelles, including the nucleus[101,104-108]. Indeed, subcellular localization of ^{125}I-labeled Ang Ⅱ in the pig kidney indicates that Ang Ⅱ generation is predominantly extracellular, followed by AT_1 receptor-mediated endocytosis leading to higher intracellular Ang Ⅱ levels[109]. In accord with this, internalization is seen to be important for AT_{1a} receptor function in polarized proximal tubule epithelial cells, where apical AT_{1a} receptor internalize before interaction with G proteins, which stimulates phospholipase C and cAMP to increase proximal tubule Na^+ reabsorption[110,111].

Within the kidney, cells from different segments can generate Ang Ⅱ or internalize Ang Ⅱ through the AT_1 receptor[109-111]. *In vitro* and *in vivo* studies showed that extracellular Ang Ⅱ accumulates within the kidney *via* AT_{1a} receptor-mediated endocytosis[82,83,107]. Although many have demonstrated different Ang Ⅱ intracellular effects, the precise role of intracellular Ang Ⅱ in nephron segments remains poorly understood. Renal intracellular Ang Ⅱ increases blood pressure and decreases 24 h urinary Na^+ excretion in rats and mices[89,105], suggesting that, like intrarenal Ang Ⅱ, intracellular Ang Ⅱ within the kidney also increases Na^+ reabsorption and blood pressure.

Endocytosis of Ang Ⅱ through the AT_1 receptor within proximal tubule cells occurs through 2 main pathways: the clathrin-dependent and the microtubule-associated pathway[106]. The canonical clathrin-dependent endocytosis pathway for Ang Ⅱ occurs in different cell types, such as vascular smooth muscle and human embryonic kidney (HEK-293) cells through the AT_1 receptor, c-Src and clathrin Adapter Protein 2[112]. In rabbit proximal tubule cells, the alternative microtubule-associated endocytic pathway rather than the clathrin-dependent pathway participates in the AT_1 receptor-mediated uptake of Ang Ⅱ[113].

Another alternative endocytic pathway for Ang Ⅱ internalization in proximal tubule cells has been described by Gonzalez-Villalobos *et al*[114], where anti-megalin antisera interferes with Ang Ⅱ binding in cell brush-border membrane vesicles extracted from mice, indicating that Ang Ⅱ internalization is a megalin-dependent process.

Angiotensin receptors are present in the intracellular organelles, including the sarco/endoplasmic reticulum, Golgi apparatus and the nucleus, indicating that Ang Ⅱ can have many intracellular effects, including modulation of gene expression[33-35]. Proximal tubule cells express angiotensinogen, renin, and ACE mRNAs, suggesting high levels of intracellular Ang Ⅱ[28,32,73]. Thus, microinjection of Ang Ⅱ directly in single rabbit proximal tubule cells increased intracellular Ca^{2+} mobilization through its intracellular AT_1 receptors and Ca^{2+} release from intracellular stores[115]. Ang Ⅱ induced transcriptional responses of mRNAs for MCP-1, NHE-3 and TGF-β1 stimulating the AT_{1a} receptor in freshly isolated intact rat renal cortical nuclei, indicating that internalized and/or intracellular Ang Ⅱ acts on nuclear receptors to mediate growth, proinflammatory responses and Na^+-retaining effects[108]. Furthermore, in isolated nuclei from kidney cortex from sheep in the absence of cytoplasm, all RAS components (angiotensinogen, ACE and renin) have been identified[116], showing that Ang Ⅱ can indeed be synthesized within the nucleus.

Another interesting role for intracellular Ang Ⅱ is encountered in pathological situations. It is thought that intracellular Ang Ⅱ levels could be altered in different diseases, such as diabetic nephropathy and cardiomyopathy, where hyperglycemia might induce intracellular Ang Ⅱ production. Indeed, a high glucose concentration induced an increase of ACE mRNA, synthesis and secretion of renin and Ang Ⅱ in an immortalized murine mesangial cell line[117-119]. Interestingly, an alternative pathway

Table 2 Most common drugs already established for clinical use and emerging drugs and new targets for the treatment of hypertension, cardiovascular and renal diseases

Target	Drug	Therapy	Clinical use
Renin	Aliskiren	HTN, RF	+
	Remikiren, enalkiren	HTN	+
ACE	Captopril, lisinopril, trandolapril	HTN, HF, LVD, DN	+
	Enalapril, enalaprilat, fosinopril, ramipril	HTN, HF	+
	Moexipril, quinapril, perindopril, benazepril	HTN	+
AT_1	Losartan, azilsartan, valsartan, ibesartan, candesartan, telmisartan, eprosartan, omesartan	HTN, HF	+
Mas	AVE 0991	HTN	-
	Ang-(1-7)-CyD	HF	-
ACE2	Xanthenone	HTN, RF, HF	-

+: Already used in clinic; –: Not used in clinic yet. ACE: Angiotensin converting enzyme; ACE2:Angiotensin converting enzyme 2; AT1: Angiotensintype 1 receptor; Mas: Ang-(1-7) Mas receptor; RF: Renal failure; HF: Heart failure; HTN: Hypertension; LVD: Left ventricular dysfunction; DN: Diabetic nephropathy.

was found for the synthesis of intracellular Ang Ⅱ in the presence of high glucose in vascular smooth muscle cells. Under normal glucose levels, Ang Ⅱ is generated by cathepsin D and ACE; however, Ang Ⅱ is obtained by cathepsin D and chymase action in the presence of high glucose[120,121].

NEW TARGETS FOR HYPERTENSION TREATMENT

RAS is important in the development of hypertension and cardiovascular diseases[1-4,90]; one of the most common treatments for these diseases is pharmacological inhibition of enzymes and blockade receptors of RAS[122]. Inhibition of renin, the enzyme that initiates RAS, presents a strategy for hypertension therapy (Table 2). Aliskiren is a more selective and potent inhibitor of human renin than other orally active renin inhibitors, remikiren and enalkiren[123]; it can block the generation of active renin in both normotensive and hypertensive human subjects[124]. Aliskiren is as effective as losartan, valsartan and ibesartan (AT_1 receptor blockers), atenolol (β blocker) and amlodipine (Ca^{2+} channel blocker), and has an antihypertensive effect comparable to other major classes of antihypertensive drugs[124,125]. Besides decreasing blood pressure, aliskiren is also renoprotective in diabetic and nondiabetic models of chronic kidney disease, preventing albuminuria in rats[126]. In humans, aliskiren significantly decreases blood pressure, and also the urinary albumin and creatinine ratio in 15 patients with type 2 diabetes mellitus[127].

ACE is another enzyme of the RAS that can be pharmacology inhibited so as to decrease hypertension (Table 2). A total of 17 small orally active ACE inhibitors have recently been synthesized for clinical use, all binding to the active site of the enzyme and interfering with ACE's ability to bind and cleave its substrates (Ang I and bradykinin, among others)[128,129]. Many ACE inhibitors were approved for hypertension treatment, heart failure and left ventricular dysfunction (*e.g.*, captopril, lisinopril, trandolapril), as also captopril for diabetic nephropathy[129].

Ang Ⅱ promotes cardiovascular disorders and hypertension *via* the AT_1 receptor, which can be blocked to treat these pathological conditions (Table 2). A total of 8 non-peptide angiotensin-receptor blockers (ARBs) orally active are used clinically for hypertension and cardiovascular diseases (namely losartan, azilsartan, valsartan, ibesartan, candesartan, telmisartan, eprosartan, omesartan), which are all well-tolerated[129,130]. Telmisartan seems more efficacious in decreasing blood pressure than the other ARBs[131,132].

Many patients with hypertension require combination regimens to achieve a significant decrease in blood pressure. In this case, the most commonly used drugs are ARBs and ACE inhibitors, Ca^{2+} channel blockers (CCB) and diuretics[130]. Long-term treatment triple therapy with olmesartan medoxomil (ARB), amlodipine besylate (CCB) and hydrochlorothiazide (diuretic) in 2112 hypertensive patients with moderate to severe hypertension resulted in 44.5%-79.8% of participants having a decreased the mean blood pressure from 168.6/100.7 mm Hg to 125.0-136.8/77.8-82.5 mmHg, reaching the blood pressure goal[133]. The same triple therapy also proved to be efficient in hypertensive Hispanic/Latin patients[134].

However, even with the successful results obtained by inhibiting the enzymes and receptors of the RAS, many patients do not respond as expected, and cardiovascular disease risks have not decreased to those in normotensive people. Due to the high death rates by heart diseases in the world, which are higher than from many cancers[135], it is important to devise new strategies for the treatment of cardiovascular diseases and hypertension.

Because of the discovery of new components in the RAS that have herein been described, novel Ang Ⅱ-derived peptides have emerged as excellent target for heart diseases. Since the ACE2/Ang-(1-7)/Mas axis has an opposite and protective effect from the deleterious ACE/Ang Ⅱ/AT_1 axis, it is now the main target for these drugs[14]. Besides inhibiting ACE activity and blocking AT_1 receptors responsible for the inhibition of ACE/Ang Ⅱ/AT_1 axis, activation of the ACE2/Ang-(1-7)/Mas axis is a promising alternative means for the treatment of the heart diseases. Nevertheless, this new strategy presents certain problems. First, as a peptide, Ang-(1-7) is proteolytically degraded in the gastrointestinal tract[18]; and second, Ang-(1-7) has a short half-life, complicating its use as an oral pharmacotherapy for hypertension and cardiovascular disease.

The difficulty was overcome after the synthesis of the first nonpeptide compound able to mimic Ang-(1-7) and bind selectively to the Mas receptor[136], the AVE 0991 5-formyl-4-methoxy-2-phenyl-1-{[4-(2-ethyl-ami-

nocarbonylsulfonamido-5-isobutyl-3-thienyl)-phenyl]-methyl}-imidazole (Table 2)[137]. Although this molecule is an antihypertensive candidate because it stimulates NO release in endothelial cells[137], promotes vasorelaxation in mouse and rat aortic rings[138], and attenuates hypertension in SHR[139], clinical trials are needed to see its effects in humans.

Another important achievement has been the inclusion of the heptapeptide in hydroxypropyl-β-cyclodextrin [Ang-(1-7)-CyD], avoiding its proteolytic degradation in the gastrointestinal tract and permitting its oral administration (Table 2)[18]. Cyclodextrins are amphiphilic oligosaccharides that increase drug stability and absorption[140]; after oral administration, they are split up into small saccharides in the colon, leaving Ang-(1-7) to be absorbed[18]. Chronic oral administration of Ang-(1-7)-CyD in isoproterenol-treated rats increases plasma Ang-(1-7) levels, with attenuation of myocardial infarction associated with cardioprotective effects[141].

Another option for the treatment of the deleterious effects of Ang Ⅱ is activation of ACE2, which, besides increasing Ang Ⅱ degradation, enhances Ang-(1-7) production; ACE2 activators are an alternative source for controlling hypertension (Table 2). Acute intravenous administration of xanthenone (XNT), which interact with ACE2 in specific sites, promotes conformational changes and increases ACE2 activity. Consequently, it decreases blood pressure, improves cardiac function and decreases renal fibrosis in SHR[142]. It also has antihypertensive effects in rats with pulmonary hypertension[143].

These results together suggest that, besides inhibition of renin and ACE, associated or not with the blocking of AT_1 receptor, activation of the ACE2/Ang-(1-7)/Mas axis and its protective effects is emerging as an excellent alternative therapy for the treatment of hypertension and cardiovascular diseases.

CONCLUSION

The data presented herein show that RAS has passed from being simply an endocrine system to one with paracrine, autocrine and intracrine functions, increasing Ang Ⅱ concentration in different tissues including the kidney. After years of research, the RAS - previously seen as a simple system with only 2 receptors (AT_1 and AT_2), and one active peptide (Ang Ⅱ), turns out to be a complex system, with many new members continuing to be described. In addition to (ACE)/Ang Ⅱ/AT_1 and AT_2 axis, other signaling pathways in the RAS, such as ACE2/angiotensin-(1-7)/Mas and Ang IV/AT_4, and other active peptide of the RAS, with physiological relevance as Ang Ⅲ, Ang-(3-4), Ang A and alamandine, are now widely recognized. These newly discovered fragments derived from Ang Ⅱ can act on the same classic Ang Ⅱ receptors, AT_1 and AT_2, or on specific receptors (Mas and AT_4) having the same or the opposite effects of Ang Ⅱ depending on the triggered signaling pathway, in the kidney and other tissues, with many roles seen in physiological and physiopathological conditions. The discovery of renin and prorenin as agonists of PRR receptor, stimulating intracellular pathways and having effects on different cells types in an Ang Ⅱ-independent manner, raised another axis for this system, namely the prorenin/PRR/MAPK ERK1/2 axis.

Finally, activation of the new ACE2/Ang-(1-7)/Mas axis with opposite and protective effects, compared with ACE/Ang Ⅱ/AT_1 axis, with different drugs such as AVE 0991, the nonpeptide compound mimicking Ang-(1-7) effects, the Ang-(1-7)-CyD, and the XNT, the activator of ACE2 activity, now leading to improved and greater fall in blood pressure creates new possibilities for patients who do not respond as expected to conventional antihypertensive drugs.

A thorough understanding of RAS and all the new possibilities described on this review will certainly contribute to the development of pharmacological approaches, discovery of new drugs and alternative treatments for hypertension, cardiovascular and kidney diseases.

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MINIREVIEWS

Therapeutic target for nephrotic syndrome: Identification of novel slit diaphragm associated molecules

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Abstract

The slit diaphragm bridging the neighboring foot processes functions as a final barrier of glomerular capillary wall for preventing the leak of plasma proteins into primary urine. It is now accepted that the dysfunction of the sit diaphragm contributes to the development of proteinuria in several glomerular diseases. Nephrin, a gene product of *NPHS1*, a gene for a congenital nephrotic syndrome of Finnish type, constitutes an extracellular domain of the slit diaphragm. Podocin was identified as a gene product of *NPHS2*, a gene for a familial steroid-resistant nephrotic syndrome of French. Podocin binds the cytoplasmic domain of nephrin. After then, CD2 associated protein, NEPH1 and transient receptor potential-6 were also found as crucial molecules of the slit diaphragm. In order to explore other novel molecules contributing to the development of proteinuria, we performed a subtraction hybridization assay with a normal rat glomerular RNA and a glomerular RNA of rats with a puromycin aminonucleoside nephropathy, a mimic of a human minimal change type nephrotic syndrome. Then we have found that synaptic vesicle protein 2B, ephrin-B1 and neurexin were already downregulated at the early stage of puromycin aminonucleoside nephropathy, and that these molecules were localized close to nephrin. It is conceivable that these molecules are the slit diaphragm associated molecules, which participate in the regulation of the barrier function. These molecules could be targets to establish a novel therapy for nephrotic syndrome.

Key words: Podocyte; Slit diaphragm; Synaptic vesicle protein 2B; Ephrin-B1; Neurexin

Core tip: The slit diaphragm located between neighboring foot processes of a glomerular podocyte functions as a final barrier to retain plasma proteins. Recently several molecules such as nephrin and podocin were identified as functional molecules of the slit diaphragm. However, the precise molecular compositions of the slit diaphragm are still unclear and the mechanism regulating its barrier function is not fully understood yet. Recently we have reported that synaptic vesicle protein 2B, ephrin-B1 and neurexin are expressed in podocyte and the decreased function of these molecules participates in the initiation of proteinuria. These molecules could be targets for a novel therapy for proteinuria.

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INTRODUCTION

A glomerular capillary wall preventing the leak of plasma proteins is consisted of three layers: an endothelial cell, a glomerular basement membrane, and a glomerular epithelial cell (podocyte). Podocyte is characterized as its

highly sophisticated shape with the primary processes and the interdigitating secondary processes. The secondary process is called as a foot process[1]. The interdigitating foot processes are bridged by a structure called slit diaphragm. In 1988, Orikasa *et al*[2] of our group reported that the murine monoclonal antibody recognizing the extra-cellular site of the slit diaphragm caused massive proteinuria if injected into rats. The finding clearly indicated that the slit diaphragm is one of the essential structures of the barrier of the glomerular capillary wall[2]. In 1998, Kestilä *et al*[3] found the responsible gene for the Finnish type congenital nephrotic syndrome, and reported that its gene product, they called nephrin, was an extracellular component of the slit diaphragm[3,4]. Podocin was identified by Bout *et al*[5] as a protein coded by the responsible gene for familial steroid-resistant nephrotic syndrome in 2000. Following nephrin and podocin, CD2 associated protein (CD2AP), NEPH1 and canonical transient receptor potential-6 (TRPC6) were identified as crucial molecules of the slit diaphragm[6-10]. Several studies showed that the functional loss of these molecules participated in the initiation of proteinuria in acquired glomerular diseases[11-18]. It is now accepted that the slit diaphragm is a final barrier of glomerular capillary wall preventing proteinuria[19-23]. To explore the targets for the novel therapy of proteinuria, the subtraction hybridization assay was done with a normal rat glomerular cDNA and cDNA of rats showing proteinuria. We identified some molecules downregulated at proteinuric states. In this article, first we review the characteristics of the critical slit diaphragm molecules previously reported, and then we introduce the novel slit diaphragm-associated molecules, synaptic vesicle protein 2 (SV2) B, ephrin-B1 and neurexin.

CRITICAL MOLECULES CONSTITUTING THE SLIT DIAPHRAGM

Zonula occludens-1

Zonula occludens-1 (ZO-1) was originally identified as a molecule of the tight junction[24]. ZO-1 belongs to the membrane-associated guanylate kinase homologue (MAGUKs)[25]. It is reported that ZO-1 was expressed at the slit diaphragm in podocyte[26]. ZO-1 is the first protein reported to constitute the slit diaphragm. Splicing variants, ZO-1 α^+ and ZO-1 α^- which lacks motif α have been reported[27]. Both are expressed in tight junctions of the tubular epithelial cells, but only ZO-1α^- is expressed at the slit diaphragm[28].

Nephrin

Nephrin is a product of gene mutated in Finish type congenital nephrotic syndrome[3]. Nephrin is now accepted as the most important component of the slit diaphragm. Nephrin is a transmembrane protein of 1241 amino acid residues of the immunoglobulin super family. Nephrin contains eight Ig-like modules and a single fibronectin type Ⅲ module. The nephrin homologues of mouse[29] and rat[12,30] were cloned. Rat nephrin has 82.2% homology to human nephrin. We have shown the anti-slit diaphragm antibody previously reported, which cause proteinuria if injected into rat, binds the extracellular site of rat nephrin[12,31,32], indicating that nephrin is an essential slit diaphragm molecule.

Podocin

Podocin was found as a protein coded by *NPHS2*, the responsible gene of autosomal recessive steroid-resistant nephrotic syndrome. Podocin is reported to bind nephrin and is accepted to be a slit diaphragm molecule[5]. Podocin is a 42 kDa protein with a single transmembrane domain. Because immunoelectron microscopic study demonstrated that both N- and C- termini were in cytoplasm, podocin is considered to have a hairpin-like structure[33]. Podocin homologues of rat and mouse were cloned. Identity between rat and mouse, mouse and human, rat and human, are 92.7%, 86%, 84.3%, respectively[14]. It is demonstrated that podocin interacts with nephrin and CD2AP[34]. It is reported that podocin is a raft-associated component of the slit diaphragm and to serve a scaffolding function.

CD2AP

CD2AP is understood to be one of critical molecules of the slit diaphragm[6]. CD2AP was originally reported to be an adaptor protein binding the cytoplasmic domain of CD2, a membrane protein on natural killer cell and T cell[35]. CD2AP is an 80 kDa protein containing an actin-binding site at the N terminus. CD2AP bound nephrin and anchored nephrin to the cytoskeleton[6]. It is reported that mice lacking CD2AP exhibit loss of foot process, a nephrotic range proteinuria and advance renal failure. It is reported that a mutation of the gene for CD2AP were detected in two human patients with focal segmental glomerulosclerosis (FSGS)[36].

NEPH1

NEPH1 is a nephrin associated protein identified by a gene trap method[7]. NEPH1 has five extracellular immunoglobulin-like domains[7]. NEPH1 interacts with C-terminal domain of podocin[8], ZO-1 and nephrin[37]. The foot processes effacement and proteinuria were detected in NEPH1 knockout mice. All NEPH1 knockout mice died before 8 week of age[7], indicating that NEPH1 is an essential molecule in podocyte.

TRPC6

In 2005, it was reported that the mutation in TRPC6 channel can cause familial FSGS[9,10]. TRPC6 belongs to the transient receptor potential superfamily of non-selective cation channels. TRPC6 is understood to be a receptor-operated channel leading to the influx of calcium in response to phospholipase C-mediated signals[38]. Immunoelectron microscopy study showed TRPC6 localized at major processes, foot processes and at the slit diaphragm[9,10,18,39]. TRPC6 is reported to be colocalized with nephrin, podocin, and CD2AP. In addition, nephrin

and podocin are co-immunoprecipitated with TRPC6 in cultured podocyte[10]. Winn *et al*[9] reported that the mutation of TRPC6, proline-to-glutamine substitution at position 112 (P112Q) which is detected in patients of familiar FSGS leads to both increased amplitude and duration of calcium influx in the over expression system. Reiser *et al*[10] showed that R895C and E897K, other TRPC mutants detected in the patients, displayed increased current amplitude in the system with HEK293 cells. It is understood that the FSGS-associated mutations could lead to be a gain-of-function alteration in activity and thus increased calcium influx. Recently, Eckel *et al*[40] showed that albuminuria caused by continuous injection of angiotensin Ⅱ was significantly less in TRPC-deficient mice than in wild type mice and discussed that TRPC6 promotes albuminuria by promoting angiotensin II-dependent increase in calcium. It is now accepted that TRPC6 channel activity at the slit diaphragm is essential for proper regulation of podocyte structure and function.

THE MAJOR SLIT DIAPHRAGM MOLECULES ARE DOWNREGULATED IN NEPHROTIC STATES

It was reported that the expression of nephrin decreased in patients of minimal change nephrotic syndrome (MCNS)[13,41]. Our group has investigated the expression of nephrin in rat puromycin aminonucleoside (PAN)-induced nephropathy[12]. PAN nephropathy is widely used as the model of MCNS. Our group showed mRNA expression for nephrin declined already at 1h after PAN injection into rats. We also observed that the immunofluorescence staining of nephrin changed to a discontinuous pattern from a continuous pattern along glomerular capillary wall on day 10 of PAN nephropathy when proteinuria peaked. The downregulation of nephrin in PAN nephropathy was reported by another group[11]. We reported that podocin is colocalized with nephrin in normal rats, whereas the podocin staining is apart from nephrin in rats with PAN nephropathy[14]. These observation showed that the molecular structure of the slit diaphragm was rearranged in PAN nephropathy, and that these molecular rearrangement led proteinuria in this model. It is conceivable that these alterations of the slit diaphragm participate in the development of MCNS. It is reported that nephrin staining changed to a discontinuous pattern in patients with membranous nephropathy[15]. It is reported that such an alteration in the staining of nephrin was detected in rats of passive Heymann nephritis model, mimic of human membranous nephropathy[42]. We observed that CD2AP and podocin were already apart from nephrin at the early phase of passive Heymann nephritis before the onset of proteinuria[43]. These reports showed that the altered localization of the slit diaphragm molecules is involved in the development of proteinuria also in membranous nephropathy. We reported that the expressions of nephrin, podocin and NEPH1 altered in rats of adriamycin (ADR)-induced nephropathy, which is accepted as a mimic of FSGS[44]. The rats with ADR nephropathy showed severe and continuous proteinuria. The dissociation of NEPH1 from nephrin was observed at the early phase of ADR nephropathy, when the dissociation of podocin from nephrin is not observed. It is postulated that the NEPH1- nephrin dissociation initiates proteinuria in this disease. In contrast to other slit diaphragm molecules, it was discussed that TRPC6 expression in podocytes is up-regulated in several diseases[18].

EXPLORING THE NOVEL FUNCTIONAL MOLECULES REGULATING THE BARRIER FUNCTION OF THE SLIT DIAPHRAGM

Although some critical molecules maintaining the barrier function of the slit diaphragm were identified, the precise molecular compositions of the slit diaphragm and the mechanism regulating its barrier function were not fully understood yet. To further analyze the molecular compositions of the slit diaphragm and to identify the target molecule for the therapy for nephrotic syndrome, we tried to purify the novel molecules and a subtraction assay with cDNA from normal rat glomeruli and nephrotic syndrome rat glomeruli was done[45]. It is plausible that, nephrin, podocin, CD2AP and NEPH1 were downregulated at the proteinuric states. Therefore, it is conceivable that the molecule whose expression decreased at the proteinuric state might be a molecule relating the development of proteinuria. We investigated the localization and the role of the molecules identified by the subtraction assay in podocyte. In addition, those of their associated molecules were also investigated. We focused on synaptic vesicle protein 2B[45], ephrin-B1[46] and neurexin[47] in these molecules. It is considered that these molecules are essential molecules of the slit diaphragm (Figure 1), and that they are the candidates for the target of novel therapy for nephrotic syndrome. The nature of these molecules is described below.

SV2B

SV2 has three isoforms, SV2A, SV2B and SV2C. Our group found SV2B was downregulated in PAN nephropathy. The expression of mRNA of SV2B was already reduced when abnormal proteinuria was not detected yet. It is considered that the observation showed the decrease of the SV2B expression is not a mere consequence of proteinuria but has an etiological significance causing proteinuria. The decrease of the expression of SV2B was also observed from the early phase in anti-nephrin antibody-induced nephropathy. The observation indicated that SV2B is the slit diaphragm-associated molecule.

SV2 is known to regulate calcium-mediated synaptic transmission. It is understood that SV2 plays an essential role in vesicle trafficking[48-50]. SV2 was understood to be exclusively expressed in the neuronal tissue. However,

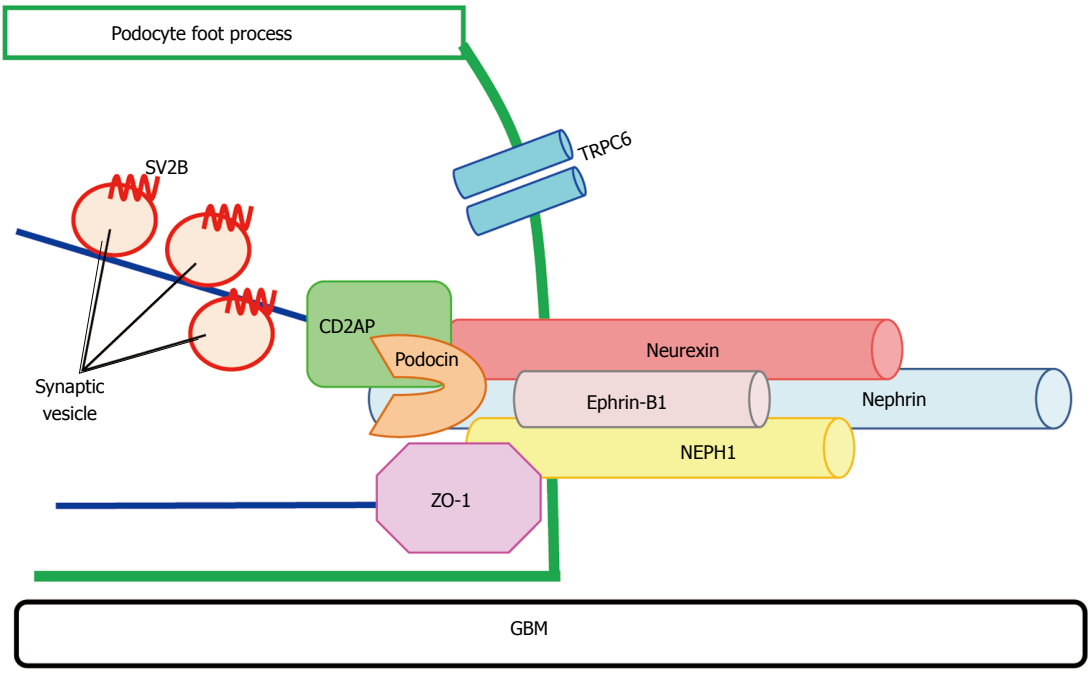

Figure 1 Molecules constituting the slit diaphragm. Nephrin and NEPH1 are the transmembrane proteins constituting the extracellular site of the slit diaphragm. Nephrin interacts with CD2 associated protein (CD2AP), podocin and NEPH1. NEPH1 interacts with ZO-1. Canonical transient receptor potential-6 (TRPC6) is located at the slit diaphragm area. SV2B is expressed at the slit diaphragm area and contributes to the proper localization of CD2AP. Ephrin-B1 and neurexin are transmembrane proteins of the slit diaphragm. GBM: glomerular basement membrane; ZO-1: Zonula occludens-1.

some reports have shown that SV2s are expressed in several other tissues[51,52]. SV2B is detected in the microvesicles of pinealocytes. Both pinealocyte and podocyte are characterized by their specialized processes[52]. These properties also suggested that SV2B plays a role in the trafficking to the terminal of processes.

To analyze the role of SV2B in podocyte, RNA silencing analysis was performed using cultured podocytes, and the expression of CD2AP, one of critical molecules of the slit diaphragm[45] was analyzed. CD2AP was detected at the tip of the process in control cells, whereas the CD2AP staining was detected mainly at the cytoplasm in the cells treated with siRNA for SV2B. From these observations, we concluded that SV2B contributed to the maintaining of the normal molecular structure of the slit diaphragm. It is postulated that the dysfunction of SV2B is involved in the development of proteinuria via the redistribution of proteins of the slit diaphragm such as CD2AP in PAN nephropathy and in other proteinuric states. Not only SV2B but also Rab3A, another synaptic vesicle molecule and rabphilin-3a, an effecter of Rab3A were expressed in the podocyte, and played an important role in maintaining the podocyte function[53]. More elucidation of the role of the synaptic vesicle like vesicle expressing SV2B and Rab3A in podocyte is awaited.

Ephrin-B1

The molecules belonging to the Eph-ephrin family were identified by the subtraction assay. Both Ephs and ephrins are transmembrane proteins and they function as ligand-receptor pairs[54-56]. Eph-ephrin family have many biological functions such as the cell migration and axon guidance[57-59]. It is also reported that the Eph-ephrin-B family play a role in the regulation of the permeability between epithelial cells[57]. These characteristics of Eph-ephrin-B prompted us to analyze a role of Eph-ephrin-B in podocyte. Ephrin-B1, ephrin-B2, EphB1 and EphB2 mRNA expressions were detected in normal rat glomeruli and in murine cultured podocytes. Our group observed that the mRNA expression and immunofluorescence findings of ephrin-B1 were found to be decreased at 24 h of the nephropathy caused by the anti-nephrin antibody injection, whereas EphB1 or ephrin-B2 was not altered. Ephrin-B1, an original name Lerk-2, is a membrane-anchored protein[54,55]. Ephrin-B1 contains a cytoplasmic tail, a single transmembrane domain and an extracellular domain[55,56]. An immunoelectron microscopic study showed that ephrin-B1 was detected at the slit diaphragm[46]. Interaction of ephrin-B1 with nephrin was observed by the immuno-precipitation assay with glomerular lysate. It is conceivable that these observations showed ephrin-B1 is a slit-diaphragm-associated protein. The expression of ephrin-B1 was decreased already at the early phase of the anti-nephrin antibody-induced nephropathy when the alteration of nephrin staining is not remarkable yet. The podocyte injury in this model is caused by the binding of antibody to nephrin. The observation that the expression of ephrin-B1 altered more rapidly than nephrin in this model is very interesting, and we believe that the findings suggested that ephrin-B1 is highly associated with nephrin.

To investigate the function of ephrin-B1 in podocyte, a knockdown system with siRNA was done in cultured podocyte. CD2AP was detected at the tip end of the pro-

Table 1 Summary of the critical slit diaphragm and the novel slit diaphragm associated molecules

Ref.	Molecules	Predicted molecule weight	Functions in the slit diaphragm
Schnabe *et al*[26]	ZO-1	225 kDa	Interact with NEPH1
Kestilä *et al*[3]	Nephrin	180 kDa	Maintaining the barrier function
Schwarz *et al*[34]	Podocin	42 kDa	A raft-associated component and interact with nephrin and CD2AP
Shih *et al*[6]	CD2AP	80 kDa	Interact with nephrin and anchor nephrin to the cytoskeleton
Liu *et al*[37]	NEPH1	110 kDa	Interact with ZO-1 and nephrin
Winn *et al*[9], Reiser *et al*[10]	TRPC6	About 110 kDa	Interact with nephrin and podocin
Miyauchi *et al*[45]	SV2B	80 kDa	The proper arrangement of CD2AP
Hashimoto *et al*[46]	Ephrin-B1	50 kDa	Maintaining the slit diaphragm structure
Saito *et al*[47]	Neurexin	150 kDa	Regulating the slit diaphragm function

ZO-1: Zonula occludens-1; CD2AP: CD2 associated protein; TRPC6: Transient receptor potential-6.

cesses in control cells, whereas the staining of CD2AP was detected in cytoplasmic area around the nuclei. The finding suggested that ephrin-B1 plays a role for the trafficking of CD2AP to the tip of process. All of these results suggest that ephrin-B1 is also a component of the slit diaphragm complex. Recently, Wnuk *et al*[60] reported that Eph-B4 and ephrin-Bs were expressed in podocyte and that the expressions were altered in glomerulonephritis model. These findings suggested that members of the Eph-ephrin-B family could be targets for a novel therapy for proteinuria.

Neurexin

After the discovery of SV2B as a critical molecule of the slit diaphragm, we analyzed the expression of several synaptic vesicle associated molecules in podocyte, and found that neurexin is expressed in rat and human podocyte[47]. Neurexin was originally identified as a cell surface receptor for α-latrotoxin, a component of black widow spider venom, and was considered to play a critical role in cell-cell interaction in across the synapse[61-65]. Neurexin has an interaction with synaptotagmin, a synaptic vesicle associated molecule, and is postulated to play a role in synaptic vesicle docking[66,67]. It is also reported that neurexin binds calcium/calmodulin-dependent serine protein kinase (CASK), a member of the MAGUK family[68]. It is reported that CASK interacts with nephrin[69]. CASK is also accepted to be an essential molecule of the slit diaphragm. Our group demonstrated that neurexin was restrictedly expressed in the glomeruli of the kidney[47]. Dual-labeling immunofluorescence studies showed that neurexin located close to CD2AP. It was also detected that some portions of the neurexin staining are coincident with the staining of Rab3A, a synaptic vesicle molecule. We observed that the staining intensity of neurexin in the glomeruli was clearly reduced, and their staining pattern shifted to a discontinuous patchy pattern in PAN nephropathy and in anti-nephrin antibody induced nephropathy. The alteration in the staining of neurexin in these models was detected more clearly and rapidly than that in the nephrin staining. These observations suggest that neurexin is one of the essential molecules regulating the slit diaphragm function. It is postulated that neurexin is a candidate of the targets for a novel therapy for nephrotic syndrome.

CONCLUSION

It is now widely understood that the dysfunction of the slit diaphragm participates in the initiation of proteinuria in several kinds of glomerular diseases. We summarized the nature of the major slit diaphragm molecules in Table 1. We reviewed the novel slit diaphragm associated molecules SV2B, ephrin-B1 and neurexin. Because proteinuria is an independent risk factor for the vascular episode in brain, heart and other organs[70], the novel more effective therapy for proteinuria should be established. It is reported that dysregulation of the synaptic vesicle function is involved in several neuronal diseases, and the drug targeting synaptic vesicle is used for the treatment for epilepsy. SV2B and other synaptic vesicle associated proteins could be novel therapeutic targets for nephrotic syndrome.

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ORIGINAL ARTICLE

Kinin B_2 receptor does not exert renoprotective effects on mice with glycerol-induced rhabdomyolysis

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Abstract

AIM: To investigate a potential protective role of the kinin B_2 receptor in a glycerol-induced rhabdomyolysis mouse model.

METHODS: We separated 28 C57Bl/6 male mice into 4 groups: untreated WT animals, untreated B_2 knockout mice, glycerol-treated WT and glycerol-treated B_2 knockout mice. Glycerol-treated animals received one intramuscular injections of glycerol solution (50% v/v, 7 mL/kg). After 48 h, urine and blood samples were collected to measure creatinine and urea levels. Additionally, kidney samples were extracted for histological evaluation, and the mRNA expression levels of kinin B1 and B2 receptors and inflammatory mediators were measured by real-time polymerase chain reaction.

RESULTS: Serum creatinine and urea levels showed differences between untreated wild-type and glycerol-treated wild-type mice (0.66 ± 0.04 *vs* 2.61 ± 0.53 mg/dL, $P < 0.01$; and 33.51 ± 2.08 *vs* 330.2 ± 77.7 mg/dL, $P < 0.005$), and between untreated B_2 knockout mice and glycerol-treated knockout mice (0.56 ± 0.03 *vs* 2.23 ± 0.87 mg/dL, $P < 0.05$; and 42.49 ± 3.2 *vs* 327.2 ± 58.4 mg/dL, $P < 0.01$), but there was no difference between the glycerol-treated wild-type and glycerol-treated knockout mice. Glycerol was able to induce a striking increase in kinin B_2 receptor expression (> 30 times, 31.34 ± 8.9) in kidney. Animals injected with glycerol had a higher degree of tubular injury than untreated animals. Wild-type and knockout mice treated with glycerol intramuscularly present kidney injury, with impairment in renal function. However, B_2 knockout mice treated with glycerol did not show a different phenotype regarding kidney injury markers, when compared to the wild-type glycerol-treated group.

CONCLUSION: We conclude that the kinin B_2 receptor

does not have a protective role in renal injury.

Key words: Kinins; acute kidney injury; Animal models; Rhabdomyolysis; Skeletal muscle

Core tip: In this work we are showing that glycerol-treated animals experienced impairment in renal function. Furthermore, we worked with kinin B_2 receptor knockout mice and our results suggest that kinin B_2 receptor does not exert renoprotective effects in this rhabdomyolysis model. In addition, we are presenting results of kidney expressions and we investigated several candidates that can participate in the kidney injury induced by glycerol.

Gattai PP, Mafra FFP, Wasinski F, Almeida SS, Cenedeze MA, Malheiros DMAC, Bacurau RFP, Barros CC, Câmara NOS, Araujo RC. Kinin B_2 receptor does not exert renoprotective effects on mice with glycerol-induced rhabdomyolysis. *World J Nephrol* 2014; 3(3): 85-91 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/85.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.85

INTRODUCTION

Acute kidney injury (AKI) is a common life-threatening disease that places a heavy burden on the health system[1]. It was reported that the mortality rate for this disease ranges from 10% to 80%. Other studies suggest that AKI may be a step in the progression toward chronic kidney disease[2], in humans and animals[3].

The main factors that predispose patients to AKI include hemodynamic instability, hypovolemia, hypoxia, ischemia and reperfusion (I/R), and burns, among others[4]. There are several models used to study this disease; one of them is the glycerol-induced rhabdomyolysis model in which AKI occurs after muscle injury. In this nephrotoxic experimental model, the waste products from metabolism, such as enzymes and other molecules (*e.g.*, uric acid and myoglobin), cause kidney injury to the proximal and distal tubules[4]. Such injuries cause an abrupt (in a matter of hours or days) decline in renal function.

In renal inflammatory conditions such as AKI, the kallikrein-kinin system (KKS) plays an important role in glucose homeostasis[5,6]. This system is also involved in kidney inflammatory and vasodilation processes[7], which are directly involved in the inflammatory response mechanism of AKI. The AKI inflammatory response involves induction of local vascular ischemia, hypoxia and tubular injury[4]. KKS exerts its actions by activating two receptors: B_1 (B_1R) and B_2 (B_2R)[8]. The activation of B_1R is inducible and occurs under pathological conditions such as in ischemia[9], while B_2R is constitutively active under normal physiological circumstances[8]. Furthermore, some authors[10] have shown that there are fewer B_2R in rat nephrons affected by renal disease. It is well established that kinins are rapidly generated after tissue injury and that they have a central role in the development and maintenance of inflammatory processes, whether they are acute or chronic[1].

There is a renal KKS[11] that can respond in a more specific way to AKI. Another system that could be involved in the mechanism of AKI, as observed in the glycerol-induced rhabdomyolysis model, is the renin-angiotensin system (RAS). A drop in blood pressure, typically observed during a hypovolemic state, will activate this system and induce renal perfusion[12]. It is important to note that, the renal KKS can activate RAS[13]. In a recent study from our laboratory[14], we showed that there is a relationship between the genetic polymorphisms of ACE and B_2R, where the former can modulate the kinins in transplanted kidney patients. In other animal models of renal injury, it has been shown that B_2 has a possible protective role[15], while others have shown that it has a deleterious role[16].

It is described in the literature that the B_2R are in all portions of the nephron, except in podocytes of human kidney[17]. Moreover, it is described that the NO levels fall in rhabdomyolysis[4], and that the B_2R activation is related with NO production[18]. Thus, the purpose of this study is to investigate the role of B_2R in the kidney in an animal model of glycerol-induced rhabdomyolysis.

MATERIALS AND METHODS

Experimental design

This study began only with approval of the ethics committee of the Federal University of Sao Paulo (UNIFESP, nº 0300/11), and experiments were performed in accordance with the guidelines established by the Brazilian College for Animal Experimentation. The animals were provided by the Center for Development of Experimental Medicine and Biology (CEDEME) at UNIFESP. Twenty-eight male 3-month-old C57Bl/6 mice were used. Animals were randomly divided into 4 groups: WT (n = 5), glycerol WT (GWT, n = 9), B_2KO (n = 6), and glycerol B_2KO (GB$_2$KO, n = 8). All animals were placed in individual cages (Alesco, Brazil) in an environment with controlled temperature (21 ℃), a light/dark cycle of 12 h for one week and water and standard chow *ad libitum*.

The WT and B_2KO groups were control groups and received no treatment. The animals in the treated groups were deprived of water for 18 h to better evidence of AKI. Animals of both treatment groups (GWT and GB$_2$KO) were then slightly sedated with an intraperitoneal injection of ketamine-xylazine solution (150 μL, Vetnil, Brazil; at 10 μL/g, Vetbrands Brazil, Brazil), and received an i.m. injection of glycerol solution (50% v/v, Merk, Brazil, 7 mL/kg), with half a dose in each gastrocnemius muscle. Then, animals were placed back in their cages in a heated environment until recovery.

The urine samples were placed in 2 mL tubes and stored at -20 ℃ until analysis. Then, 48 h after the injec-

tions, the animals were anesthetized with *i.p.* ketamine-xylazine solution (300 μL at 10 μL/g), and blood samples were collected by intracardiac puncture into non-heparinized 1.5 mL tubes. These samples were incubated for approximately 20 min at room temperature and were then centrifuged at 4 ℃ at 4000 rpm for 10 min. The serum was collected and stored in a 1.5 mL tube at -20 ℃ until analysis. After blood sample collection, the animals were sacrificed by cervical dislocation, and each kidney was removed, weighed and transversally cut. One piece was placed in one 2 mL tube and immediately immersed in liquid nitrogen and then stored at -80 ℃ until analysis, while the other half was placed in a 2 mL tube with 10% buffered formaldehyde solution for 24 h, then placed into a new tube with a 70% ethanol solution and stored until sectioning.

Renal function analysis

For renal function analysis, we measured serum creatinine (S_{Cr}) levels and urine creatinine levels (U_{Cr}) according to the method described by Jaffé with slight modifications[19]. Briefly, we deproteinized the samples by adding 100 μL of each serum sample to a 1.5 mL tube containing 200 μL of 1.84 % H_2SO_4 and 300 μL of sodium tungstate and thoroughly mixed the resulting solution. The tubes were maintained at room temperature for 15 min and then centrifuged for 10 min at 4000 rpm at room temperature. The supernatant was then collected and placed in another 1.5 mL tube. Then, 5.28 mL of picric acid was mixed with 1.32 mL of a 10% NaOH solution in a 14 mL falcon tube. Next, 100 μL of picric acid (Labtest Diagnostica SA, Brazil) was added to 63 wells of a 96-well flat-bottomed ELISA microplate (Cral plast, Brazil), followed by pipetting and mixing of 200 μL of Milliq H_2O (Millipore, United States) in triplicate for blank samples, 200 μL of standard protein at 5 mg/mL in triplicate, and 200 μL of each deproteinized sample in duplicate. The plate was incubated for 20 min and then read in an EPOCH spectrophotometer (Biotek Instruments Inc., United States) at 450 nm. To measure U_{Cr} levels, we used the same method, but we did not deproteinize the samples. Instead, we diluted the samples (1:125) and multiplied the results by 25. The blood urea nitrogen (BUN) level were measured by colorimetric reaction kit (Labtest diagnostica, Brazil) in spectrophotometer at 600 nm, following the manufacturer's instructions.

Urine collection

Urine was collected by direct punction in bladder, for measure of creatinine and urea levels. The urine was collected in 1.5 mL tubes.

We also quantified the total urine protein (mg/dL) by colorimetric assay at 660 nm using the Pierce Protein Assay Kit (Thermo Scientific, United States).

Gene expression quantification

Total RNA was extracted using the TRIzol Reagent method (Invitrogen, United States) from tissues and stored at -80 ℃. The samples were run on 1% agarose gels to evaluate the integrity of the samples, and the samples were then quantified using a nanoDrop (NanoDrop Technologies, Inc., United States). Single-stranded cDNA was synthesized for each sample, using MML-V reverse transcriptase (Promega, United States). The qRT-PCR was carried out using Taqman (Applied Byosystem, United States) probes for GAPDH, B_1R and B_2R gene expression, and SYBR Green Real-time polymerase chain reaction (PCR) (Applied Byosystem, United States) probes for β-actin (sense - 5' CTG GCC TCA CTG TCC ACC TT 3' , antisense - 5' CGG ACT CAT CGT ACT CCT GCT T 3'), TGF-β1 (sense - 5' TTA GGA AGG ACC TGG GTT GG 3' , antisense - 5' AAG TTG GCA TGG TAG CCC TT 3') and IL-1β (sense - 5' AGG AGA ACC AAG CAA CGA CA 3' , antisense - 5' CGT TTT TCC ATC TTC TTC TTT 3') gene expression. The plates were placed in a 7500 real-time PCR system (Applied Biosystems, United States) for reading. The fold change was calculated by taking the $2^{-\Delta CT}$ of each sample and dividing it by the wild type (WT) group average.

Histological analysis

Slices 5 μm thick were cut from the samples stored in 70% ethanol solution and stained with HE. The samples were analyzed using an optical microscope at 200 × magnification. The criteria used to analyze the tubular injury in the samples included epithelium desquamation, cellular debris in the lumen, flattening of epithelium, the presence of cylinders and dilation of the lumen. Based on these criteria, an expert classified and scored the injuries: grade Ⅰ (< 10%), grade Ⅱ (10%-25%), grade Ⅲ (25-50%), and grade Ⅳ (> 50%).

Statistical analysis

The values are expressed as the mean ± SE. Statistical analyses were carried out by one-way ANOVA (analysis of variance) followed by Tukey's *post-hoc* test, and associations were made by the Spearman correlation test. P values < 0.05 were considered statistically significant. The statistical software used for graphs and analysis was GraphPad Prism 5.

RESULTS

Renal function evaluation

The S_{Cr} levels were different between the control wild-type and glycerol, and knockout control group and glycerol, with glycerol groups greater about 4 times than control groups (Figure 1A). There were no significant differences in the U_{cr} levels, despite the wild-type showed about 11 times higher levels than glycerol wild-type group (Figure 1B). The BUN levels were different about 10 times between the control wild-type and glycerol, and about 8 times between control knockout mice and glycerol (Figure 1C). Urinary urea content differed between the control and glycerol groups, about 24 times for wild-type groups and about 33 times for knockout groups

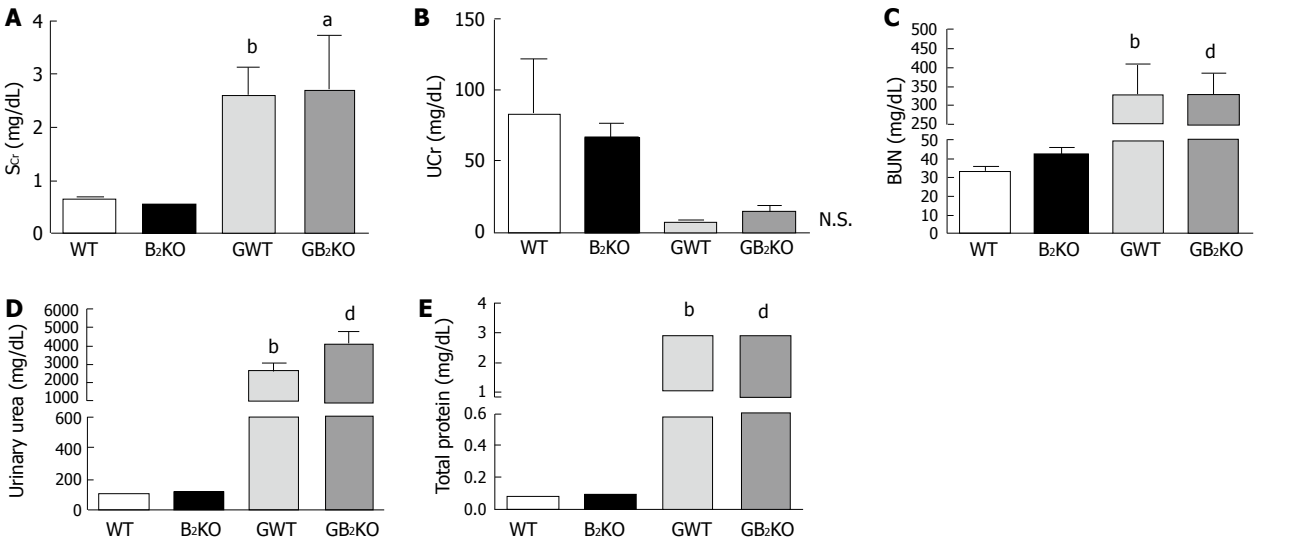

Figure 1 Serum and urinary parameters. A: S_{Cr} levels ($^aP < 0.05$ *vs* B_2KO, $^bP < 0.01$ *vs* WT); B: U_{Cr} levels; C: BUN levels ($^bP < 0.005$ *vs* WT, $^dP < 0.01$ *vs* B_2KO); D: Urinary urea ($^bP < 0.01$ *vs* WT, $^dP < 0.005$ *vs* B_2KO); E: Total urine proteins ($^bP < 0.005$ *vs* WT, $^dP < 0.005$ *vs* B_2KO). WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; B_2KO: Glycerol kinin B2 receptor knockout mice; BUN: Blood urea nitrogen.

Table 1 Serum and urinary creatinine

Variable	Groups			
	WT	B_2KO	GWT	GB_2KO
S_{Cr}	0.6621 ± 0.041	0.5584 ± 0.027	2.613 ± 0.536[b]	2.233 ± 0.867[a]
U_{Cr}	50.31 ± 29.24	32.80 ± 15.44	4.540 ± 2.137	9.084 ± 3.931

$^aP < 0.05$ *vs* B_2KO; $^bP < 0.01$ *vs* WT. B2KO: Kinin B2 receptor knockout mice; WT: Wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice.

(Figure 1D). The levels of total urine proteins were different between the wild-type and glycerol group (around 42 times), while the difference between knockout and glycerol group was approximately 36 times. There were no difference between wild-type glycerol and knockout glycerol group (Figure 1E).

Gene expression evaluation

The fold changes in B_1R, B_2R, β-actin, IL-1β and TGF-β1 renal expression are shown in Figure 2. Also shown are the associations between these expression levels. The fold change of the B_2R was different between the control and glycerol groups about 30 times (Figure 2A). The B_1R fold change was different between the control and glycerol groups about 14 times, and between glycerol groups almost 2 times (Figure 2B). The associations were not different between the B_1R and B_2R (Figure 2C), but showed difference between B_2R and IL-1β, where greater expression of B_2R results in lower expression of IL-1β (Figure 2D), and between B_1R and TGF-β1 groups, with greater expression of B_1R when the expression of TGF-β1 is lower (Figure 2E).

Histological evaluation and histomorphometry

The histological evaluation and histomorphometry shown in Figure 3 demonstrate that both sets of animals (WT and B_2KO) had a high degree of tubular injury when injected with glycerol (Figure 3F and H), but WT had a higher degree IV compared with B_2KO.

DISCUSSION

It is described that the NO levels fall in rhabdomyolysis[4], and that the B_2R activation is related with NO production[18] we considered investigate the role of kinin B2 receptor in glycerol-induced rhabdomyolysis model.

Our findings demonstrate that the renal expression of B_2R in animals that received glycerol is 30 times greater than that of the controls. Another study showed a 5-fold increase of B_2R expression[15]. These results suggest that B_2R may be involved in the kidney inflammatory process. Interestingly, the peak renal expression of B_2R coincided with a significant reduction in renal function, which could suggest that the renal upregulation of these receptors occurs in response to renal injury.

Renal function was evaluated by measuring serum creatinine and BUN levels. In mice, particularly male C57Bl/6 mice, the normal serum creatinine level (S_{Cr}) is approximately 0.8 ± 0.1 mg/dL[20], which is similar to the values found in the control groups (Table 1). In a recent study[21], the authors showed using the glycerol model in rats, that the peak of renal function impairment occurs between 48 and 72 h, as evidenced by S_{Cr} levels. In our study, we investigated the peak of injury.

Kidney injury in the glycerol model is due to the release of nephrotoxic molecules and proteins (*e.g.*, myoglobin) into the bloodstream, causing damage upon reaching the kidney[4]. The literature describes, using rat and cell culture models[22-27], the deleterious role of this protein, specifically the heme prosthetic group, in the tubular endothelium. This role is associated with the production of reactive oxygen species (ROS) and free radicals in the mitochondria, which initiate lipid peroxidation reactions.

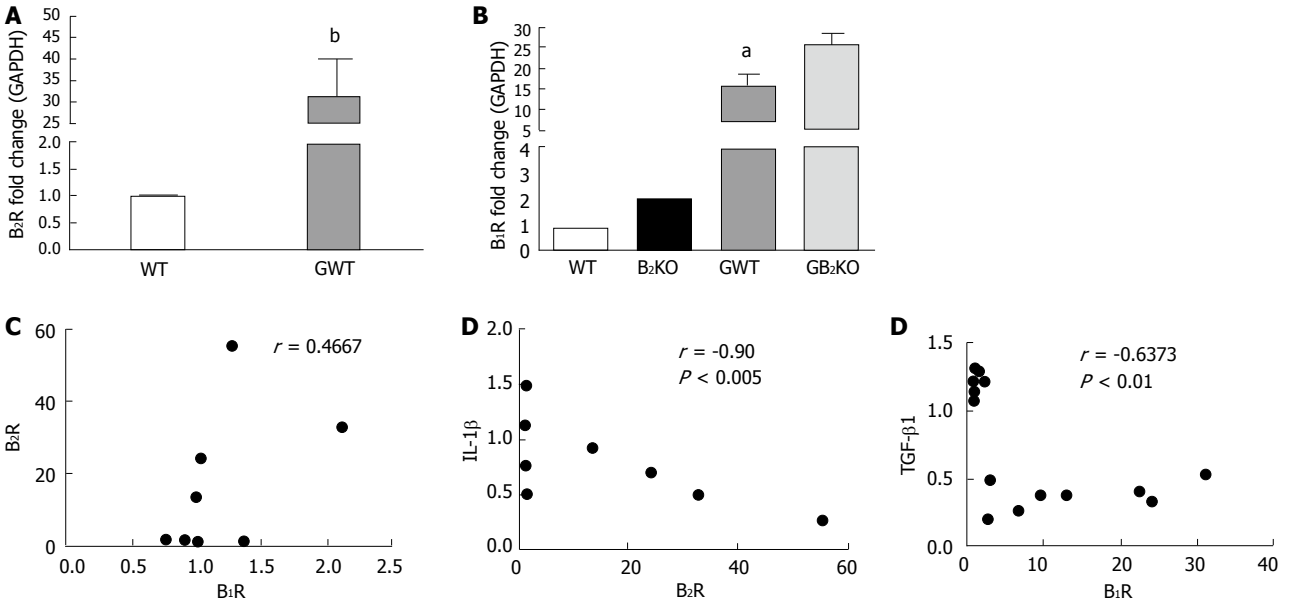

Figure 2 Renal gene expression and association between genes. A: B_2R fold change ($^bP < 0.01$ *vs* WT); B: B_1R fold change ($^aP < 0.05$ *vs* B_2KO); C: Association between B_2R and B_1R; D: Association between IL-1β and B_1R; E: Association between TGF-β1 and B_1R. WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice; BUN: Blood urea nitrogen.

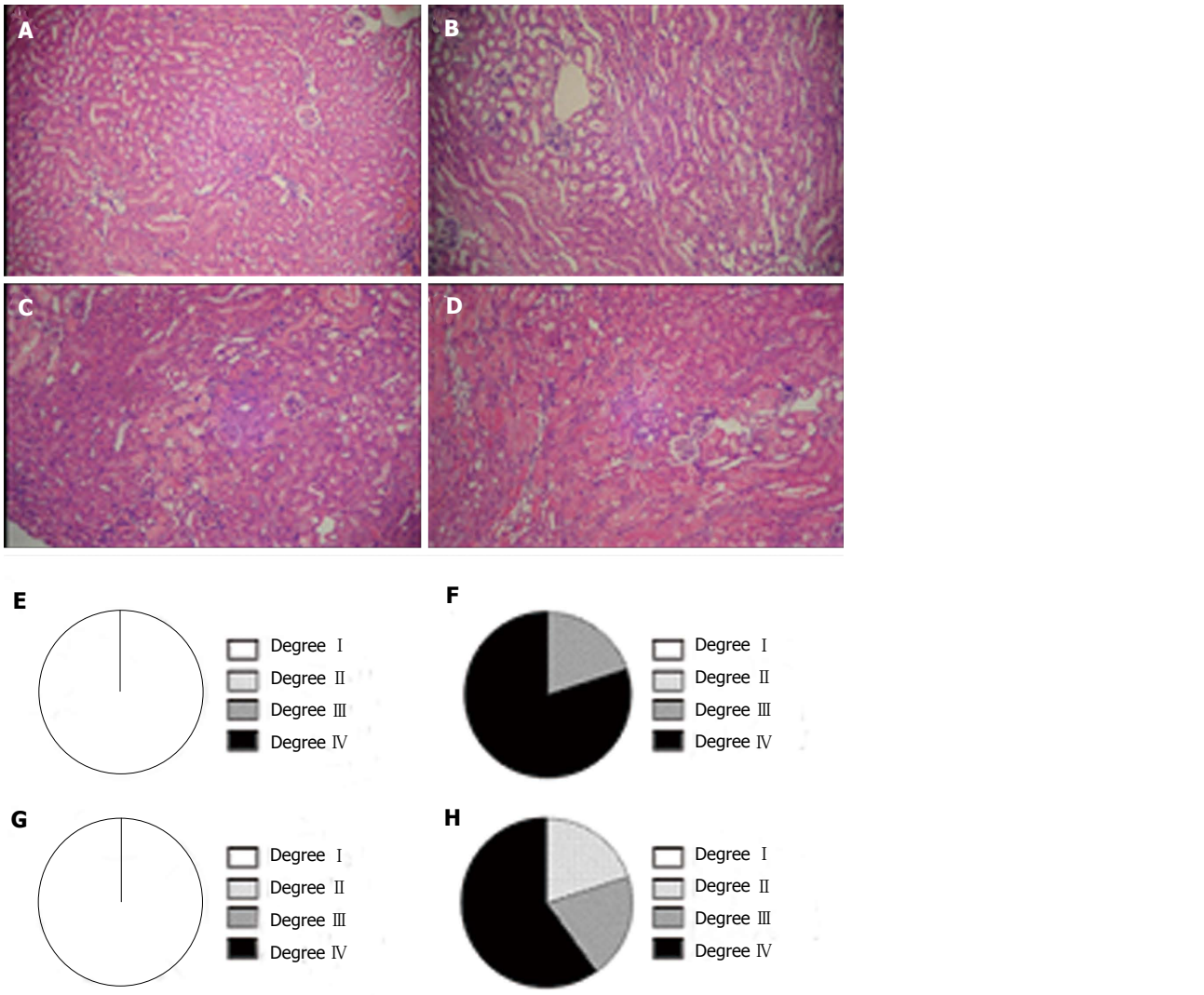

Figure 3 Histological evaluation and Graphs show the degrees of renal injury. A: Degree Ⅰ; B: Degree Ⅱ; C: Degree Ⅲ; D: Degree Ⅳ; E: WT; F: GWT; G: B_2KO; H: GB_2KO. WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice;

It has been shown that human skeletal muscle can survive for up to 3 or 4 h during circulatory ischemia[28]. In other studies[24], the authors showed that in the rat glycerol-induced rhabdomyolysis model, the renal mitochondria were already markedly degenerated 3 h after the glycerol application. Twenty-four hours after treatment, there were clear signs of tubular necrosis (proximal and distal). The authors attributed this to the formation of intra-tubular aggregates. This conclusion suggests that disruption of skeletal muscle can occur approximately 3 h after the glycerol administration.

Recent studies[28-31] show that deletion of B_1R and B_2R exacerbates the renal phenotype in diabetic mouse models, suggesting that both receptors have a protective effect on diabetic nephropathy by suppressing oxidative stress *via* NO and prostaglandins. However, because the absence of one of the receptors causes increased expression of the other, it is difficult to determine the precise function of each receptor[28]. In other models, such as the I/R model[15] the receptors' role was also studied. Blocking B_1R showed an antifibrotic effect, which therefore has a protective effect. Some authors[16] have demonstrated that renal injury by ischemia and reperfusion is significantly increased by B_2R activation and that this activation is related to increased production of ROS, suggesting that B_2R activation is deleterious.

Kinin receptors in renal tissue were studied in the I/R model, where it was shown that both receptors have a protective role in this type of injury[30]. However, a different study[15] found that during I/R in mice, the double knockout showed an extremely high S_{Cr} along with a proinflammatory profile. The renal B_2R expression alternated, beginning with low expression after 4 h, high expression after 24 h, and 48 h after the reperfusion onset, its expression level was similar to that observed at 4 h and then increased to values similar to those observed at 24 h post-reperfusion.

An important difference between the previous two studies[15,24] and ours is that in the other studies, ischemia and reperfusion were induced exclusively in the kidneys, whereas in our work, nephrotoxic kidney injury occurs after induction of primary skeletal muscle injury. Furthermore, in the model we use, there is no time for reperfusion to occur because the peak of injury would be 48 h later, allowing the kidneys to therefore remain in an ischemic state.

Regarding the release of proinflammatory cytokines (*e.g.*, IL-1β) and growth factors with a known profibrotic effect (TGF-β1), some authors[21] have shown that in the rat glycerol model, the mRNA expression levels of IL-1β peak 48 h after treatment, while in our work, the same expression levels were observed in GB_2KO animals, whereas GWT animals showed expression levels similar to those found 24 h post-treatment by these authors. In our study, we found a strong negative association between the expression of B_2R and IL-1β, which may indicate that B_2R does not induce inflammation in the kidneys.

Previous studies show[21] that renal TGF-β1 expression 48 h after *im* glycerol injection in mice is 1.5 times greater than our results indicate. It is important to note that the lineage of the animals used was different and that the endogenous gene studied was also different. Furthermore, in our work, we did not find any association between renal expression of B_2R and TGF-β1 (data not shown). Meanwhile, B_1R and TGF- β1 had a strong negative association.

Another important issue regarding analysis of TGF-β1 expression is the methodological difference between the studies. Although both studies use rodents, rats and mice have differences in several genes, including TGF-β1; the rat version is on chromosome 1, while the murine version is located on chromosome 7.

In conclusion, our results suggest that B_2R does not have a renoprotective role in mice with glycerol-induced rhabdomyolysis.

COMMENTS

Background

Acute kidney injury (AKI) is a common life-threatening disease that places a heavy burden on the health system and may be a step in the progression toward chronic kidney disease. It was reported that the mortality rate for this disease ranges from 10% to 80%, depending on the population studied. According to some authors, prevention is the key to avoiding the morbidity and mortality associated with AKI.

Research frontiers

Kinins is related with several models of inflammatory process in different organs as well in kidney. However, there is a lack of acknowledgments about kinins and rhabdomyolysis. The kinins are related with pathologies as well in physiology process. However, the absence of B2 receptor can induces the expression of the kinin B1 receptor and it difficult the determination of the precise function of each receptor. In this study, the authors demonstrated that the overexpression of kinin B_2 receptor could be involved with kidney injury once that the peak renal expression of kinin B_2 receptor coincided with a significant reduction in renal function.

Innovations and breakthroughs

This is the first study reporting that kinin B_2 receptor is over-expressed in kidney in rhabdomyolysis model. Furthermore, these results suggest that the overexpression may be one of the causes of kidney injury in this animal model.

Applications

Kinin B2 receptor antagonist could be tested in future to avoid kidney injury in rhabdomyolysis process.

Terminology

Rhabdomyolysis is a skeletal muscle disease where the muscle tends to break, that commonly result in acute kidney injury. Bradykinin is a nonapeptide messenger that is enzymatically produced from kallidin and that act via activation of two membrane receptors: kinin B_1 receptor and kinin B_2 receptor. Kinin B_1 receptor is a bradykinin receptor that is induced in response to inflammation, it may play a role in chronic inflammation. Kinin B_2 receptor is a bradykinin receptor that is constitutively expressed and may play a role in the acute phase of inflammation.

Peer review

Gattai *et al* reported that the kinin B_2 receptor knockout. The authors provided detailed data on this and the manuscript is well written.

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RETROSPECTIVE STUDY

Role of endoscopic ultrasound fine-needle aspiration evaluating adrenal gland enlargement or mass

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Abstract

AIM: To report the clinical impact of adrenal endoscopic ultrasound fine-needle aspiration (EUS-FNA) in the evaluation of patients with adrenal gland enlargement or mass.

METHODS: In a retrospective single-center case-series, patients undergoing EUS-FNA of either adrenal gland from 1997-2011 in our tertiary care center were included. Medical records were reviewed and results of EUS, cytology, adrenal size change on follow-up imaging ≥ 6 mo after EUS and any repeat EUS or surgery were abstracted. A lesion was considered benign if: (1) EUS-FNA cytology was benign and the lesion remained < 1 cm from its original size on follow-up computed tomography (CT), magnetic resonance imaging or repeat EUS ≥ 6 mo after EUS-FNA; or (2) subsequent adrenalectomy and surgical pathology was benign.

RESULTS: Ninety-four patients had left (n = 90) and/or right (n = 5) adrenal EUS-FNA without adverse events. EUS indications included: cancer staging or suspected recurrence (n = 31), pancreatic (n = 20), mediastinal (n = 10), adrenal (n = 7), lung (n = 7) mass or other indication (n = 19). Diagnoses after adrenal EUS-FNA included metastatic lung (n = 10), esophageal (n= 5), colon (n = 2), or other cancer (n = 8); benign primary adrenal mass or benign tissue (n = 60); or was non-diagnostic (n = 9). Available follow-up confirmed a benign lesion in 5/9 non-diagnostic aspirates and 32/60 benign aspirates. Four of the 60 benign aspirates were later confirmed as malignant by repeat biopsy, follow-up CT, or adrenalectomy. Adrenal EUS-FNA diagnosed metastatic cancer in 24, and ruled out metastasis in 10 patients. For the diagnosis of malignancy, EUS-FNA of either adrenal had sensitivity, specificity, positive predictive value and negative predictive value of 86%, 97%, 96% and 89%, respectively.

CONCLUSION: Adrenal gland EUS-FNA is safe, minimally invasive and a sensitive technique with significant impact in the management of adrenal gland mass or enlargement.

Key words: Adrenal gland neoplasms/diagnosis; Adrenal glands/pathology; Adrenal gland/ultrasonography; Adrenal gland neoplasms/secondary; Endosonography; Biopsy; Fine-needle

Core tip: Studies evaluating endoscopic ultrasound fine-needle aspiration (EUS-FNA) of the adrenal gland generally include patients with underlying malignancy only and most lack follow-up for benign lesions. We report the clinical utility of adrenal gland EUS-FNA in a retrospective study that included 94 patients who underwent EUS-FNA of either adrenal for various indications and provide follow-up information for those with benign EUS-FNA cytology results. For the diagnosis of malignancy, EUS-FNA of either adrenal had sensitivity, specificity, positive predictive value and negative predictive value of 86%, 97%, 96% and 89%, without

serious adverse events.

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INTRODUCTION

The development of modern imaging techniques such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), has led to increased detection of adrenal masses, which are found in up to 5% of patients undergoing CT of the abdomen[1]. The incidence of an adrenal incidentaloma (detection of an otherwise unsuspected adrenal mass on imaging), ranges from 0.2%-7% as reported in autopsy series[2]. Most of these incidentally found lesions are non-functioning adenomas, but 2% are metastatic lesions[3].

About 75% of adrenal masses identified during staging of patients with cancer are metastatic lesions which are most commonly metastases from lung, breast, stomach and kidney, as well as, melanomas and lymphomas[2]. The sensitivity and specificity of imaging techniques are currently insufficient to differentiate benign from malignant masses, therefore, patients with a high index of suspicion for malignancy are often referred for percutaneous biopsy[4].

Image-guided fine needle aspiration (FNA), using either ultrasound (US) or CT and percutaneous approach, have traditionally been used for sampling of the adrenal glands[5,6]. However, this technique yields non-diagnostic samples in up to 14% of patients and is associated with adverse events in 0.4%-12%[7,8].

Endoscopic ultrasound guided-fine needle aspiration (EUS-FNA) of either adrenal offers a minimally invasive and accurate method for sampling the adrenals with a low risk profile[3,9-12]. However, studies to date have mostly included patients with underlying malignancy and the great majority lack follow-up imaging for benign lesions or include follow-up for few patients[4,12,13]. This study reports the utility of EUS-FNA in patients with known adrenal gland enlargement or a mass, and the impact of the EUS-FNA cytology result on patient care, final diagnosis and adverse events from the procedure.

MATERIALS AND METHODS

This retrospective single-center case series was approved by the institutional Review Boards at the Indiana University Health School of Medicine in Indianapolis Indiana. Cytology and EUS databases between October 1997 and December 2011 were reviewed to identify all patients who underwent EUS-FNA of either adrenal gland. The original 38 patients were previously described in a 2007 publication from our hospital[3]. Medical records were reviewed and results of imaging (CT and MRI) prior to the procedure, EUS indications and findings, cytological investigations and complications were recorded. In addition, follow-up clinical information and any repeat adrenal imaging or surgery of the adrenal gland was abstracted. For patients without available follow-up on our medical records, referring physicians were contacted by phone to obtain this information. Through institutional protocol, all patients were called within 48 h after EUS to assess for any short-term adverse events not already identified. Adverse events were defined as: systolic blood pressure less than 80 mmHg at any time during the procedure, hypoxemia (oxygen saturation less than 85% on room air or on baseline oxygen supplementation), bradycardia (heart rate less than 50 beats per minute), bleeding recognized during EUS or subsequent imaging studies with hemoglobin drop of $\geqslant$ 2 g/dL from baseline, need for blood transfusion within 48 h of the procedure, pneumothorax, abdominal pain, hypertensive urgency and, requirement for hospitalization.

EUS

After obtaining written informed consent, patients received conscious sedation using various combinations of intravenous midazolam, meperidine, fentanyl or propofol under appropriate cardiorespiratory monitoring. All procedures were done by or under the supervision of one of seven attending endoscopists. Radial endosonography (Olympus GFUM-20, GFUM-130, GFUM-160 or GFUE160-AL5; Olympus America, Center Valley, PA; United States), was performed initially in some patients. Linear EUS (Olympus GF-UC30P, Olympus GF-UC140P, or Pentax 32-UA or 36-UX; Pentax Medical, Montvale, NJ; United States), was performed in all patients.

The left adrenal gland was visualized by one of 2 methods. First, the descending aorta was followed to the celiac axis; once this was seen, the left adrenal gland was visualized after a slight clockwise rotation and withdrawal movement. Alternatively, the splenic vein posterior to the body of the pancreas was identified by transgastric imaging; clockwise rotation and withdrawal of the echo endoscope following the splenic vein laterally then permitted the identification of the left adrenal gland superior to the upper pole of the left kidney. Transduodenal imaging of the right adrenal gland with EUS was performed with the echoendoscope in the long position along the greater curvature of the stomach. The inferior vena cava or the right kidney was then visualized, and then right adrenal gland was uniformly present between the superior pole of the right kidney, the liver and the inferior vena cava. EUS exams for patients in this study attempted to image a known or suspected adrenal mass or enlargement and did not routinely attempt to visualize both adrenal glands

The size of the adrenal gland for study purposes was the maximal cross-sectional diameter of the gland. An adrenal gland mass was considered to be a focal enlarge-

ment of the gland with a notable discrete mass, whereas, adrenal gland enlargement was considered when the gland was diffusively increased size without a visible discrete mass.

EUS-FNA was performed using a 19, 22 or 25 gauge, 8 cm needle (Cook-Medical, Winston-Salem, NC; United States or Boston Scientific, Natick, MA; United States). Minimal clotting parameters required to perform EUS-FNA were a platelet count of $\geqslant$ 50000 and INR $\leqslant$ 1.5. Color Doppler imaging was used to ensure the absence of intervening vascular structures along the anticipated needle path. After needle puncture of the adrenal gland, the stylet was removed. At the discretion of the endosonographer, suction was applied to the proximal end of the needle with a vacuum containing syringe. If excess blood was present in the initial specimen, subsequent passes with the same needle were attempted without suction. There was no maximum number of biopsy attempts allowed. Biopsy attempts were performed at the discretion of the endosonography until considered that useful clinical information was provided, or that further attempts would be futile. According to our routine endoscopy unit protocol, patients were monitored in the recovery area after EUS imaging for at least 60 min before discharge. No additional monitoring was performed after adrenal biopsy.

Cytological examination

Aspirates were expressed and smeared onto 2 glass slides. One slide was air-dried and stained with a modified Giemsa stain for on-site interpretation, while the other slide was alcohol-fixed and stained using the Papanicolaou method. A cytotechnologist and/or cytopathologist, not blinded to the patient's clinical history, were available on-site for real-time preliminary interpretation for all procedures; this added an additional 2-3 min to the procedure for each FNA pass. Additional aspirates were submitted for immunocytochemical analysis at the discretion of the cytopathologist to confirm metastatic malignancy when required.

Cytology reports were characterized as "diagnostic for malignancy", "benign adrenal tissue", or "non-diagnostic". The following were considered to be cytologic features of benign adrenocortical tissue: clusters of cells with a foamy cytoplasm and smoothly contoured, round to oval nuclei, all within a vacuolated or foamy background with occasional single cells[13]. Diagnostic cytology specimens were considered to include any of the following: benign-appearing cytologic features of the adrenal gland, primary adrenal neoplastic tissue, or metastatic malignant cells. Non-diagnostic cytology specimens had none of these three features but did show any of the following: amorphous debris, blood, or gastric contaminant.

Study definitions

The final diagnosis was made on the basis of the surgical pathology if resection was performed, unequivocal cytology from EUS-FNA, clinical follow-up, or the stability of lesion size as assessed by subsequent imaging studies. An adrenal lesion was considered stable (and therefore benign) if size was within 1 cm by follow-up imaging (CT or MRI) obtained at least 6 mo after EUS-FNA[14]. EUS-FNA of either adrenal gland was considered to have had an impact on patient care if the cytology resulted in either: (1) benign cytology which excluded adrenal metastasis and permitted resection of the primary tumor; or (2) initial diagnosis of malignancy, distant metastasis, tumor recurrence or primary adrenal neoplasm.

Statistical analysis

For analysis, continuous variables were described as means and standard deviations, and dichotomous variables were expressed as simple proportions, with or without 95%CI. Student's *t* test and Fisher's exact tests were used to test for differences in comparisons between continuous and dichotomous variables, respectively. For calculating test characteristics of EUS-FNA for the diagnosis of malignancy, only aspirates interpreted as diagnostic for malignancy on cytological examination were considered as true positives. Patients with subsequent adrenalectomy, percutaneous adrenal biopsies or follow-up abdominal imaging of the adrenal at least 6 mo after EUS were utilized to calculate the test characteristics of EUS-FNA for the diagnosis of non-malignant (benign or non-diagnostic) specimens. 95% confidence intervals were calculated when appropriate. A *P* value less than 0.05 was considered statistically significant.

RESULTS

94 consecutive patients (52% men; median age: 66 years, range 32-86) underwent 95 attempted EUS-FNA of the left (n = 90) and/or right (n = 5) adrenal gland during the study period. There were no adverse events related to these procedures. Patient characteristics and EUS findings by results of diagnostic and non-diagnostic biopsies are summarized in Table 1. Patients with diagnostic malignant biopsies had smaller lesions than those with diagnostic benign lesions (P = 0.027) otherwise the clinical and EUS features of the two groups were similar. Indications for EUS in all 94 patients are summarized in Table 2. Known adrenal gland enlargement, fullness or mass according to previous imaging was present in 55 (59%). A previous diagnosis of cancer was present in 40 patients (42%) (Table 3).

Prior attempt with percutaneous CT-guided approach for adrenal biopsy was performed and unsuccessful in 3 patients, two of them subsequently had a diagnostic adrenal EUS-FNA (1 malignant, 1 benign); the third patient had a non-diagnostic EUS-FNA of the adrenal gland.

EUS findings and cytology

The mean maximal diameters for the right and left adrenal masses were 3.5 $\pm$ 0.88 cm and 2.72 $\pm$ 1.36 cm, respectively. EUS identified an adrenal mass in the 5 (100%) patients who underwent right adrenal EUS-FNA and in 75/90 (83%) who underwent left EUS-FNA. The left 15 adrenals

Table 1 Patients characteristics and endoscopic ultrasound findings *n* (%)

Characteristics	Diagnostic (*n* = 85)		Non-diagnostic (*n* = 9)	*P* value
	Benign (*n* = 60)	Malignant (*n* = 25)		
Age (mean ± SD)	67 ± 11	63 ± 14		0.16[1]
	66 ± 12		66 ± 11	0.99[2]
Race				
White	57 (95)	25 (100)	7 (78)	
African American	3 (5)	0 (0)	2 (22)	
Hispanic	0 (0)	0 (0)	0 (0)	
Gender				⩾ 0.99[3]
Male	26 (27)	19 (20)	5 (5)	
Female	34 (36)	6 (7)	4 (5)	
Adrenal biopsied				⩾ 0.99[4]
Left	58 (61)	23 (24)	9 (10)	
Right	2 (2)	3 (3)	0 (0)	
EUS image of adrenal				
Mass	49 (52)	25 (26)	6 (6)	0.14[5]
Diffuse enlargement	11 (12)	1 (1)	3 (3)	0.09[6]
Size by EUS, cm				
Mean ± SD	3.4 ± 1.6	2.6 ± 1.2		0.027[7]
Mean ± SD	2.8 ± 1.4		2.4 ± 1.2	0.41[8]
Range	0.7-5.2	1.3-7.0	1.0-4.0	
Echogenicity				
Hypoechoic	40 (42)	22 (24)	4 (4)	
Hyperechoic	1 (1)	0 (0)	1 (1)	0.14[9]
Not reported or unavailable	19 (20)	4 (4)	4 (4)	
Number of FNA passes				
Mean ± SD	3.0 ± 1.7	3.0 ± 1.3	3.1 ± 1.5	0.461

[1]Mean age diagnostic vs non-diagnostic; [2]Diagnostic *vs* non-diagnostic cytology result based on gender; [3]Adrenal Gland FNA side and Diagnostic vs non-diagnostic cytology result; [4]Presence or absence of an adrenal mass and diagnostic *vs* non-diagnostic cytology result; [5]Presence of absence of an adrenal mass and benign *vs* malignant FNA cytology; [6]Median size by EUS (cm) and malignant *vs* benign FNA cytology; [7]Median size by EUS (cm) and Diagnostic *vs* non-diagnostic cytology; [8]Adrenal Echogenicity on EUS and Diagnostic *vs* non-diagnostic cytology; [9]Number of FNA passes; and Diagnostic *vs* non-diagnostic FNA; [10]Mean age benign vs malignant. EUS: Endoscopic ultrasound; FNA: Fine-needle aspiration.

without mass demonstrated only diffuse enlargement (one patient had bilateral adrenal EUS-FNA) (Table 1).

Nine aspirations were non-diagnostic (9.5%). Four of these, had a previous diagnosis of cancer and 6 had an identified adrenal mass during EUS with a mean mass diameter of 2.4 ± 1.2 cm. Non-diagnostic aspirations occurred mostly before 2004, however the frequency before and after 2004 was not different ($P = 0.14$), and this was considered to be related to operator's learning curve (Table 4).

Diagnostic cytology was obtained in 86 biopsies after a mean of 3.2 ± 1.4 needle passes. There was no statistical significance between the number of needle passes for diagnostic biopsies and non-diagnostic biopsies ($P = 0.98$). All nondiagnostic biopsies were from the left adrenal gland; all right adrenal biopsies were diagnostic. Ninety-one fine-needle aspirations were performed with a 22G needle and included all the specimens that yielded a non-diagnostic sample. Only 3 and 1 biopsies on these series were obtained with a 25 G and a 19 G needle, respectively.

Table 2 Indications for endoscopic ultrasound

Indication for EUS	*n* (%)
Cancer staging[1]	26 (27)
Suspected cancer recurrence[2]	5 (6)
Abnormal CT/PET-CT or MRI	
Pancreatic mass	20 (21)
Mediastinal mass	10 (11)
Lung mass	7 (7)
Adrenal mass	7(7)
Gastric mass	2 (2)
Liver mass	3 (3)
Kidney mass	1 (1)
Retroperitoneal mass	1 (1)
Other[3]	12 (13)
Total of patients	94

[1]Esophageal cancer (*n* = 3), gastric cancer (*n* = 2), breast (*n* = 1), jejunal adenocarcinoma (*n* = 1), renal cell cancer (*n* = 2), cholangiocarcinoma (*n* = 1), lung cancer (*n* = 16); [2]Suspected recurrence of oral cancer (*n* = 1), breast cancer (*n* = 1), hepatoma (*n* = 1), lung adenocarcinoma (*n* = 1), esophageal adenocarcinoma (*n* = 1); [3]Chronic pancreatitis (*n* = 3), abnormal upper endoscopy (*n* = 3), common bile duct stricture (*n* = 2), celiac nerve block (*n* = 1), suspected metastatic disease on imaging (*n* = 1), Barrett's esophagus with high grade dysplasia (*n* = 1), ectatic pancreatic duct (*n* = 1). EUS: Endoscopic ultrasound; PET: Positron emission tomography; CT: Computed tomography.

Table 3 Previous diagnosis of cancer in patients undergoing endoscopic ultrasound guided fine-needle aspiration

Previous diagnosis of cancer (*n* = 40)	Benign cytology on EUS-FNA (*n* = 21)	Malignant cytology on EUS-FNA (*n* = 15)	Non-diagnostic cytology on EUS-FNA (*n* = 4)
Penile cancer	0	1	0
Oral SCC	0	1	0
Lung cancer	15	3	1
Renal cell carcinoma	0	2	1
Esophageal ADC	1	3	0
Breast cancer	1	1	0
Gastric ADC	1	1	0
Hepatocellular carcinoma	0	1	0
Pulmonary carcinoid	0	0	1
Colon ADC	0	1	1
SCC of the duodenum	1	0	0
Basal cell cancer of the skin	1	0	0
Bladder cancer	1	0	0
Melanoma	0	1	0

EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma.

Adrenal gland FNA was malignant in 26% (*n* = 25) and benign in 64% (*n* = 60). Details about adrenal gland EUS-FNA cytology results are summarized in Table 5.

Clinical follow-up

Follow-up was available for 36/60 (60%) patients with benign adrenal cytology. The remaining 24 patients either were: lost to follow-up (*n* = 4), did not get repeat adrenal gland imaging (*n* = 5) or died (*n* = 15). The 15 patients died a mean of 28 ± 36 mo after EUS without follow imaging.

Table 4 Timing of diagnostic and non-diagnostic biopsies *n* (%)

Timing of EUS-FNA	Diagnostic EUS-FNA	Non diagnostic EUS-FNA	Total EUS-FNA
Before 01/2004	31 (33)	6 (7)	37
After 2004	54 (57)	3 (3)	57
Total	85 (90)	9 (10)	94

Non diagnostic EUS-FNA before 2004 vs after 2004 (P = NS). EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration.

Available follow-up for 5/9 (55%) patients with non-diagnostic biopsies, demonstrated a stable adrenal lesion on repeat CT or MRI; the remaining four died before follow-up imaging (Figure 1). Median follow-up for benign and non-diagnostic biopsies was 24 mo (range 4-96) and 12 mo (range 7-36), respectively.

In 36 patients with benign adrenal cytology, available follow-up from imaging in 28 showed a stable adrenal lesion on CT (n = 27) or repeat EUS (n = 1). Five additional patients underwent adrenalectomy and without repeat imaging in 4. In these five, surgical pathology was benign in 4 and demonstrated an adrenocortical carcinoma in 1 (Table 6). For the remaining three, 2 had subsequent CT-guided adrenal biopsy showing metastatic non-small cell lung cancer in one (4 mo after EUS) and large cell neuroendocrine tumor in another (EUS-FNA biopsy of the pancreas had previously showed neuroendocrine tumor). Finally, one patient had follow-up CT 6 mo after EUS that demonstrated a new contralateral adrenal mass with findings of metastatic disease to the adrenals (Table 6).

In one additional patient with history of melanoma, CT scan for surveillance revealed a left adrenal mass. EUS-FNA of the mass was malignant, however, adrenalectomy 1 mo later showed benign pathology.

Clinical impact of EUS-FNA

For the diagnosis of malignancy EUS-FNA of the adrenal gland had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 86% (95%CI: 68%-95%), 97% (95%CI: 83%-100%), 96% (95%CI: 79%-100%) and 89% (95%CI: 74%-96%), respectively. The sensitivity, specificity, positive predictive value and negative predictive value of adrenal gland EUS-FNA for benign lesions was 97% (95%CI: 83%-100%), 86% (95%CI: 68%-95%), 89% (95%CI: 74%-96%) and 96% (95%CI: 79%-100%), respectively.

The diagnostic accuracy of adrenal gland EUS-FNA was 92% for both benign and malignant lesions.

Only 2 patients died within 6 mo of the procedure. If these two were hypothetically included as false-negative biopsies, test characteristics for the diagnosis of malignancy would change to: sensitivity 80%, specificity 97%, positive predictive value 96% and negative predictive value to 84%.

In patients with benign adrenal gland cytology, EUS-FNA ruled out adrenal metastasis in 10 patients with underlying malignancy available follow-up (adrenalectomy or follow-up imaging). EUS-FNA of the adrenal gland made the initial diagnosis of stage Ⅳ cancer in 18 patients (lung cancer in 10, undifferentiated carcinoma in 1 and, esophageal in 4, colon in 2 and pancreatic adenocarcinoma in 1), and initial diagnosis of cancer recurrence in 6 patients (RCC in 2, oral SCC in 1, HCC in 1, esophageal cancer in 1 and breast cancer in 1).

Table 5 Cytology results from adrenal gland endoscopic ultrasound guided fine-needle aspiration

EUS-FNA cytologic diagnosis	*n*
Malignant EUS-FNA cytology (26%, n = 25)	
Metastatic lung cancer	10
Metastatic esophageal adenocarcinoma	5
Metastatic colon adenocarcinoma	2
Metastatic renal cell carcinoma	2
Metastatic breast adenocarcinoma	1
Metastatic pancreatic adenocarcinoma	1
Metastatic melanoma	1
Metastatic oral squamous cell carcinoma	1
Metastatic hepatocellular carcinoma	1
Undifferentiated carcinoma	1
Benign EUS-FNA cytology (64%, n = 60)	
Benign adrenal tissue	57
Aldosteronoma	1
Paraganglioma	1
Pheochromocytoma[1]	1

[1]Previously negative normal plasma catecholamines and, 24-h urine normetanephrines, vanillylmandelic acid and metanephrines. EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration.

Benign cytology and exclusion of metastases in 10/36 patients with malignancy or a precancerous lesion (non-small cell cancer in 7, gastrointestinal stromal tumor in 1, esophageal adenocarcinoma in 1, and gastric adenocarcinoma in 1) permitted subsequent surgery. EUS-FNA of the adrenal gland confirmed an initial diagnosis of unsuspected pheochromocytoma in one patient. Finally, unnecessary surgery was avoided in 18 patients with metastatic disease and 6 patients with cancer recurrence.

DISCUSSION

Adrenal gland adenomas are discovered in 5% of abdominal CT exams, in 2%-9% of autopsy studies and up to 4%-7% of patients with potentially resectable lung cancer, therefore accurate characterization of these lesions in cancer patients is essential[12,15]. Unfortunately, sensitivity and specificity of imaging techniques are currently insufficient to differentiate benign from malignant masses and, false-negative and false-positive rates by CT scan both average 10%[4].

Distinguishing a metastatic lesion from a primary adrenal tumor is aided by the knowledge of past cancer type and contrast-enhanced CT of the chest, abdomen and pelvis. Fluorodeoxyglucose (FDG)-positron emission tomography (PET)/CT is increasingly used in re-staging protocols for FDG-avid malignant tumors and can aid to document other extra-adrenal metastatic lesions[16]. According to the AACE/AAES (American As-

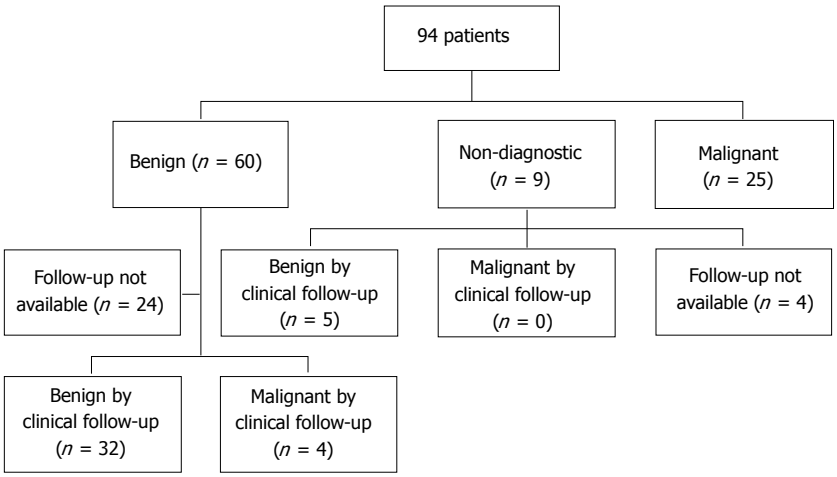

Figure 1 Patient flowchart .

Table 6 Final diagnosis for patients with non-malignant biopsies for who follow up was available

Final diagnosis	Benign FNA	Non-diagnostic FNA
Confirmed benign on follow up	32[1]	5[1]
Confirmed malignant on follow up	4[2]	0
Total of patients with follow up	36	5

[1]Follow up CT; [2]Subsequent CT-guided adrenal biopsy (*n* = 2), enlargement on repeat CT (*n* = 1) or adrenalectomy (*n* = 1). FNA: Fine-needle aspiration; CT: Computed tomography.

sociation of Clinical Endocrinologists and American Association of Endocrine Surgeons) guidelines, CT-guided FNA of an adrenal lesion can be performed to confirm metastatic disease if a definitive diagnosis is needed for oncologic treatment planning[16].

In our series, the sensitivity, specificity, PPV and NPV of adrenal gland EUS-FNA for the diagnosis of malignancy was of 86%, 97%, 96% and 89%, respectively. These results are similar to other series which report adrenal gland EUS-FNA sensitivity and negative predictive value rates ranging from 86%-100% and 70%-100%, respectively yet most of these studies have only included patients with underlying lung cancer[6,13].

With the widespread availability of CT and therefore percutaneous CT-guided fine-needle aspiration of the adrenals, the use of EUS-FNA to obtain adrenal gland biopsy could be questioned. While percutaneous CT-guided adrenal gland EUS-FNA of lesions of 2.8-5 cm in size, has been reported to be reliable and predict a benign course on long term follow up in patients with a benign cytology result[17], the reported rate of complications from percutaneous CT-guided adrenal gland EUS-FNA ranges from 0%-12% with an overall rate of 5.3%[7,15]. The most frequent adverse events related to percutaneous adrenal biopsies include hemorrhage and pneumothorax. Less common adverse events are pain, pancreatitis, and rarely needle-tract seeding. In our study we identified no short term (< 48 h) adverse events in any patient and no adverse events in those with available long term follow-up. In the current series, we performed diagnostic left adrenal biopsies in 2 of 3 patients in whom percutaneous approach of the left adrenal gland had been previously attempted unsuccessfully. These findings have been reported by others and emphasize that EUS-FNA may be utilized as a rescue procedure for those in whom percutaneous biopsies are contraindicated or unsuccessful[9]. Taken together, EUS-FNA appears to be a safe procedure and an acceptable alternative to percutaneous sampling of the adrenal glands.

About 5% of all incidentally discovered adrenal lesions are pheochromocytomas, and 25% of all pheochromocytomas are discovered incidentally. Typical features of pheochromocytomas include paroxysmal hypertension, headaches, sweating and palpitations; but, patients may not present with classical symptoms and up to 8% may be asymptomatic[18]. Sood *et al*[18] reported 3 cases of patients with catecholamine secreting tumors who underwent CT-guided percutaneous mass biopsy, including one with a pheochromocytoma and did not experience any adverse events related to the biopsy. In our series, one patient was unexpectedly diagnosed with pheochromocytoma by EUS-FNA and did not experience any adverse events from the procedure.

EUS shows a normal or minimally enlarged left adrenal gland in 98% of patients compared with only a 69% by transabdominal ultrasound[19]. A normal or minimally enlarged right adrenal gland, however, is seen in only 30% of patients on EUS, whereas transabdominal ultrasound permits detection in nearly all patients. Therefore, left adrenal EUS-FNA is attempted more often than right adrenal biopsies[19]. Recently, Uemura reported a rate of visualization of the right adrenal gland of 87.3% (*n* = 150) on EUS[13]. To date, there have been only a few reports of successful right adrenal gland EUS-FNA, but no large case-series[9-12,20]. The utility of EUS-FNA of right adrenal masses requires further clarification.

In our case series, the median adrenal gland diameter was higher in patients with diagnostic benign biopsies compared to malignant FNA specimens. This is in contrast with the results reported by Eloubeidi *et al*[12] who found larger masses in patients with malignancy (3.1 cm) compared to those with benign lesions (2.3 cm). A potential reason for this difference is that our group has

Table 7 Comparison of different Studies evaluating adrenal gland endoscopic ultrasound guided fine-needle aspiration

Ref.	Year	Number of patients	Patient population	EUS-FNA Left adrenal, *n*	Patient population	EUS-FNA Left adrenal, *n*	EUS-FNA Right adrenal, n	Benign EUS-FNA cytology, *n*	Malignant EUS-FNA cytology (*n*)	Non-Diagnostic rate	Sensitivity	Specificity	PPV	NPV	F/U for benign lesions	Method for F/U
Current research	2014	94	Patients undergoing EUS-FNA of either adrenal	94	Patients undergoing EUS-FNA of either adrenal	90	5	60	25	10%	86%	97%	96%	89%	Available on 36/60	CT/MRI, repeat EUS at ⩾ 6 mo or surgical pathology from adrenalectomy
[1]Uemura *et al*[13]	2013	150	Potentially resectable lung cancer	150	Potentially resectable lung cancer	91	51	7	4	0%	100%	100%	100%	100%	Available in 4/7	F/U CT at 6 months
Schuurbiers *et al*[17]	2011	85	Lung cancer	150	Lung cancer	85	0	25	55	6%	86%	96%	91%	70%	Available in 23/30	Clinical (n = 11) or F/U CT (n = 10)[2]
Eloubeidi *et al*[12]	2010	59	Known or suspected malignancy	59	Known or suspected malignancy	54	5	37	22	0%	NR	NR	NR	NR	Clinical F/U for 37	Not part of study protocol
Bodtger *et al*[4]	2009	40	Known or suspected lung cancer	40	Known or suspected lung cancer	40	0	29	11	0%	94%	43%	91%	55%	Available	Survival at 2 yr
Ang *et al*[21]	2007	119	Lung cancer	119	Lung cancer	4	0	2	2	0%	NR	NR	NR	NR	N/A	N/A

[1]EUS-FNA was done in 11 patients, 3 had bilateral EUS-FNA; [2]Two patients had CT at 3 mo. EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration; PPV: Positive predictive value; NPV: Negative predictive value; FNA: Fine-needle aspiration; F/U: Follow up; N/A: Not available; NR: Not reported; CT: Computerized Tomography.

aggressively biopsied adrenal masses over 3 cm in size in the following patients: (1) a history of malignancy; (2) a new diagnosis of cancer; or (3) a suspected recurrence due to the significant impact a diagnosis of metastatic malignancy has in this population.

Various techniques have been used to estimate the probability of malignancy of an adrenal mass, including its size, imaging characteristics and growth rate on serial imaging[16]. Asymptomatic patients with an indeterminate initial imaging study are advised to have follow-up imaging in 3-12 mo to assess for growth[16]. Surgical resection is recommended for lesions that grow; however, the threshold increase in size and growth rate that triggers resection have not been determined[16]. Guidelines from the AACE/AAES in 2009 on the management of adrenal incidentalomas recommend that benign appearing lesions smaller than 4 cm should have repeat adrenal imaging at 3-6 mo and then annually for 1-2 years. These same guidelines recommend surgery for growth rate more than 1cm or development of a hormonally active lesion (grade 3, Level C evidence)[16]. Based on these recommendations above, we utilized adrenal growth rate of ⩽ 1 cm at follow imaging 6 mo or longer after EUS to correct confirm benign cytology as a benign lesion.

Other studies evaluating adrenal gland EUS-FNA and its clinical impact in patients with established or suspected malignancy, have either used survival at ⩾ 2 years as confirmation for benignancy or not reported follow-up for benign lesions[4,12,21]. Schuurbiers *et al*[17] reported follow-up imaging for 10/30 patients with either benign or non-diagnostic EUS-FNA of the left adrenal gland. Similarly, Uemura *et al*[13] reported follow-up imaging at 6 mo for 4/7 patients with benign EUS-FNA and underlying lung cancer. To our knowledge, our series represents the first large study to utilize growth rates to confirm benign adrenal lesions and utilize these data to calculate test characteristics of EUS-FNA in non-cancer patients undergoing right and/or left adrenal gland EUS-FNA (Table 7).

False positive results for malignancy have been reported with EUS-FNA and its incidence varies anywhere from 1% to 15%[22]. Our rate was 1% and it was considered to be secondary to cytological misinterpretation.

Potential limitations of this study include limited assessment of long-term adverse events after EUS-FNA due to inability to contact patients within weeks of the pro-

cedure. Nevertheless, a careful review of the available records was performed and all patients were contacted for short term events within 48 h of the procedure. Secondly, many patients with benign adrenal gland FNA cytology had underlying cancer and died before follow-up CT or never followed up, which could have affected the final diagnosis of the nature of the adrenal gland abnormality. However, because follow-up imaging was not available for these patients, they were excluded from the sensitivity analysis.

Another potential limitation is that during several years of the study time period, PET scan was not available and therefore is not applicable to this case series. With the advent of PET, any decision to pursue a biopsy for a positive or indeterminate PET scan is generally at the discretion of the referring physician. With widespread metastatic disease, a positive scan within either adrenal is likely considered as diagnostic for metastatic disease and therefore a biopsy would not be necessary. However, in a patient with known or suspected malignancy and a positive adrenal gland on PET in isolation, we advocate EUS-FNA of the adrenal as this may signify novel metastatic disease which may merit additional or novel chemotherapy or possibly adrenalectomy.

In conclusion, EUS-FNA of the adrenal is a safe, minimally invasive and sensitive technique with significant impact in the management of patients with malignancy diagnosed either prior or during the procedure. It permits surgical treatment for cancer in patients with localized malignancy and a benign adrenal lesion. This technique also diagnoses metastatic disease and cancer recurrence, avoiding unnecessary invasive surgical procedures in patients with established metastatic disease by adrenal biopsy.

COMMENTS

Background

Different modalities can be used to sample the adrenal glands. Image guided fine-needle aspiration using either CT and ultrasound guidance have traditionally been used. With the advent of new endoscopic techniques, endoscopic ultrasound guidance for fine-needle aspiration (EUS-FNA) of either adrenal gland has become a very plausible technique for this matter. There have been reports of adrenal gland EUS-FNA and this has shown to be a very safe and minimally invasive procedure.

Research frontiers

When sampling adrenal gland lesions, especially in patients with known or suspected underlying malignancy, it is of supreme importance not only the technique used possesses a great deal of diagnostic accuracy, but also to understand how did previous studies obtain that diagnostic accuracy; this relates to the method for follow up of lesions with benign cytology results. This is a very important area of research in this subject.

Innovations and breakthroughs

Most publications regarding EUS-FNA have universally included patients with underlying malignancy and, have had small patient numbers and/or have not included repeat imaging to document follow up on lesions with benign cytology results. According to the recommendations of the American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons, adrenal lesions with benign appearance and smaller than 4cm, should have repeat adrenal imaging at 3-6 mo. These same guidelines also recommend surgery if the growth rate exceeds 1cm or if the lesion becomes hormonally active. In our study, we included 94 patients that had EUS-FNA of either adrenal gland, reviewed records and, abstracted information about EUS indication, EUS findings, EUS-FNA results, clinical and follow up imaging if this was available. A true diagnosis of a benign lesion was considered when there was a benign EUS-FNA result and the lesion had not grown more than 1 cm from its original size on follow up CT/MRI or repeat EUS or if the patient underwent adrenalectomy when the surgical pathology was benign. The clinical impact of adrenal EUS-FNA was analyzed on a case by cases basis. In the present study, the authors showed that adrenal gland EUS-FNA is a sensitive, specific, and safe minimally invasive diagnostic technique that has a great impact in patient care. Adrenal gland EUS-FNA ruled out metastatic disease in patients with underlying malignancy, therefore permitting surgery for primary tumor; it also made the initial diagnosis of stage Ⅳ cancer or recurrent malignancy in others.

Applications

This study suggests that adrenal gland EUS-FNA is a clinically useful, accurate and a safe technique in patients with adrenal gland mass or enlargement regardless or the presence of underlying malignancy.

Terminology

Endoscopic ultrasound (EUS) or echo-endoscopy is a procedure in which endoscopy is combined with ultrasound to obtain images of the internal anatomy. Combined with Doppler imaging, nearby blood vessels can be evaluated. During the performance of this procedure, abnormal structures can be biopsied using a fine-needle aspiration technique.

Peer review

This is a retrospective single-center case-series evaluating the impact of EUS-FNA (Endoscopic ultrasound guided fine-needle aspiration) in the evaluation of patients with left and/or right adrenal gland lesions discovered at EUS as part of a staging procedure or incidentally for other indications. The authors should be congratulated in their effort to present real clinical impact of EUS-FNA in patients with both malignant and benign adrenal lesions/findings that has never been done before, where patient population were mainly patients with cancer who were undergoing EUS-FNA for staging purposes.

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OBSERVATIONAL STUDY

Gait speed and hospitalization among ambulatory hemodialysis patients: USRDS special study data

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Abstract

AIM: To assess the association of measured gait speed with hemodialysis (HD) patients' hospitalization, in conjunction with, and apart from, recent fall history.

METHODS: Gait speed was measured by a standard protocol and falls during the past 12 mo were ascertained for a prevalent multi-center HD cohort (n = 668) aged 20-92. Hospitalization during the past 12 mo was identified in the patient's clinic records, and the first hospitalization after gait speed assessment (or the competing event of death) was identified in the 2013 United States Renal Data System Standard Analysis Files.

RESULTS: Slow gait speed, defined as < 0.8 m/s, characterized 34.7% of the patients, and 27.1% had experienced a recent fall. Patients with slow gait speed but without a history of recent falls were 1.79 times more likely to have been hospitalized during the past 12 mo (OR = 1.79, 95%CI: 1.11-2.88, P = 0.02), and patients with slow gait speed and a history of recent falls were over two times more likely to have been hospitalized (OR = 2.10, 95%CI: 1.19-3.73, P = 0.01), compared with patients having faster gait speed and no recent fall history. Prospective examination of gait speed/fall history status in relation to first hospitalization (or death) incurred by the end of follow-up December 31, 2011 also showed that slow gait speed was associated with these events in conjunction with a history of falls (HR = 1.54, 95%CI: 1.04-2.30, P = 0.03).

CONCLUSION: The International Task Force on Nutrition and Aging reported that gait speed is a powerful predictor for older adults of adverse outcomes such as hospitalization. In our data, gait speed--apart from, as well as in conjunction with, recent fall history--was associated with HD patients' hospitalization for multiple causes. Gait speed may be a sensitive health indicator among HD patients across the age spectrum.

Key words: Falls; Gait speed; Hemodialysis; Hospitalization; Walking disability

Core tip: Walking places demands on the heart, lungs, circulatory, nervous, and musculoskeletal systems. Studies of older adults support the prognostic importance of slowed gait speed for the risk of poor health and function, including hospitalization. However, little is known about the association of gait speed with hemodialysis (HD) patient outcomes. The usual gait speed of 668 HD patients was measured in a United States Renal Data System special study. Slowed gait speed--apart from, as well as in conjunction with, recent fall history--was associated with HD patients' hospitalization for

multiple causes. Gait speed may be a useful monitoring tool in the HD clinical setting.

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INTRODUCTION

The rate of hospital admissions among dialysis patients is high[1]. Hospitalization consumes a large portion of healthcare expenditures and is associated with risk for the patient. Identifying and understanding factors that may lead up to hospitalization is important for multiple quality improvement objectives[2].

In the geriatric population, a deficit in gait speed is recognized as a relatively easily measured and consistent prognostic indicator of patient outcomes, including hospitalization risk[3-5]. Studenski *et al*[5] have provided compelling evidence of the prognostic importance of slow gait speed for multiple health outcomes among older adults and have identified 0.8 m/s as a cutpoint below which gait speed connotes significantly increased likelihood of poor health and function and even mortality. End-stage renal disease (ESRD) patients appear to represent a model of early aging in many ways. While it is recognized that average gait speed is slower among ESRD patients compared with age-adjusted norms[6,7], the association of gait speed with ESRD patient outcomes has received limited attention.

A recent study by Beaubrun *et al*[8] of 52408 hemodialysis (HD) patients who were followed 2000-2009 reported that over one-fifth of patients who incurred a non-fracture-related hospitalization, and more than half of those who were hospitalized following a fracture, were characterized by "walking disability/history of falls". Medicare claims diagnoses of "difficulty walking" and "abnormal gait" define walking disability in administrative data[9]. Walking disability and falls are frequently associated[9]. At the same time, "walking disability" does not specify a quantitative measure of gait speed, and the potential association of walking disability with hospitalization apart from falls remains unknown when walking disability and history of falls are treated as one combined variable.

In this study of a large contemporary cohort of patients aged 20-92 undergoing maintenance HD therapy, we measured patients' gait speed and investigated its association with hospitalization in conjunction with, and apart from, patients' recent fall history.

MATERIALS AND METHODS

ACTIVE/ADIPOSE (A Cohort Study to Investigate the Value of Exercise in ESRD/Analyses Designed to Investigate the Paradox of Obesity and Survival in ESRD) is a multi-center study of prevalent patients on HD coordinated by the United States Renal Data System (USRDS). An overview of the study design and measures is available in the 2011 USRDS Annual Data Report[10]. Institutional review boards at Emory University and the University of California-San Francisco approved the study. A total of 668 study participants had gait speed and fall history information and are the focus of the analyses reported in this paper.

Usual gait speed was measured two times over a 15-feet walkway, and the average speed of the two trials was determined. Coordinators observed whether the participant used an assistive device for walking and whether an assistive device was used to perform the walk. For the analyses reported in this paper, slow gait speed was defined as < 0.8 m/s. Hospitalization during the past 12 mo was identified in the patient's clinic records, and the first hospitalization after gait speed assessment (or the competing event of death) was identified in the 2013 USRDS Standard Analysis Files.

Statistical analysis

Participants' sociodemographic and clinical characteristics were described by percentage or mean (SD) and compared using chi-square or t-test. A four-level variable was used to summarize participants' gait speed and fall history status, i.e. slow gait speed + no fall history; slow gait speed + fall history; faster gait speed + no fall history; faster gait speed + fall history. The association of this variable with hospitalization during the past 12 mo was examined in a multivariable logistic regression model, and time to first hospitalization (or death) through December 31, 2011 was examined in a multivariable Cox proportional hazards analysis using the USRDS 2013 Hospitalization Standard Analysis File; patients were censored at the end of follow-up. Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC, United States).

RESULTS

Overall mean (SD) age of study participants was 57.1 (14.3); the median age was 57.5. The primary cause of ESRD was diabetes or hypertension in 72% of the cohort, similar to the total United States in-center HD population[1]. Forty-one percent were women, and median time since ESRD treatment start (ESRD vintage) was 3 years. Consistent with the study sites, African-American patients were more heavily represented than in the overall United States HD population, and the average age of study participants was correspondingly younger.

There were 103 patients who could not be included in the analysis due to lack of gait speed assessment or fall information. Compared with patients who had gait speed and fall information, patients missing this information were more likely to be women, older, and white; to have diabetes, congestive heart failure (CHF), coronary artery disease/myocardial infarction (CAD/MI), cerebrobascular accident/transient ischemic attack

Table 1 Characteristics of patients included (n = 668) and not included (n = 103) in the analysis

	Measured walk and fall information (n = 668)	Missing measured walk or fall information (n = 103)[1]	P value
Male (%)	61.2	45.6	0.003
Age (yr), mean ± SD	56.3.(14.1)	62.2 (14.1)	< 0.001
Race (%)			< 0.001
White	22.2	31.1	
Black	63.6	45.6	
Native American	0.5	1	
Asian	10.9	12.6	
Other (Native Hawaiian, other Pacific Islander, other)	2.8	9.7	
ESRD vintage, yr, mean ± SD	5.0 (5.2)	4.4 (4.1)	0.24
Diabetes (%)	48.1	73.5	< 0.001
COPD (%)	7.5	11.8	0.14
Cancer (%)	7.7	9.8	0.46
CHF (%)	27.3	42.2	0.002
CAD/MI (%)	26	39.2	0.005
CVA/TIA (%)	9.3	15.7	0.047
PVD (%)	6.5	31.4	< 0.001
Other cardiac diseases (%)	23.9	43.1	< 0.001
KDQOL-CF score, mean ± SD	88.0 (16.3)	86.3 (18.2)	0.35
Hemoglobin, g/dL, mean ± SD	11.6 (1.3)	11.3 (1.3)	0.1
Assistive walking device (%)	19.5	91.7	< 0.001
History of recent fall(s) (%)	27.1	37.2	0.04

[1]One patient lacked fall history information only; no physical performance information was obtained for 19 patients; and study coordinators reported that 83 patients were not able to walk 15 feet to perform the walk test. CAD: Coronary artery disease; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; ESRD: End-stage renal disease; KDQOL-CF: Kidney Disease Quality of Life-Cognitive Function; MI: Myocardial infarction; m/s: Meters per second; Other cardiac disease: Cardiac dysrhythmia, atrial fibrillation, tachycardia, pericarditis, cardiac arrest; PVD: Peripheral vascular disease; TIA: Transient ischemic attack.

Table 2 Characteristics of patients with measured walk and fall information, by slow gait speed (< 0.8 m/s) and faster gait speed (⩾ 0.8 m/s)

	Gait speed < 0.8 m/s (n = 232)	Gait speed ⩾ 0.8 m/s (n = 436)	P value
Male (%)	47	68.8	< 0.001
Age, yr, mean ± SD	62.1 (13.4)	53.2 (13.5)	< 0.001
Race (%)			< 0.001
White	13.8	26.6	
Black	75	57.6	
Native American	0.4	0.5	
Asian	8.2	12.4	
Other (Native Hawaiian, other Pacific Islander, other)	2.6	3	
ESRD vintage, yr, mean ± SD	4.5 (4.9)	5.2 (5.3)	0.1
Diabetes (%)	59.9	41.7	< 0.001
COPD (%)	12.1	5.1	0.001
Cancer (%)	8.2	7.4	0.71
CHF (%)	31.5	25.1	0.08
CAD/MI (%)	33.2	22.1	0.002
CVA/TIA (%)	12.5	7.6	0.04
PVD (%)	9.5	4.8	0.02
Other cardiac diseases (%)	29.3	21	0.02
KDQOL-CF score, mean ± SD	84.3 (18.0)	90.0 (14.9)	< 0.001
Hemoglobin, g/dL, mean ± SD	11.5 (1.3)	11.6 (1.3)	0.67
Assistive walking device (%)	45	6	< 0.001
Assistive walking device used when gait speed measured (%)	26.8	2.3	< 0.001
History of recent fall(s) (%)	36.2	22.3	< 0.001
Gait speed, m/s, mean ± SD	0.61 (0.15)	1.04 (0.18)	< 0.001

CAD: Coronary artery disease; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; ESRD: End-stage renal disease; KDQOL-CF: Kidney Disease Quality of Life-Cognitive Function; MI: Myocardial infarction; m/s: Meters per second; Other cardiac disease: Cardiac dysrhythmia, atrial fibrillation, tachycardia, pericarditis, cardiac arrest; PVD: Peripheral vascular disease; TIA: Transient ischemic attack.

(CVA/TIA), peripheral vascular disease (PVD) and other cardiac diseases; to use an assistive device for walking; and to report having fallen in the past 12 mo. Patients who lacked gait speed or fall information and could not be included in the analyses reported in this paper did not differ from the included patients with respect to ESRD vintage, chronic obstructive pulmonary disease (COPD), cancer, Kidney Disease Quality of Life-Cognitive Function (KDQOL-CF) score, or hemoglobin level (Table 1).

Characteristics of the 668 study participants who had gait speed measured are shown in Table 2. Corresponding mean (SD) gait speed values were 0.61 (0.15) m/s for those with slow gait speed *vs* 1.04 (0.18) m/s for those with faster gait speed; $P < 0.001$. Participants with slow gait speed were more likely to be women, older, and black. They were more likely to have diabetes, COPD, CAD/MI, CVA/TIA, PVD, and other cardiac diseases. Their average score on the KDQOL-CF scale was lower, they were more likely to use an assistive device for walking and to have used such a device to perform the walk speed test, and they were more likely to report having fallen during the past 12 mo. Participants with slow gait speed and those with faster gait speed did not differ with respect to ESRD vintage, cancer, CHF, and average hemoglobin level.

Gait speed < 0.8 m/s characterized 34.7% of patients, and falling during the past 12 mo was reported by 27.1% of patients. Slow gait speed was more likely to characterize patients with a history of falls than patients without a history of falls (46% *vs* 30%, $P < 0.001$).

Almost half of the study cohort had been hospitalized in the past 12 mo, and 41% of those hospitalized had slow gait speed, compared with 29.3% of those who had not been hospitalized ($P = 0.001$). Patients with slow gait speed but no history of recent falls (n = 148) were 1.79 times more likely to have been hospitalized compared with patients with faster gait speed and no recent falls (n = 339) (OR = 1.79, 95%CI: 1.11, 2.88, $P = 0.02$). Patients with slow gait speed and a history of recent falls (n = 84) were over two times more likely to have been hospitalized compared with patients having faster gait speed and no recent fall history (OR = 2.10, 95%CI: 1.19, 3.73, $P = 0.01$). The risk of hospitalization among patients with faster gait speed and recent fall history (n =

Table 3 Characteristics associated with hospitalization in past 12 mo from multivariable logistic regression analysis

	[1]Adjusted odds ratios (95%CI) for Hospitalization	*P* value
Gait speed/fall history		
[2]Faster walk/no fall history (reference)	1	
[3]Slower walk/no fall history	1.79 (1.11, 2.88)	0.02
Slower walk/fall history	2.10 (1.19, 3.73)	0.01
Faster walk/fall history	1.46 (0.90, 2.39)	0.13
Female	0.55 (0.39, 0.78)	< 0.001
Age, yr	0.98 (0.97, 1.00)	0.01
ESRD vintage, yr	0.95 (0.92, 0.98)	0.003

[1]Adjusted also for race, diabetes, CHF, CAD, CVA, PVD, other cardiac diseases, COPD, cancer, hemoglobin level, KDQOL-CF score, assistive device use (variables not significantly associated in this logistic regression model with hospitalized/not hospitalized in past 12 mo); [2]Faster walk = gait speed ⩾ 0.8 m/s; [3]Slower walk = gait speed < 0.8 m/s. ESRD: End-stage renal disease.

Table 4 Multivariable Cox proportional hazards model predicting index hospitalization/death[1] from baseline gait speed assessment through December, 2011

	[2]Adjusted hazard ratios (95%CI)	*P* value
Gait speed/fall history		
[3]Faster walk/no fall history (reference)	1	
[4]Slower walk/no fall history	1.11 (0.79, 1.56)	0.53
Slower walk/fall history	1.54 (1.04, 2.30)	0.03
Faster walk/fall history	1.47 (1.05, 2.05)	0.02
CAD	1.34 (1.01, 1.78)	0.04
Black race	2.09 (1.48, 2.94)	< 0.001
ESRD vintage, yr	1.02 (1.00, 1.04)	0.03

[1]Hospitalization events = 271; deaths = 17; [2]Adjusted also for age, diabetes, CHF, CVA, PVD, other cardiac diseases, COPD, cancer, hemoglobin level, KDQOL-CF score, assistive device use; [3]Faster walk = gait speed ⩾ 0.8 m/s; [4]Slower walk = gait speed < 0.8 m/s. ESRD: End-stage renal disease.

97) was not significantly higher than the hospitalization risk among patients with faster walk and no recent fall history. Female sex, one-year increment in age, and one-year increment in ESRD vintage were associated with a lower risk for hospitalization (Table 3).

Patients with slower gait speed and a history of falls were more likely to incur subsequent hospitalization (or death) compared with the reference group of patients who had faster walk/no fall history (HR = 1.54, 95%CI: 1.04, 2.30, $P = 0.03$). In the Cox analysis, increased risk of these events was also evident for patients with faster walk and fall history, those with a history of CAD, and blacks, and a one-year increment in ESRD vintage was associated with increased likelihood of hospitalization/death (Table 4).

DISCUSSION

Slow gait speed, defined in this analysis as < 0.8 m/s, characterized one-third of the ambulatory HD patients whom we studied. Patients with gait speed < 0.8 m/s were more likely than those with gait speed ⩾ 0.8 m/s to have been hospitalized during the past 12 mo, even in the absence of a history of recent falls. When we prospectively examined gait speed/fall history status in relation to first hospitalization (or death), patients with gait speed < 0.8 m/s and a history of falls were more likely to incur hospitalization/death than those with faster gait speed and no history of falls.

The causes of hospitalization among patients with slow gait speed varied widely, *e.g.*, cardiac issues, respiratory distress, mini-stroke, neuropathy, leg pain. Studenski *et al*[5] have emphasized that gait speed is a sensitive marker of health because it may reflect known and unrecognized disturbances in multiple organ systems. Walking places demands on the heart, lungs, circulatory, nervous, and musculoskeletal systems, and slowed gait may reflect damaged systems as well as a high-energy cost of walking[5]. Mobility limitations can be early indicators of muscle weakness, pain or discomfort, and shortness of breath, as well as potential falls[7]. In addition, decreasing mobility may induce a cycle of reduced physical activity and deconditioning[5].

Slow gait speed may have contributed to "walking disability" in the Beaubrun *et al*[8] 2013 analysis of HD patients who were hospitalized following fracture. In their study, walking disability/history of falls characterized 55.6% of the study population[8]. The number of patients in our study cohort who were hospitalized following fracture was small ($n = 24$), but slow gait speed and fall history were prominent; 45.5% of patients hospitalized post-fracture had slow gait speed and 70.8% had recently fallen.

The International Task Force on Nutrition and Aging concluded that gait speed is strongly associated with adverse outcomes, including falls and hospitalization[11]. However, there has been little investigation of the association of gait speed with hospitalization in the dialysis population. As we have noted, the recent study by Beaubrun *et al*[8] links walking disability/history of falls with hospitalization risk, but that study did not have information about gait speed.

The frailty index developed by Fried *et al*[12] includes slow walk as one component. Several studies of chronic kidney disease patients, both dialysis-dependent and non-dialysis-dependent, have measured gait speed and used the Fried index to classify patients as frail[13-18]. One of these studies investigated HD patients' hospitalization in association with frailty. McAdams-DeMarco *et al*[17], in a study of 146 HD patients in one dialysis center, found that 42.6% of frail participants, compared with 28.2% of nonfrail participants, had two or more hospitalizations in the year following study enrollment, and frailty was associated with 1.4 times (95%CI: 1.00-2.03, $P = 0.049$) more hospitalizations independent of age, sex, comorbidity, and disability[17]. The Fried index classi-

fies individuals as frail who have three or more of the five criteria that comprise the index (recent weight loss, reported exhaustion, weak grip strength, slow walk, and low physical activity)[12], which does not necessarily include having slow walk.

Our study has several strengths. Data were supplied by a large multi-center study cohort. Performance-based gait speed was carefully assessed, along with a large number of patient characteristics and treatment-related factors. The fall prevalence that we observed was very similar to 12-mo fall prevalence estimates from other studies[19]. We acknowledge, however, that although the ACTIVE-ADIPOSE cohort shares many similarities with the general ESRD population, the rate of fracture-related hospitalization would be expected to be higher in a cohort with higher representation of whites and older patients[8]. In addition, having been hospitalized over the past 12 mo could have influenced participants' gait speed, and prospective examination of hospitalization events was truncated for study participants whose baseline gait speed assessments were obtained near the end of the study enrollment period in 2011. These also represent potential study limitations.

We used the cutoff of < 0.8 m/s to define slow gait speed, the cutoff that has been most often observed to predict adverse health outcomes among older adults[11]. However, 0.6 m/s and 1.0 m/s have been used as cut-offs in other studies[5,7]. Most of the existing evidence about gait speed and outcomes is derived from studies of older, community-dwelling populations. Investigation of other gait speed cut points may be useful in dialysis cohorts that have a different sociodemographic and/or clinical profile compared with the cohort we studied. Studenski *et al*[5] noted that further work is needed to examine associations of gait speed with outcomes such as disability and health care use, especially in populations based in clinical practice.

Gait speed provides a straightforward and informative indicator of health status. It may offer the clinician a tool for assessing expected outcomes and tailoring goals of care[5]. Compared with patient-reported mobility difficulty, a gait speed test provides a quantitative marker that facilitates tracking mobility changes[7]. Referral to a specialist (physical therapist, clinical exercise specialist, cardiac rehabilitation) for further evaluation and intervention may be indicated. Gait speed performance can be assessed relatively easily and quickly (less than two minutes) in the clinical setting, and poor performance may indicate that there are underlying health problems placing the patient at increased risk for hospitalization, as well as for other potential adverse outcomes.

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The interpretation and reporting of the data presented here are the responsibility of the authors and in no way should be seen as an official policy or interpretation of the United States government. The results presented in this paper have not been published previously in whole or part, except in abstract format.

COMMENTS

Background

Usual gait speed has been shown to be a powerful predictor of outcomes, including hospitalization, among older persons, and hemodialysis (HD) patients represent a model of early aging. Frailty, which may include slow gait speed, has been shown to be associated with HD patients' hospitalization risk, as has the syndrome of "walking disability/history of falls," but the utility of gait speed alone as a predictor of HD patients' hospitalization has not been studied.

Research frontiers

There is growing recognition of the significance of physical performance deficits among chronic kidney disease (CKD) patients, including increased mortality risk. Measures of lower extremity function may capture a complex set of skeletal muscle and neurologic impairments that develop in individuals with CKD and may substantially affect their survival as well as their independent physical functioning. Research frontiers include identifying mechanisms that underlie decreased physical performance and evaluating whether interventions that improve physical performance may also positively influence comorbidity and clinical outcomes in CKD.

Applications

Gait speed can be considered a vital sign, and measuring gait speed is simple, quick, reproducible, inexpensive, and feasible in clinical settings.

Terminology

Gait speed is typically measured as the time it takes an individual to walk at a usual pace over a measured distance (usually 4 to 6 meters). The time for that distance is then converted into a speed in m/s.

Peer review

Clearly stated study indicating that slow measured walking speed in dialysis patients can be used as a predictor of subsequent fracture and hospitalization for all causes.

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RANDOMIZED CONTROLLED TRIAL

Long-term outcome of ketoconazole and tacrolimus co-administration in kidney transplant patients

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Abstract

AIM: To study the long-term outcome of ketoconazole and tacrolimus combination in kidney transplant recipients.

METHODS: From 2006 to 2010, ketoconazole was given in 199 patients and was continued for at least 1 year or until graft failure (Group 1), while 149 patients did not receive any ketoconazole (Group 2). A combination of tacrolimus, mycophenolate and steroid was used as maintenance therapy. High risk patients received basiliximab induction.

RESULTS: Basic demographic data was similar between the 2 groups. The 5-year cumulative incidence of biopsy-confirmed and clinically-treated acute rejection was significantly higher in Group 1 than in Group 2 (34% *vs* 18%, $P = 0.01$). The 5-year Kaplan-Meier estimated graft survival (74.3% *vs* 76.4%, $P = 0.58$) and patient survival (87.8% *vs* 87.5%, $P = 0.93$) were not different between the 2 groups. Multivariable analyses identified ketoconazole usage as an independent risk of acute rejection (HR = 2.33, 95%CI: 1.33-4.07; $P = 0.003$) while tacrolimus dose in the 2nd month was protective (HR = 0.89, 95%CI: 0.75-0.96; $P = 0.041$).

CONCLUSION: Co-administration of ketoconazole and tacrolimus is associated with significantly higher incidence of acute rejection in kidney transplant recipients.

Key words: Kidney transplant; Rejection; Survival; Tacrolimus Ketoconazole; Pharmacokinetics; Cytochrome P450

Core tip: Tacrolimus is mainly metabolized by cytochrome P450 enzymes and ketoconazole is a potent inhibitor of P450. Transplant programs often use ketoconazole to reduce the tacrolimus dose and financial cost. Small short-term studies had previously supported such practice, but the long-term outcome are still lacking. We hereby report our center's experience of this combination in kidney transplant recipients. Our study suggests that co-administration of ketoconazole and tacrolimus is associated with significantly higher incidence of acute rejection in kidney transplant recipients.

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INTRODUCTION

Tacrolimus is a macrolide antibiotic produced from

Streptomyces tsukubaensis. It binds to FK506-binding protein to form a complex that inhibits calcineurin phosphatase in Tlymphocytes [commonly referred as calcineurin inhibitor (CNI)]. Tacrolimus has been widely used as the primary immunosuppressive agent in kidney transplant patients[1]. Due to its narrow therapeutic index, optimal dosing with therapeutic monitoring is necessary. Tacrolimus is mainly metabolized by cytochrome P450-3A in the liver and a substrate of P-glycoprotein, an efflux pump, in both liver and intestine[2,3]. The inhibition of P450-3A and P-glycoprotein can slow down its metabolism and ketoconazole is such a potent inhibitor. Transplant programs often use ketoconazole to reduce the tacrolimus dose and cost. The financial benefit and short-term safety of such a practice had been reported previously by a few small studies in Egypt[4,5]. But the long-term outcomes of such a practice are still lacking.

Our interest in examining this issue comes from our participation in the National Institutes of Health funded pilot study of solid organ transplants in human immunodeficiency virus (HIV) infected patients[6-8]. There were unexpectedly higher incidences of acute rejection (by a factor of 2 to 3) in both kidney and liver recipients. About half of the rejections were early and aggressive[6,7]. In these patients, the targeted trough levels of CNIs were consistent with those in non-HIV recipients, but their required doses of CNI were significantly lower due to the necessary treatment with anti-retroviral protease inhibitor, which also inhibits P450-3A and P-glycoprotein. Due to the altered pharmacokinetics, their total exposure to CNI [area under curve (AUC)] likely was considerably low. This may be more plausible than other proposed viral and/or immunological factors to explain the higher-than-expected rejection rates in HIV infected patients[9-11]. In the current study, we analyze the long-term outcome of patients who received co-administration of ketoconazole and tacrolimus in non-HIV infected kidney transplant recipients.

MATERIALS AND METHODS

Study population

This is a retrospective review, and patients were identified using the transplant center database at Tulane University Hospital and Clinic. During the period between 2006 and 2010, there were consecutive 450 non-HIV infected adult patients who underwent a primary kidney transplant at our center. Among them, ketoconazole was given in 199 patients after transplant surgery and was continued for at least 1 year or until graft failure (Group 1), while 149 patients did not receive any ketoconazole (Group 2). A combination of tacrolimus, mycophenolate and steroid was used as maintenance therapy in all patients. Factors for excluding patients from this study included primary graft non-function (n = 3), death in first week of transplant surgery (n = 2), incomplete data due to lost fellow-up (n = 19), different combination of maintenance immunosuppressives (n = 48) or usage of other drug that inhibits (such as diltiazem, amiodarone, *etc.*) or reduces (such as phenytoin, isoniazid, *etc.*) P450 enzymes (n = 9). The patients, whose ketoconazole was stopped before 1 year of kidney transplant (n = 17) or before the graft failure (n = 4) were also excluded from analysis. All patients were followed up closely at our center, including scheduled routine labs and clinic visits. Patients with acute illness were usually directly admitted or transferred to our center, and the outside events were recorded in a timely manner.

Immunosuppressive therapy

High risk patients, defined as having peak panel reactive antibody > 10%, human leukocyte antigen mismatch > 4, donor cold ischemia time > 18 h or expanded criteria donor kidneys, received induction therapy with 2 doses of basiliximab (20 mg per dose). Corticosteroids were administrated as methylprednisolone 500 mg IV intraoperatively, then tapered on postoperative days 1 to 3, and changed to oral prednisone 60 mg on postoperative day 4. The patients typically continued oral prednisone 20 mg daily for the first month, then 10 mg daily for the 2nd month and 5 mg daily thereafter. Each patient was started mycophenolate, either mycophenolate mofetil at 1 g or enteric-coated sodium mycophenolate at 720 mg, twice daily after transplant. Oral tacrolimus was started immediately after transplant, and doses were adjusted to keep the 12-h trough levels at 8 to 12 ng/mL for the first year. The target of tacrolimus trough levels was then maintained at 4 to 6 ng/mL after the first year. Whole blood tacrolimus concentrations were measured with liquid chromatography-tandem mass spectrometry in our hospital. The decision of adding ketoconazole was typically made within the first week of transplant surgery in order for patients to achieve the targeted trough levels prior to discharge. The dose of ketoconazole was started at100mg per day in all patients, and further increased to 200 mg per day in 4 patients.

Infection prophylaxis

All patients received one tablet of sulfamethoxazole/trimethoprim DS three times per week for the first year as prophylaxis for pneumocystis pneumonia and bacterial infections. Three-month antifungal prophylaxis with oral nystatin was given to patients who did not receive ketoconazole. Cytomegalovirus (CMV) prophylaxis was given to CMV seronegative recipients who received organs from a CMV seropositive donor. The regimen included IV ganciclovir during the transplant hospitalization followed by oral ganciclovir or valganciclovir for 3 mo.

Rejection

Acute rejection was presumed when patients had a sudden increase of serum creatinine that could not be explained by other clinical causes. Kidney biopsy was performed before the treatment. The severity of rejection was defined according to Banff criteria. Acute cellular rejection of grade 1 or below was initially treated with IV methylprednisolone for 3 d. Thymoglobulin was used for steroid resistant cellular rejection, or as the initial therapy

Table 1 Demographic characteristics of transplant patients between Group 1 (with ketoconazole) and Group 2 (without ketoconazole)

	Group 1 (n = 199)	Group 2 (n = 149)	P value
Age, mean ± SD (yr)	47.2 ± 13.2	48.8 ± 14.4	0.21
Gender (%)			
Male	56	61	0.47
Female	44	39	
Race (%)			
Black	64	55	0.19
Non-black	36	45	
BMI (kg/m2)	28.3 ± 5.4	27.4 ± 5.7	0.34
Peak PRA (%)	15.5 ± 25.3	13.8 ± 27.0	0.27
HLA mismatch	4.1 ± 1.4	3.9 ± 1.6	0.52
Causes of ESRD (%)			0.75
Diabetes	25	31	
Hypertension	38	35	
Nephritis	19	15	
PCKD	8	6	
Others	10	13	
Induction (%)	55	51	0.51
Donors (%)			0.63
Living	26	29	
Deceased	74	71	
CIT (h)	17.8 ± 7.2	18.5 ± 6.4	0.24

SD: Standard deviation; BMI: Body mass index; PRA: Panel reactive antibodies; HLA: Human leukocyte antigen; ESRD: End stage renal disease; PCKD: Polycystic kidney disease.

for rejection of Banff grade 2 or higher. Acute antibody mediated rejection (AMR) was diagnosed with positive C4d staining in the peritubular capillaries and/or demonstration of donor specific antibody. AMR was treated with a course of 5 to 7 daily plasmapheresis and intravenous immunoglobulin (IVIG) (150 mg/kg) in addition to IV methylprednisolone and thymoglobulin.

Statistical analysis

The outcome measures included: (1) incidence of biopsy-confirmed and clinically-treated acute rejection; (2) patient and kidney graft survival; (3) quality of graft function; and (4) incidence of clinically treated infections. Statistical analyses were performed using Statistics Analysis System (SAS) version 9.3 software (SAS Institute Inc, Cary, NC, United Statas). Chi-squared or Fisher exact test was used for count data, t test was used for continuous measures. Product-limit estimates of survival curves were generated by the Kaplan-Meier method and the survival difference was analyzed by log-rank test. Multivariable logistic regression analysis with a stepwise variable selection was used for examining risk factors of acute rejection. A P value < 0.05 was considered statistically significant. If there was no data at or around the particular time point, the previous or next available measure was used for analysis.

RESULTS

From 2006 to 2010, a total of 450 adult patients received a primary kidney transplant in our center. All of them were transplanted 3 to 7.5 years ago as of July 31, 2013, which is the end of the study period. Table 1 summarizes the demographic characteristics at the time of kidney transplants, and shows that there was no significant difference between the 2 groups. The total daily tacrolimus dose, 12-h trough level and graft function (serum creatinine) are summarized in Table 2. Both groups achieved similar targeted trough levels at all times according to our immunosuppressive protocol. Compared to Group 2, Group 1 initially required higher dose of tacrolimus during the first week of transplant. With administration of ketoconazole, their daily tacrolimus dose decreased. Subsequently, Group 1 required significantly lower dose of tacrolimus in the first month and in all times after that. The graft function remained comparable between the 2 groups.

Table 2 Tacrolimus dose, trough level and kidney function in the two groups

	1 wk	1 mo	2 mo	1 yr	3 yr	5 yr
Tacrolimus dose (mg/d)						
Group 1	10.9 ± 5.6	7.5 ± 4.8	6.0 ± 3.6	5.6 ± 3.8	5.3 ± 3.1	4.9 ± 2.8
Group 2	8.6 ± 4.1	8.3 ± 3.7	8.1 ± 3.2	7.8 ± 3.0	7.0 ± 2.2	6.2 ± 2.5
P value	0.004	0.03	< 0.001	< 0.001	< 0.001	< 0.001
Tacrolimus trough level (× 10 mg/L)						
Group 1	11.3 ± 2.1	9.7 ± 1.9	9.3 ± 1.7	8.6 ± 2.1	6.4 ± 1.9	5.3 ± 1.4
Group 2	10.7 ± 1.8	10.2 ± 2.0	9.5 ± 1.6	9.0 ± 1.8	5.8 ± 1.7	4.8 ± 1.5
Serum Cr (× 10 mg/L)						
Group 1	2.2 ± 1.3	1.7 ± 1.1	1.5 ± 0.9	1.6 ± 1.2	1.6 ± 0.8	1.7 ± 0.9
Group 2	1.9 ± 1.1	1.7 ± 0.9	1.6 ± 0.7	1.5 ± 0.8	1.5 ± 0.7	1.6 ± 0.8

The key post transplant events are summarized in Table 3. The delayed graft function (DGF) was defined by an inadequate renal function that required dialysis support in the first week after transplant. In both groups, the percentage of patients who had DGF was similar. Acute rejections were the biopsy-confirmed and clinically-treated ones. The 5-year cumulative incidence of acute rejection was significantly higher in Group 1 than Group 2, but the types of rejection were not different. There was no significant difference in the incidence of CNI toxicity or infectious disease between the 2 groups. Here, the CNI toxicity was the renal toxicity confirmed by kidney biopsy and required CNI dose reduction.

There was no statistical difference in graft survival by Kaplan-Meier analysis between the two groups (Figure 1A). The estimated graft survivals at 1, 3 and 5 years were 92.4%, 82.4% and 74.3% in Group 1, and 94.6%, 83.8% and 76.4% in Group 2 (Log-Rank P = 0.58). There was no difference in patient survival between the 2 groups (Figure 1B). The Kaplan-Meier estimated 1, 3, and 5 years patient survivals were 96%, 91.4% and 87.8% in Group 1, and 96.6%, 90.5%, 87.5% in Group 2 (Log-Rank P = 0.93). The causes of graft loss and death were listed in Table 3. There was no statistical difference in the overall causes of graft loss or patient death between the 2 groups.

The risk factors for acute rejection were examined by multivariable logistic regression analyses. The identified significant factors are listed in Table 4. We found that ke-

Table 3 Post transplant events and causes of graft loss and patient death

	Group 1 (*n* = 199)	Group 2 (*n* = 149)	*P* value
Posttransplant events, *n* (%)			
Delayed graft function	56 (28)	39 (26)	0.77
Acute rejection	68 (34)	27 (18)	0.01
Type of rejection			0.49
Cellular rejection	49	17	
Antibody rejection	14	6	
Both rejections	5	4	
CNI toxicity	8 (4)	15 (10)	0.09
Infectious diseases	63 (32)	54 (36)	0.37
Type of infection			0.67
CMV	32	22	
BKV	14	13	
HSV	5	6	
Bacteria	7	10	
Fungus	5	3	
Total graft loss, *n* (%)	52 (26)	35 (23)	0.57
Causes of graft loss			0.88
DWFG	22	16	
CAN	17	10	
Rejection	9	5	
Infection	2	3	
Others	2	1	
Total patient death, *n* (%)	27 (14)	18 (12)	0.68
Causes of death			0.88
CVD	14	10	
Infections	6	5	
Malignancy	2	2	
Others	3	1	

CNI: Calcineurin inhibitor; CMV: Cytomegalovirus; HSV: Herpes simplex virus; CVD: Cerebrovascular disease.

Table 4 Multivariable analysis of risk factors for acute rejection

	Hazard ratio	95%CI	*P* value
Race (black *vs* non-black)	2.68	1.67-6.73	0.032
Donor (living *vs* deceased)	0.32	0.11-0.94	0.038
Ketoconazole (yes *vs* no)	2.33	1.33-4.07	0.003
Delayed graft function (yes *vs* no)	2.14	1.22-3.73	0.008
Infection (yes *vs* no)	1.89	1.04-3.48	0.038
Tacrolimus dose (mg/d) in 2nd month	0.89	0.75-0.96	0.041

toconazole usage was an independent risk of acute rejection ((HR = 2.33, 95%CI: 1.33-4.07; P = 0.003). Tacrolimus dose at each time point was also tested. The daily dose of tacrolimus in the 2nd month after transplant was a significant factor in determining the risk of rejection (HR = 0.89, 95%CI: 0.75-0.96; P = 0.041), *i.e.*, the higher the daily dose, the lower the risk of rejection. Other commonly described risk factors in literatures, such as black ethnicity, DGF and infectious complications were also demonstrated in our study, while live donor kidneys were associated with lower risk of rejection compared to the deceased donor kidneys.

DISCUSSION

Tacrolimus remains a backbone of modern immunosuppressive therapy in solid organ transplants. Due to the numerous adverse effects, narrow safety margin and large intra- and inter-individual variability in pharmacokinetics, therapeutic monitoring is mandatory[1-3]. The normal pharmacokinetic curve of tacrolimus has a peak-and-trough pattern. A rapid peak phase reflects the absorption after an oral dose while a slow slope towards trough level reflects the drug metabolism. Ideally, tacrolimus dosing should be based on a 12 h area under the curve (AUC) that indicates the extent of systemic exposure. In clinical practice, oral doing is usually guided by monitoring 12 h trough levels, because of the convenience for blood sampling and the assumed correlation between trough level and AUC[2,3,12,13]. However, this correlation varies considerably and the best sampling time for a spot tacrolimus level to predict its total body exposure remains controversial[2,3,12-14]. The advance in pharmacogenetics has led the discovery of several gene polymorphisms in P450 family, which explains the inter-individual variability of tacrolimus metabolism[13,15,16]. Transplant patients

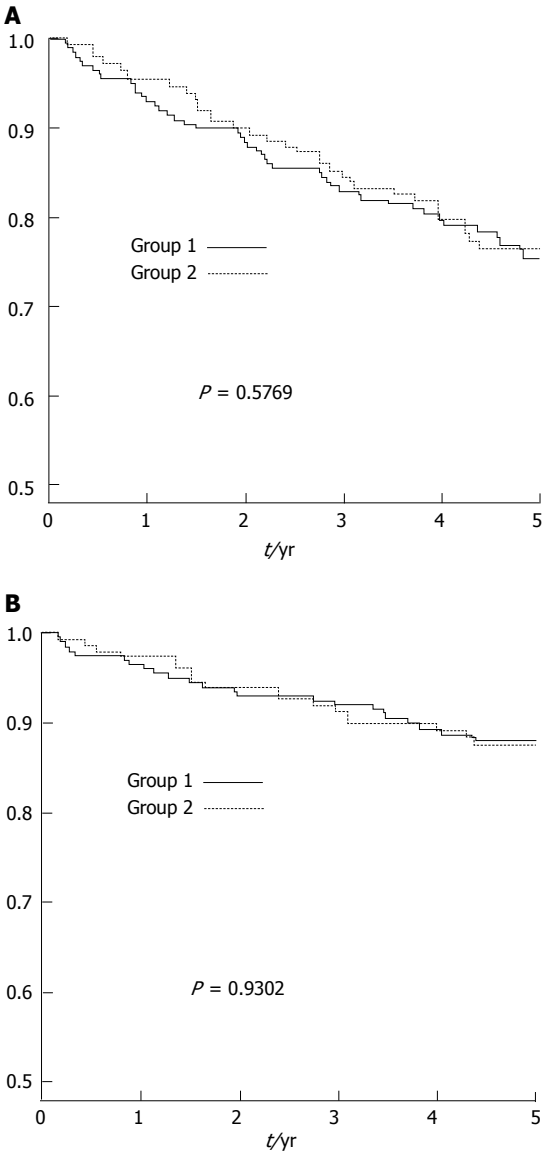

Figure 1 Kaplan-Meier estimated survival. A: Kidney graft survival; B: Patient survival.

expressing P450-3A5 (expressers) were shown to need higher doses of tacrolimus than non-expressers to reach similar trough levels[13,16].

Our study suggests that co-administration of ketoconazole with tacrolimus increase the risk of acute rejection. The 5-year cumulative incidence of acute rejection was significantly higher in Group 1 (34%) than Group 2 (18%), although the types of rejection were not different. Both groups achieved similar targeted trough levels at all time-points according to protocol. Compared to Group 2, Group 1 initially required higher dose of tacrolimus in the first week of transplant. As expected, their daily tacrolimus dose decreased with addition of ketoconazole. Subsequently, group 1 had significantly lower dose of tacrolimus in the first month and at all times after that. The risk factors for acute rejection were examined and we found that the use of ketoconazole was an independent risk of acute rejection (OR = 2.33, 95%CI: 1.33-4.07; P = 0.003). The daily dose of tacrolimus in the 2nd month after transplant was protective from rejection (HR = 0.89, 95%CI: 0.75-0.96; P = 0.041), *i.e.*, the higher the daily dose, the lower the risk of rejection. This suggests that higher incidence of rejection may be directly related to the reduced dose of tacrolimus from the co-administration of ketoconazole.

Previous studies from Egypt reported long-term safety and financial savings of coadministration of ketoconazole with cyclosporine in 51 patients after living-related kidney transplants[17,18]. The same group further studied the coadministration of ketoconazole with tacrolimus[4,5]. A total of 70 live donor kidney transplant recipients were randomized into ketoconazole group (100 mg/d) and control group (without ketoconazole). By six months, ketoconazole group experienced significant reduction of tacrolimus dose (by 58.7%) and cost (by 56.9%)[4]. After 2 years, ketoconazole group still had a remarkable reduction of tacrolimus dose (by 53.8%) and financial cost (by 52.9%). There was no adverse effect of ketoconazole throughout the 2 years[5]. None of the Egyptian studies noted higher incidence of acute rejection with ketoconazole. Our current study is different from theirs in many aspects. In addition to a larger population and longer follow-up, our transplant recipients were more heterogeneous where the majority of patients were African Americans (64% in Group 1). Deceased donor kidneys (74%) rather than living-donor kidneys were the dominant allografts and about 30% of patients experienced DGF after transplants. Many patients were also highly sensitized and/or poorly matched with donors. Therefore, our patients would be considered to have higher risk for acute rejection[19,20]. This may explain the difference in the results. Similar to their studies, we did not found any toxic side effect of using low dose of ketoconazole in kidney transplant patients.

In HIV-infected transplant patients, we have experienced difficulties in dosing CNI. As reported previously, the acute rejection rates were unexpectedly high (31% at 1 year, and 41% at 3 years) in HIV-infected kidney recipients despite the fact that their targeted trough levels of CNI were similar to non-HIV patients. More than half (52%) of acute rejection episodes did not respond to steroid therapy[6]. Even in HIV-infected liver transplant recipients, about half of the acute rejections occurred within the first 3 wk of transplant[7]. The protease inhibitor (also a potent inhibitor of P450-3A and P-glycoprotein) used to control HIV infection in these patients likely changed the normal pharmacokinetic curve of CNI. Jain *et al*[11] found that the pharmacokinetic curves of tacrolimus in these patients did not show a normal peak-and-trough pattern, but rather resembled a flat line. Recently, van Maarseveen *et al*[21] studied the pharmacokinetics of tacrolimus in patients receiving ritonavir. It was found that their pharmacokinetic curves lacked an absorption peak every 12 h. When similar trough level was targeted, their mean 12-h AUC was approximately 44% lower than the AUC in HIV-negative recipients. Therefore, the authors suggested that the trough levels of tacrolimus in the HIV-positive patients receiving ritonavir should be approximately 40% higher compared to HIV-negative recipients in order to achieve an equivalent exposure (AUCs) of tacrolimus. Indeed, the previous study also noted that a higher tacrolimus trough level was associated with a decreased risk of first rejection (HR = 0.90; 95%CI: 0.81-1.00; P = 0.04) in HIV-infected transplant patients[6].

Interestingly, a recent study found that HIV could infect the transplanted renal allografts despite undetectable viremia. The reinfection of HIV in tubular cells was hypothesized to stimulate immune responses and increase the risk of rejection[9]. A dysregulated immune response in HIV-infected host was also proposed by others[6,7]. However, a French report showed similar rejection rates (15%) after kidney transplants in HIV-infected patients *vs* non-infected patients[22]. They attributed the lower rejection rate to the use of raltegravir (an integrase inhibitor)-based antiviral therapy, which does not inhibit P450 system. Subsequently, their patients had "normal" exposures to CNI. Lack of higher incidence of rejection in the French study does not support the hypothesis of either viral infection or dysregulated immune response as the predominant mechanism for the higher rejection rates observed in the United States study. Taken together, a more plausible explanation appears to be lower exposure to CNI due to co-administration of protease inhibitor in these HIV-infected recipients.

We speculate that similarly altered pharmacokinetic phenomenon would exist in our patients, which could explain our result. Co-administration of ketoconazole lowered the dose of tacrolimus and flatted the normal peak-and-trough curve, therefore, decreased the AUC of tacrolimus and increased the risk of acute rejection.

Our study is limited by its retrospective nature, single center data, and lack of peak level monitoring and AUC data of tacrolimus. Nevertheless, it is the first report of high risk of rejection associated with coadministration of ketoconazole with tacrolimus in HIV-negative transplant recipients. This is consistent with the results in HIV-infected transplant patients. It is an important issue for car-

ing those patients with financial difficulty. This issue may be particularly relevant in the developing countries where co-administration of an inexpensive P450-3A inhibitor is a common practice to cut costs associated with expensive CNI. Our data suggests that high vigilance and careful monitoring is necessary, especially if other risk factors of rejection are present. Clearly, a prospective, randomized or a self-controlled study is needed to characterize the pharmacokinetic curves of tacrolimus with ketoconazole, so that a higher trough level can be proposed for clinical practice.

COMMENTS

Background

Tacrolimus is mainly metabolized by cytochrome P450 enzymes and ketoconazole is a potent inhibitor of P450. Transplant programs often use ketoconazole to reduce the tacrolimus dose and financial cost. But the long-term safety of this combination in kidney transplant recipients remains to be studied.

Research frontiers

This experience in human immunodeficiency virus (HIV) infected patients has noted unexpected high incidences of acute rejection after kidney and liver transplants. In these patients, the targeted trough levels of calcineurin inhibitors (CNIs) were consistent with those in non-HIV recipients, but their required doses of CNI were significantly lower due to the necessary treatment with antiretroviral protease inhibitor, which inhibits P450 enzymes.

Innovations and breakthroughs

Due to the altered pharmacokinetics of CNI by P450 inhibition from protease inhibitor, the total exposure to CNI (area under curve) is considerably low. This may explain the higher-than-expected rejection rates in HIV infected patients. In the current study, the authors have reported similar outcome from the coadministration of ketoconazole with tacrolimus in non-HIV infected kidney transplant recipients.

Applications

Coadministration of a P450 inhibitor to cut the dose and cost of tacrolimus may increase the risk of graft rejection. High vigilance and careful monitoring is necessary. A prospective, randomized or a self-controlled study is needed to characterize the pharmacokinetic curves of tacrolimus with ketoconazole, so that a higher trough level can be proposed for clinical practice.

Terminology

Kidney transplant: remove a kidney from a donor and put it into a patient with kidney failure; Rejection: recipient body attacks donor kidney as a foreign object; tacrolimus: a key drug to prevent rejection; ketoconazole: a cheap antibiotics that inhibits the breakdown of tacrolimus, therefore, saves the dose and cost of tacrolimus.

Peer review

This is a needed study exploring the outcome of attempting to reduce the dose of tacrolimus by adding ketoconazole to the regimen in first kidney transplant recipients to reduce the cost of immunosuppressive drugs. The interest of this work lies primarily in the observation of the authors regarding the five-year cumulative incidence of acute rejection in the group with ketoconazole and the consequently conclusion that the use of ketoconazole was an independent risk of acute rejection. The overall study is correct and the literature is to date.

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CASE REPORT

Recurrent epiploic appendagitis and peritoneal dialysis: A case report and literature review

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Abstract

Epiploic appendagitis (EA) is rare cause of acute or subacute abdominal pain in patients on peritoneal dialysis (PD), where the diagnosis can be challenging as the clinical features, laboratory markers and imaging characteristics have not been described previously in this group of patients. Here, we present the management of a case of EA in a patient on PD and review published literature pertinent to the subject. The importance of establishing the diagnosis early by laparoscopy is emphasised.

Key words: Epiploic appendagitis; Peritoneal dialysis; Abdominal pain; Diagnosis; Laparoscopy

Core tip: The diagnosis and management of a patient with epiploic appendagitis (EA), who presents with acute abdominal pain, can be challenging. A high index of suspicion, exclusion of other causes of acute abdominal pain by computerised tomographic scan and an a low threshold for an early diagnostic laparoscopy is the way forward in establishing the diagnosis of EA and preservation of peritoneal dialysis catheter.

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INTRODUCTION

Epiploic appendagitis (EA) is a rare cause of acute or subacute abdominal pain, where the diagnosis can be challenging, particularly in a patient on peritoneal dialysis (PD)[1]. Lack of pathognomonic clinical, laboratory and radiological findings can pose significant difficulty in diagnosis and differentiation of EA from common acute abdominal pathologies. EA is a self-limiting condition with a benign clinical course, which can be managed conservatively, if the diagnosis can be made through exclusion. A Medline search on the management of EA on PD patients showed no publications on this subject. Here, we present the management of patient on PD presenting with left iliac fossa pain (LIF), where the diagnosis of EA was confirmed by laparoscopy and review the pertinent published literature.

CASE REPORT

A 61-year-old female, who was on peritoneal dialysis for 2 years for renal failure secondary to *Escherichia coli* sepsis in the past, had presented with sudden onset of continuous severe LIF in the early hours of the morning which was exacerbated by movements. There was no associated fever, vomiting, change in bowel habit or haemodynamic instability. There was marked tenderness and guarding in the LIF inferolateral to the exit site of the PD catheter. However, there was no clinical evidence of infection involving the exit site, catheter tunnel or peritonitis. The complete blood count was normal, but the C-reactive protein (CRP) was raised to 16 mg/L (normal range, 0-5

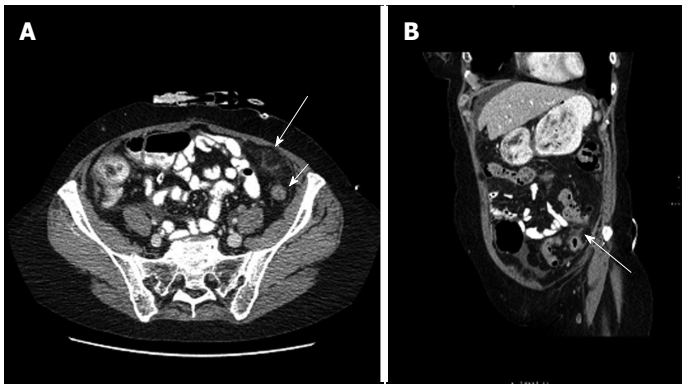

Figure 1 Computed tomography. A: Axial computed tomography (CT) scan of the abdomen showing a fat density area with some surrounding inflammation (large arrow) anterior to the descending colon (small arrow) in the left iliac fossa just below the abdominal wall at the site of epiploic appendagitis; B: A coronal oblique CT image showing the fat density area with surrounding inflammation (arrow) adjacent to the descending colon.

mg/L). The cytology of PD fluid showed a white blood cell count of 251/mm^3 with 94% polymorphonuclear leucocytes with negative culture. An ultrasound (US) scan and a non-contrast computerised tomographic (CT) scan of abdomen were unremarkable. She was treated with analgesics, intraperitoneal Vancomycin (2 gm) and Gentamicin (32 mg) on a presumed diagnosis of low-grade peritonitis. Five days later her abdominal pain had subsided significantly, hence she was discharged home.

Two weeks later, she presented with recurrent pain in the LIF, which on one occasion was very intense mimicking acute mesenteric ischaemia requiring repeated administration of morphine. Abdominal examination revealed tenderness and guarding in the LIF. The white cell count was normal, but the CRP was elevated to 67 mg/L. The culture of the PD fluid was sterile. Arterial blood gas and blood lactate were normal. A repeat contrast enhanced CT scan of abdomen showed patent coeliac axis, superior and inferior mesenteric arteries with normal perfusion of the intestine. However there was small amount of fluid in the pelvis and a fat density area with some surrounding inflammation anterior to the descending colon in the left iliac fossa just below the abdominal wall, which was suggestive of the possible diagnosis of epiploic appendagitis (Figure 1). A flexible sigmoidoscopy and transvaginal US scan were normal.

As the abdominal pain was intractable and persistent after 6 wk of treatment, under general anaesthesia, the PD catheter was removed and a laparoscopy was carried out using the PD catheter entry site. The greater omentum was adherent in the LIF where a small area on the anterior surface of the middle of the sigmoid colon was adherent to anterior abdominal wall over the site of maximum tenderness. There was evidence of inflammation on the anterior surface of the sigmoid colon where appendix epiploicae were present at the site of fibrinous adhesion, indicating epiploica appendagitis (Figure 2). Rest of the sigmoid colon and the abdominal organs were normal. The adhesion was released laparoscopically after inserting a 5 mm port in the right iliac fossa and a new PD catheter was replaced. This led to complete resolution of symptoms and restoration of PD.

DISCUSSION

Epiploic appendages are peritoneal outpouching of adipose tissue on the colonic surface, 50-100 in number, measuring 0.5-5 cm in length. The majority of them are situated on the sigmoid and descending colon, which explains the preponderance of EA in the left side of the abdomen, compatible with the finding in our case. Each appendage is supplied with one or two arteries from the colonic vasa recta and drained by a single vein[2]. These vascular structures are extremely mobile and susceptible to ischaemia, inflammation and necrosis if torsion, kink or stretching occurs[3].

Since the first report of EA in 1959 by T Case, several case series have been described in the normal population[4], but the clinical features and management of EA in a PD patient has not been reported previously. The primary EA is believed to occur spontaneously, whereas secondary EA occurs as a result of adjacent inflammatory diseases such as diverticulitis, appendicitis and cholecystitis[5,6].

The presenting symptom of EA is a localised non-migratory pain on the LIF, in the absence of severe illness, which is exacerbated by movements. Localised and rebound tenderness are usually present. There is lack of significant inflammatory response, particularly at the time of initial presentation. In PD patients, the nature of pain may be different if the inflamed appendage is adherent to the parietes and repeated distension of the abdominal wall during PD fluid exchanges may lead to stretching of the sensitive parietal peritoneum and severe pain. A high index of suspicion of EA after exclusion of acute diverticulitis, appendicitis, haemorrhagic ovarian cyst, torsion of an ovarian cyst, pelvic inflammatory diseases and mesenteric lymphadenitis, delayed perforation of bowel by the PD catheter or ischaemia of intra-abdominal organs, is necessary. Epiploic appendagitis adherent to the abdominal wall can lead to complications such as intestinal obstruction form torsion of the small intestine, strangulation, ileus and catheter drainage problems[7-10].

The patients on PD are more prone to serious intra-abdominal pathology than patients on haemodialysis due to an effect of PD to impair the normal physiological response of the peritoneal membrane[11]. An early diagnosis of EA by excluding all causes of surgical peritonitis is paramount in PD patients. An undiagnosed peritonitis in PD patients caused by perforation of intestine or intestinal ischaemia can prove fatal. The presence of pneumo-

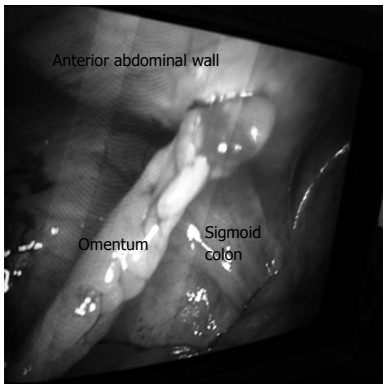

Figure 2 Laparoscopic appearance showing the omentum and anterior surface of the sigmoid colon adherent to the peritoneum lining the anterior abdominal wall in the left iliac fossa.

peritoneum visible in erect chest-X-ray and CT scan may not be sensitive or specific for a perforated hollow viscus since this may be present in 3.7% of healthy PD patients and in 0%-11% in patients with bowel perforation[12].

Laboratory findings specific to EA could be insignificant according to several studies. Mild leucocytosis and a low-grade increase in CRP is accepted as markers of mild inflammation[13]. The diagnostic yield of radiological investigations such as US, CT and magnetic resonance (MR) scan is considered to be high, although the findings are not specific for EA. Epiploic appendages cannot usually be seen during radiological imaging unless inflamed or associated with gross ascites or haemoperitoneum. EA may be diagnosed with US scan, with findings of solid, oval, non-compressible hyperechoic mass under the site of maximal tenderness, and lack central blood flow on Doppler US scan[14]. The CT features of EA are characteristic according to several studies, which show a pericolonic ovoid mass with hyperattenuating rim surrounded by fat stranding[15,16]. The anterior localisation of EA in relationship to the colonic lumen is mentioned as a usual finding in making a confident diagnosis. The other fatty lesions that can mimic US and CT findings of acute EA are chronic calcified EA, omental infarction, post-operative changes, and peritoneal carcinomatosis[17,18]. The MR scan findings include an oval shaped fat intensity mass with a central dot on T1- and T2-weighted images, which possessed an enhancing rim on post-gadolinium T1-weighted fat saturated images. The lesion is best visualized on post-contrast T1-weighted fat saturated images[19]. The only concern about MR scan is the risk of nephrogenic systemic sclerosis due to gadolinium.

The majority of patients with EA recover spontaneously within less than four weeks with conservative management[20]. Recurrence of symptoms have been reported in up to 40% of cases, where surgery in the form of laparoscopic or conventional open excision of the inflamed epiploic appendix may be needed[13]. Establishment of an early and definitive diagnosis of EA may not be straightforward, in PD patients, as it happened in our case. There was a delay of 6 wk before a diagnostic laparoscopy was performed. We believe that a low threshold for diagnostic laparoscopy should be maintained if the symptoms fail to resolve within a week or the diagnosis remains uncertain[21,22]. We did remove the original catheter and used the catheter entry site to introduce a port and telescope. In retrospect, laparoscopy without removal of catheter could have been performed, thereby avoiding the need of removal and replacement of PD catheter.

In a conclusion, establishment of the diagnosis of EA in patients on PD can be challenging, hence a diagnostic laparoscopy should be undertaken early to establish the diagnosis, exclude any other abdominal conditions and retain the PD catheter.

COMMENTS

Case characteristics

A 67-year-old female, on peritoneal dialysis (PD), presented with recurrent attacks severe left iliac fossa pain.

Clinical diagnosis

There was marked tenderness and guarding in the left iliac fossa.

Differential diagnosis

Consideration of acute diverticulitis, appendicitis, haemorrhagic ovarian cyst, torsion of an ovarian cyst, pelvic inflammatory diseases and mesenteric lymphadenitis, delayed perforation of bowel by the PD catheter or ischaemia of intra-abdominal organs, in the differential diagnosis is necessary.

Laboratory diagnosis

An elevated C-reactive protein was present.

Imaging diagnosis

Computed tomography scan of the abdomen showed inflammatory changes in the left iliac fossa (LIF) suggesting the possibility of epiploic appendagitis (EA).

Pathological diagnosis

Laparoscopy revealed adhesion of the sigmoid colon to the anterior abdominal wall with an appendix Epiploica was present.

Treatment

Laparoscopy and adhesiolysis led to complete resolution of symptoms.

Experiences and lessons

Recurrent pain over the LIF is patient on PD in the absence of classical clinical and radiological features should prompt a clinician to consider the diagnosis of EA and perform a diagnostic laparoscopy to establish the diagnosis.

Peer review

This case report is suitable for publication.

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CASE REPORT

Innocuous-looking skin scab over an arteriovenous fistula: Case report and literature review

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Author contributions: Shrestha B managed the patient and prepared the manuscript; Simon Boyes was involved in preparing the manuscript; Brown P performed radiological investigations and contributed to manuscript preparation.
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Abstract

Little is written on the management of an innocuous-looking skin scab over an autogenous arteriovenous fistula (AVF) used for haemodialysis. The seriousness of the underlying pathology can be under-estimated, and this may lead to early loss of the AVF, and major-life-threatening haemorrhage. We describe the management of a 78-year-old patient presenting with an innocuous-looking scab over an AVF and review the pertinent literature on this subject.

Key words: Arteriovenous fistula; Scab; Duplex scan; Bleeding; Treatment

Core tip: An innocuous looking scab may develop over an arteriovenous fistula after repeated punctures. There may be serious underlying damage to the arterialised vein. This case report emphasizes the importance of an early diagnostic colour Doppler examination in these circumstances and timely surgical intervention, to prevent potential life-threatening haemorrhage or other complications.

Shrestha B, Boyes S, Brown P. Innocuous-looking skin scab over an arteriovenous fistula: Case report and literature review. *World J Nephrol* 2014; 3(3): 118-121 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/118.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.118

INTRODUCTION

An arteriovenous fistula (AVF) is a crucial life line for patients with end-stage renal failure, and its preservation is paramount. Complications related to AVF such as puncture haematoma, pseudoaneurysm formation, venous hypertension, arterial steal, high-output cardiac failure, and ischemic neuropathy, can not only lead to the early loss of an AVF but also can be life-threatening[1,2]. The prevalence of AVF compared to arteriovenous grafts, has risen steadily as a result of the National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF/KDOQI) recommendations[3,4]. Little has been written on the pathological anatomy, natural history and management of visible scabs in the skin overlying AVF cannulation sites. We describe the management of a 78-year-old patient presenting with an innocuous-looking scab over the cannulation site of an AVF, emphasise the importance of early recognition and intervention, and review the pertinent literature related to the subject.

CASE REPORT

A 78-year-old male with renal failure secondary to obstructive uropathy had been on haemodialysis (HD) for two years using a brachiocephalic AVF. The AVF was cannulated using rope-ladder technique three times a week for 3 h in each session leading to excellent dialysis (pA: -71, pV: 73, blood flow: 360 mL/min, Kt/V: 1.4, pre-HD serum creatinine: 600 μmol/L and post-HD serum creatinine: 142 μmol/L). His haemoglobin was 12.2 gm/dL, white cell count of 7.5×10^6 / mL and C-reactive

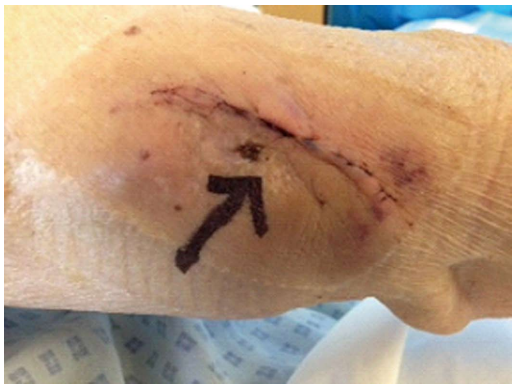

Figure 1 A 3 mm size scab over the arteriovenous fistula (black arrow).

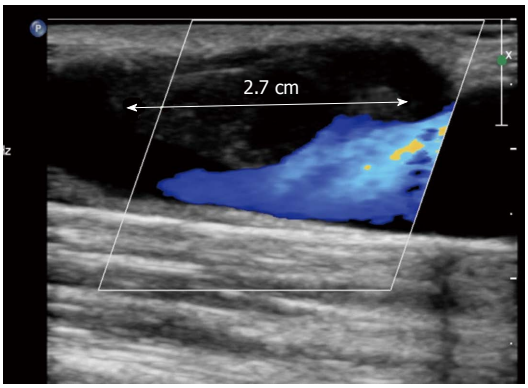

Figure 2 Colour Doppler scan showing a 2.7 cm long thrombus (white arrow) partially occluding the lumen of the arteriovenous fistula.

protein of 25 mg/L. His regular medication was erythropoietin, alphacalcidol and simvastatin.

The patient, who normally dialysed at a satellite unit, was referred to our tertiary referral teaching hospital because the dialysis staff were concerned about a visible black scab on the distal needling site which had developed over the previous 2 wk. There was no history of bleeding post-dialysis.

On examination, there was a 3 mm-sized black scab on the skin at the distal cannulation site without surrounding cellulitis or discharge (Figure 1). There was no clinical evidence of pseudo-aneurysm or subcutaneous haematoma. A palpable thrill and a low-pitched bruit were present along the course of the arterialised upper arm cephalic vein. The initial clinical impression was that the scab represented needle tract thrombus, which could be managed conservatively. However, based on previous experience of managing similar cases, a Doppler ultrasound scan was carried out. This showed a thrombus under the scab measuring 2.7 cm in length narrowing the vein significantly (Figure 2). It was suspected that although the size of the scab on the skin surface was about 3 mm in diameter, the defect in the cephalic vein wall could be more than 2 cm. A decision was made to explore the fistula and repair the defect.

Under general anaesthesia, through an incision parallel to the AVF, the arterialised cephalic vein was skeletonised and controlled proximal and distal to the scab. The skin containing the scab was dissected off the vein, and this revealed dark thrombus bulging out through a 2.5 cm long defect on the anterior wall of the vein. The thrombus was causing partial narrowing of the vein (Figures 3 and 4). The thrombus was removed and the vein filled proximally and distally with heparinised saline. The edge of the defect on the vessel wall was debrided and closed using continuous 4/0 prolene suture. The thrombus was sent for culture and remained sterile after 48 h of incubation. The AV fistula was used for HD after 24 h.

DISCUSSION

Dialysis access procedures and access complications are the leading causes of morbidity among HD patients, often resulting in an increased number of hospitalizations and missed dialysis sessions. These complications, such as erosion, infection, thrombosis, and pseudoaneurysm formation, burden the health care system and disrupt patient care. As such, limiting these complications can significantly improve the quality of life of dialysis patients whilst decreasing health care costs[5,6]. The trauma of recurrent cannulation of a functioning AVF through the same site can lead to a tear of the wall of the vein. Simultaneous heparinisation during HD allows blood to flow out through the tear leading to the formation of a small pseudoaneurysm. Thrombus can form within the pseudoaneurysm in hypotensive and hypercoagulable states. The size of the pseudoaneurysm may be difficult to estimate on physical examination. The incidence of pseudoaneurysms complicating AVF ranges from 2% to 10%[7]. Generally, investigation of an innocuous-looking scab over an AVF is seldom initiated unless there is bleeding post-HD from the cannulation site, an obvious pseudoaneurysm, recirculation or venous hypertension[8].

Our case illustrates the fact that although the scab was only 3 mm in diameter with no evidence of surrounding inflammation, it was associated with a 2.5 cm long defect in the vein wall. The defect was filled with a thrombus which was also narrowing the lumen of the vein. The appearances were consistent with a thrombosed pseudoaneurysm.

Repeated needling of thrombus within a pseudoananeurysm may cause infective complications which may lead to bacteraemia and systemic sepsis. There is also a risk of necrosis of the overlying skin, ulceration and a life-threatening haemorrhage[9,10]. Dislodgement of the thrombus can lead to fatal pulmonary embolism. Recognition of the potential danger of small skin scabs was important in the timely investigation and intervention in our patient. Not only was the AVF salvaged, but the potential hazard of massive bleeding was prevented.

When assessing patients with skin scabs, a history of bleeding post-dialysis, adequacy of dialysis and systemic sepsis should be obtained. Physical examination should include the inspection of skin overlying the AVF for the extent of skin necrosis, cellulitis, pulsatility and haematoma, as well as assessment of the nature of the bruit along the length of the AVF. Duplex ultrasound is the

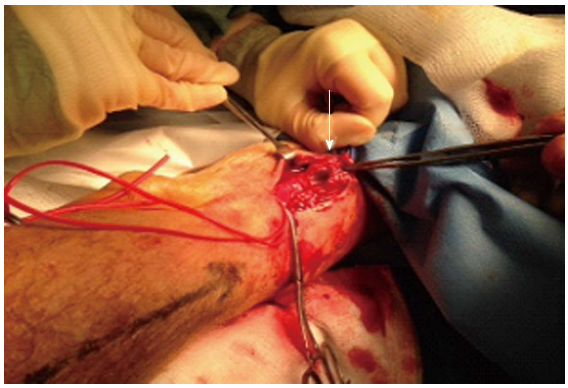

Figure 3 A 2.5 cm long defect on the anterior wall of the vein (white arrow).

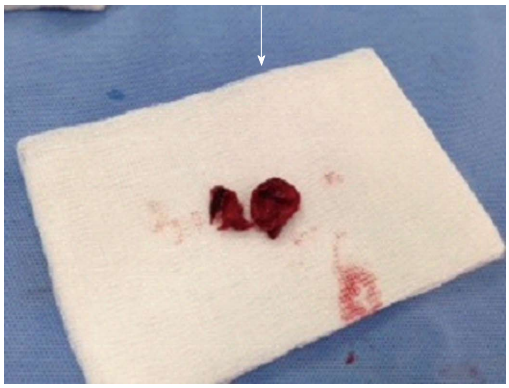

Figure 4 Thrombus removed from the defect in the vessel wall.

primary diagnostic modality, with a sensitivity of 94% in the assessment of the size of the defect, and complications such as the presence of thrombus, reduced blood flow, stenoses and pseudo-aneurysms[11]. Ultrasound-guided compression of pseudoaneurysms can be curative[12]. If a defect is detected in the wall of the AVF, the patient should be prepared for exploration of the AVF under brachial block or general anaesthesia. Broad-spectrum antibiotic cover should be provided at the time of surgery. Surgical repair of the defect can be done by direct suture, patch angioplasty using saphenous vein or a Dacron patch, excision followed by end-to-end anastomosis, excision and interposition of a Polytetrafluroethylene graft or insertion of a bypass graft[13]. A large skin defect due to necrosis may require a locally rotated full-thickness Limberg skin flap[14]. Any thrombus extracted from the AVF should be sent for microbiological assessment. In majority of the patients, the AVF can be salvaged and used for HD within 24 h. If a bypass graft is inserted, the reported primary-assisted patency at 12 mo is 50%[15].

Every effort should be made to prevent the development of pseudoaneurysms, venous stenoses, skin erosions, haemorrhage and reduced access patency. The cannulation technique of an AVF has been a subject of debate. The rope-ladder (RL) technique is the most common technique, although button-hole cannulation (BHC) or constant-site technique is recommended by the NKF/KDOQI vascular access guidelines. Previous studies have shown less haematoma formation and less intervention in BHC group compared to the RL group[16-19]. However, a recent study comparing access patency, intervention rate, bacteraemia and mean scores from kidney disease-specific health-related quality of life (KD HRQOL-36) survey between RL and BHC groups failed to show a significant difference in outcome between the two groups[20].

In conclusion, awareness of the possibility of a large defect in the vein wall under an innocuous-looking scab, with the potential for life-threatening haemorrhage and access failure, should be raised among patients and health care workers[21]. If there is clinical concern an urgent Doppler ultrasound should be arranged, with urgent surgical intervention if indicated. It is also essential that trained staff follow established cannulation techniques to reduce the risk of puncture site complications.

COMMENTS

Case characteristics

A 78-year-old male, who was on haemodialysis using a brachiocephalic arteriovenous fistula (AVF), was referred by the dialysis staff raising concern for a scab over the cannulation site.

Clinical diagnosis

On examination, there was a 3 mm-sized black scab on the skin on the distal cannulation site without surrounding cellulitis or discharge.

Differential diagnosis

A pseudoaneurysm and a haematoma underlying the scab were entertained in the differential diagnosis.

Laboratory diagnosis

The white cell count and C-reactive protein were within normal limits.

Imaging diagnosis

The Duplex ultrasound scan showed a 2.7 cm large thrombus and a 2 cm defect in the vessel wall.

Pathological diagnosis

A 2.5 cm defect in the wall of the cephalic vein was occupied by a thrombus.

Treatment

The vessel was explored and the defect was closed with 4/0 prolene suture.

Experiences and lessons

The size of the visible scab over an autogenous AVF is misleading as in our case where there was a 2.5 cm defect in the vessel wall filled with a thrombus although the size of the scab was of 3 mm diameter. A Duplex san should be performed early to assess the AVF for prevention of bleeding.

Peer review

The authors described the management of a 78-year-old patient who presented with an innocuous-looking scab over the cannulation site of an AVF which turned out to be a thrombus on the outer wall of the vein. This a good case report.

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Acknowledgments

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