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EDITORIAL

Nephroprevention in the oldest old with chronic kidney disease: Special considerations

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Abstract

Nephroprevention strategies are crucial for handling chronic kidney disease (CKD) complications, and slowing its progression. However, these preventative measures should be guided by major geriatrics principles in order to help nephrologists to adequately handle the oldest old with CKD. These geriatric concepts consist of taking into account the relevance of choosing an individualized therapy, handling clinical frailty, and keeping a geriatric perspective which means that a good quality of life is sometimes a more important therapeutic objective in octogenarians than merely prolonging life. Even though nephroprevention strategies for treating the oldest old with CKD are basically similar to those applied to younger patients such as low sodium and protein diet, optimized hemoglobin levels, blood pressure and metabolic control, the treating physician or care provider must at all times be ready to make fundamental adjustments and tweak patient care paradigms and objectives if and when the initial therapeutic options applied have caused unintended clinical consequences and complications. Additionally, the sarcopenia status should also be evaluated and treated in very old CKD patients.

Key words: Oldest old; Very old; Nephroprevention; Chronic kidney disease; Chronic nephropathy

Core tip: Even though nephroprevention in the oldest old is basically similar to those applied to younger patients, it should be performed applying a geriatric perspective, where good quality of life is sometimes a more important therapeutic objective than merely prolonging life.

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INTRODUCTION

Nephroprevention strategies are crucial for handling chronic kidney disease (CKD) complications, and slowing its progression[1,2]. The term "CKD" is inter-

preted as a estimated glomerular filtration rate < 60 mL/min per 1.73 m² measured by the modification of diet in renal disease equation and/or presence of proteinuria, at least 1+, on dipstick urinalysis[3]. The term "nephroprevention" is defined as all those habits, diets, and medications which are currently proposed as useful therapeutic tools for achieving this purpose, such as avoidance of sedentary lifestyles, smoking, high sodium and high protein diets, as well as effectively managing disease states such as hypertension, dyslipidemia, hyperglycemia, and hyperparathyroidism[1,2]. However, these preventative measures should be guided by major evidence-based geriatrics concepts in order to help nephrologists to adequately handle oldest old people with CKD[2,4-9]. In this article, the "oldest old" is defined as people older than 79 years, according to the definition adopted by the most relevant literature in this field[10,11]. The first geriatric concept involved in this care model consists of the relevance of choosing an individualized therapy since treatment outcomes in the oldest old are influenced by many clinical variables which can persuade nephrologists to use alternate therapeutic approaches for treating patients in this category. Such variables include changes secondary to ageing (immunesenescence, reduced glomerular filtration rate and reduced hepatic metabolism), polypharmacy (use of $\geqslant$ 6 medications), prevailing elderly diseases (depression, visual and hearing impairment), and the concomitant presence of other geriatric syndromes (delirium, falls, and postration)[7,12-16].

The second geriatric concept consists of prescribing treatment paradigms under a geriatric perspective. This means that a good quality of life is sometimes a more important therapeutic objective in octogenarians, than merely achieving a lower mortality[2]. This does not mean that very old patients should be undertreated but that their treatment should be adjusted to their real biological expectations, while being cognizant of the increased potential for therapeutic adverse effects[2]. Finally, the third geriatric concept refers to the imperative for taking into account the notion of clinical frailty in the elderly. Frailty is an entity which appears as a consequence of many causes and it is characterized by a reduction in strength and endurance, making people prone to lose autonomy and to die[9]. Such therapeutic strategies are based on the prescription of low intensity resistance and aerobic physical exercises, together with adequate nutrition, appropriate vitamin D supplementation, and the avoidance of polypharmacy, all of which measures may help prevent or delay the onset of this syndrome[9,15,17].

In the present article, we have expanded on the following particular therapeutic targets to include the following-dietary salt, serum hemoglobin, blood pressure, glycemic control and lipid management-in the oldest old with CKD patients (Table 1).

Table 1 Therapeutic targets for oldest old chronic kidney disease patients

	Targets
Diet	Low-normal sodium Low-normal protein
Hemoglobin (g/dL)	11-12
Blood pressure (mmHg)	150/140-80
Hemoglobin A1C (%)	7-8.5

DIETARY SALT

There is a trend to sodium urine loss in the elderly due to their reduced sodium reabsorption capability at the thick ascending limb of the loop of Henle and collecting tubules[18]. Consequently, it is important to take into account that when the oldest old become salt restricted (50 mmol/d), they may develop hyponatremia (senile sodium leakage hyponatremia), volume depletion (ortostatism, hypotension), and even acute renal failure[19,20]. Whereas low sodium diet is one of the cardinal features of nephroprevention[19], this paradigm of care, when applied to the oldest old should be followed by monitoring blood pressure, serum sodium level, and renal function in order to rule out any of the above mentioned complications. If and when such unintended consequences are detected, a normal sodium diet would then be a better prescription for the specific oldest old patient with CKD[19,20].

SERUM HEMOGLOBIN

It has been reported in the literature that the presence of anemia can exacerbate several existing geriatric syndromes together with exaggerating neurocognitive dysfunction[21]. Therefore the oldest old often do not tolerate reduced serum hemoglobin levels as such low hemoglobin levels could negatively impact on gerontological functional test [activities of daily living (ADL) and instrumental ADK], and furthermore lead to an increased tendency to develop delirium and/or falls[22,23]. Thus, the application of a target serum hemoglobin level of 11 g/dL, or less, as part of nephroprevention strategy in the oldest old must be followed by monitoring their cognition status and gait pattern in order to exclude de novo appearance of those geriatric syndromes that are enhanced by anemia[21-24]. If this situation is documented in a particular older old with CKD, a higher serum hemoglobin target (11.5-12 g/dL) will therefore represent a reasonable alternative of care.

BLOOD PRESSURE

Meta-analysis of observational studies indicate that the incidence of stroke, myocardial infarction, and overall mortality increased with increasing blood pressure in

old and vey old patients, although the observed relative risk decreased with increasing age[25]. Additionally, the INVEST study highlighted a J-shaped relationship between systolic and diastolic blood pressure and outcomes in hypertensive old people suffering from coronary arterial disease[25]. The risk of mortality in patients aged ⩾ 80 years increased when systolic blood pressure was < 140 mmHg or diastolic blood pressure < 70 mmHg[25]. Although it has been documented that anti-hypertensive treatment in the oldest old was associated with a reduction in the frequency of strokes and major cardiac events, there was however no benefit in cardiovascular death nor in general mortality[26]. Furthermore, the evidence-base provided by several studies (INVEST, STONE, HYVET) is reassuring regarding targeting relatively higher blood pressure levels in the very elderly-blood pressure target < 150-80 mmHg-although these aforementioned studies did not specifically address CKD patients[25]. Nonetheless, it has been recommended that target blood pressure in the oldest old with CKD should be < 150/90 mmHg in non-albuminuric patients, and < 140/80 mmHg in abuminuric ones[25]. Very importantly, these blood pressure goals should be reached gradually, and the treating physician must always take into account each individual patient´s comorbidities[7,17]. This is to avoid the interdependence phenomenon between diseases (comorbidities worsen each others) usually observed in the elderly and their reduced tolerance to medication; since treatment of hypertension in the oldest old can induce orthostatic hypotension, falls with bone fractures, and the exacerbation of renal failure which sometimes then is not reversible on drug discontinuation[20,27-46]. In this sense, it has been reported in the literature a clinical entity termed "normotensive acute renal failure" which consists of an acute GFR deterioration in CKD elderly when their blood pressure is reduced to normal range. This phenomenon has been attributed to reduced kidney perfusion secondary to senile renal dysautonomy[47]. Moreover, it is worth noting here that concomitant sodium sensitivity and endothelial dysfunction are increased in the very elderly population, and therefore low sodium diet (used with caution) and exogenous nitric oxide donors are often useful for treating resistant hypertension in this group[17]. Other antihypertensive drugs such as thiazides, angiotensin converting enzyme inhibitors (ACEI), angiotensin Ⅱ receptor blockers (ARB), and aldosterone antagonists should be used with caution in this population, more so when GFR is below 30 mL/min per 1.73 m² due to the risk of further GFR reduction precipitating renal failure which may be irreversible, together with the complicating electrolytes and acid-base disorders[20,25-48]. The syndrome of rapid onset end stage renal disease (SORO-ESRD), a new syndrome of unanticipated acute yet irreversible ESRD, which we first described in 2010, is known to be more prevalent in the older CKD patient, and is associated with exposure to nephrotoxic agents including ACEIs and ARBs[37,38,42,43].

HEMOGLOBIN A1C

Elderly people are at high risk for developing diabetes mellitus because of the following two mechanisms: insulin resistance and pancreatic islet senile dysfunction[49,50]. Besides, ageing alters the counter-regulatory responses to hypoglycemia in non-diabetic people; furthermore during hypoglycemic episodes, symptoms begin at higher levels of glycemia and they are more intense in young people, while psychomotor coordination is more affected in old individuals[50] Additionally, diabetes mellitus is usually associated with high comorbidity in old people, and this subgroup cannot obtain cardiovascular benefit from strict glycemia control[49,50]. Moreover, therapeutic strategies with less stringent A1c levels are therefore needed in the oldest old diabetic patients since this subgroup of patients assist double to the hospital due to hypoglycemia episodes than the general diabetic patients, and it has also been documented that hypoglycemia is related to cognitive impairment in the elderly[49,50]. Thus, the consensus recommendation is a hemoglobin A1c target < 8% for elderly patients (not < 7% as is usually recommended for young adults) or for those patients with major complications and/or comorbid conditions[50]. Finally, a hemoglobin A1C target of 8%-9% has been recommended for patients with low life expectancy (⩽ 5 years)[50].

LIPID METABOLISM

Regarding lipid lowering therapy in this population, an interesting study in very elderly patients documented a 15% reduction in coronary events with pravastatin. This suggests, that this drug can be prescribed in the oldest old suffering from diabetes mellitus except in those with very poor life expectancy[50].

MISCELLANEOUS-PROTEIN DIET AND EXERCISE

Although energy needs decline with age, very elderly people can be exposed to malnutrition because of anorexia, impaired taste and smell, chewing and swallowing problems, geriatric syndromes and senile prevalent comorbidities which lead to difficulties for cooking and eating[24,50]. Because of the above predicated reasons, caution must be employed when overly restrictive eating patterns (including renal sparing low protein diet) are applied since such practices may further contribute to malnutrition in the oldest old with CKD. The interventions for improving nutritional status in the oldest old patients consist of using smaller but more frequent and fortified portions of food, and/or adding nutrition supplements between meals[24,50]. Additionally,

senile sarcopenia is a prevalent entity which can worsen with low protein ingestion, as well as with other ageing-associated comorbidities, such as diabetes mellitus[51]. Conversely, adequate physical activity, adjusted to the individual patient´s clinical situation, can further improve functional status even in patients with poor health status[50,51].

OVERALL MORTALITY IN VERY ELDERLY PEOPLE

Even though, there is some limitation of ascribing a single pathology as the cause of death in very elderly people since concomitant multiple diseases are very common in this age group, many studies have documented cardiovascular (31%-54%), oncologic (20%-25%), and respiratory (10%-15%) diseases as the first, second, and third causes of death, respectively[10,24]. However, studies performed in centenarians (people older than 90 years) have documented respiratory disease (48%-52%), particularly pneumonia, as the main cause of death. Besides, it has been observed that the higher cardiovascular causes of death in the very elderly people are acute myocardial infarction, and cardiac insufficiency secondary to cumulative damage from ischemic heart disease. Regarding oncologic diseases, malignancies of the digestive tract: primarily gastric, esophageal, and colorectal cancer, are the most frequent oncologic causes of death in very old people. Additionally, the remaining major causes of death in very elderly people are: cerebrovascular disease, Alzheimer´s disease and related dementias of later life[10,24].

CONCLUSION

Even though nephroprevention strategies for treating the oldest old with CKD are basically similar to those applied to younger patients, it is recommended that the managing physician must always individualize patient care. The treating physician or care provider must at all times be ready to readjust and tweak care paradigms and objectives if and when the initial therapeutic options applied have caused unintended clinical consequences and complications.

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REVIEW

Combined functional and anatomical diagnostic endpoints for assessing arteriovenous fistula dysfunction

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Abstract

Failure of arteriovenous fistulas (AVF) to mature and thrombosis in matured fistulas have been the major causes of morbidity and mortality in hemodialysis patients. Stenosis, which occurs due to adverse remodeling in AVFs, is one of the major underlying factors under both scenarios. Early diagnosis of a stenosis in an AVF can provide an opportunity to intervene in a timely manner for either assisting the maturation process or avoiding the thrombosis. The goal of surveillance strategies was to supplement the clinical evaluation (*i.e.*, physical examination) of the AVF for better and earlier diagnosis of a developing stenosis. Surveillance strategies were mainly based on measurement of functional hemodynamic endpoints, including blood flow (Q_a) to the vascular access and venous access pressure (VAP). As the changes in arterial pressure (MAP) affects the level of VAP, the ratio of VAP to MAP (VAPR = VAP/MAP) was used for diagnosis. A Q_a < 400-500 mL/min or a VAPR > 0.55 is considered sign of significant stenosis, which requires immediate intervention. However, due to the complex nature of AVFs, the surveillance strategies have failed to consistently detect stenosis under different scenarios. VAPR has been primarily developed to detect outflow stenosis in arteriovenous grafts, and it hasn't been successful in accurate diagnosis of outflow lesions in AVFs. Similarly, AVFs can maintain relatively high blood flow despite the presence of a significant outflow stenosis and thus, Q_a has been found to be a better predictor of only inflow lesions. Similar shortcomings have been reported in the detection of functional severity of coronary stenosis using diagnostic endpoints that were based on either flow or pressure. This limitation has been associated with the fact that both pressure and flow change in the presence of a stenosis and thus, hemodynamic diagnostic endpoints that employ only one of these parameters are inherently prone to inaccuracies. Recent attempts have resulted in development of new diagnostic endpoints that can combine the effects of pressure and flow. These new hemodynamic diagnostic endpoints have shown to be better predictors of functional severity of lesions as compared to either flow or pressure based counterparts. In this review article, we discussed the advantages and limitations of current functional and anatomical diagnostic endpoints in AVFs.

Key words: Arteriovenous fistula; Dysfunctional arteriovenous fistulas; Stenosis; Surveillance; Flow rate;

Pressure

Core tip: Current surveillance strategies are based on either flow (Q_a) or pressure (VAPR) measurements. The Q_a has only shown to be a good predictor of inflow stenosis in arteriovenous fistulas (AVFs). The VAPR was primarily developed to detect outflow stenosis in arteriovenous grafts and has shown to be a poor predictor of stenosis in AVFs. These limitations have been associated with the fact that both pressure and flow change in the presence of a stenosis and thus, hemodynamic diagnostic endpoints that employ only one of these parameters are inherently prone to inaccuracies. Thus, diagnostic endpoints that can combine both effects of pressure and flow can provide better assessment of stenosis severity in AVFs.

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INTRODUCTION

Around 600000 Americans have end-stage renal disease, among whom approximately 415000 patients are being treated by hemodialysis through surgically created vascular access (VA)[1]. Failure in maintaining a functional VA has been the leading cause of hospitalization in the hemodialysis population and has resulted in more than $1 billion annual cost to the health care system in the United States[1]. The most preferred form of the VA is the arteriovenous fistula (AVF); however, this type of access has still a significantly high failure rate (20% to 50% in the United States). AVF failure requires placement of central venous catheter which is the least desirable form of VA due to its significant morbidity and mortality[2,3].

Thrombosis is the major cause of failure in AVFs, which requires endovascular or surgical intervention of the access[4-7]. The majority of AVFs with thrombosis had an underlying stenosis[8-10]. A developing stenosis gradually reduces the blood flow to the access and alters the pressure in the AVF. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI)[11,12] has recommended routine surveillance to detect the stenosis early enough to allow preemptive interventions. Current surveillance strategies[13] include device-based measurements, such as blood flow to AVF or venous access pressure. Under the current KDOQI guidelines[12], an AVF with blood flow rate < 400-500 mL/min or a ratio of venous access pressure to main arterial pressure > 0.55 has to be referred to fistulography for assessing the grade and location of the stenosis.

The accuracy of the current surveillance strategies for detecting a stenosis is debatable, especially in AVFs[14-17]. These limitations can be associated with specific pathophysiology of AVFs[8,10]. AVFs can have a significant stenosis and still maintain a relatively high blood flow[18]. In such scenarios, blood flow to AVF gradually reduces over time. Thus, sequential (longitudinal) measurements of Q_a over time were expected to provide better clinical decision making than a single Q_a measurement. Also in case of a significant outflow stenosis, the venous access pressure may retain its normal levels at the cannulation site due to development of downstream collateral pathways[8,10,15,19]. It is noteworthy that similar shortcomings have also been reported in functional (hemodynamic) diagnosis of coronary stenosis. Such diagnostic endpoints for coronary artery disease were either based on pressure or flow; however this neglects the fact that both flow and pressure change in the presence of a stenosis. Recent studies have attempted to shift the current paradigm into introducing new diagnostic endpoints that can account for both pressure and flow variation for assessing the functional severity of a stenosis[20-24]. This review article describes the advantages and limitations of current surveillance strategies including functional and anatomical diagnostic endpoints in AVFs.

This review has covered the most important and pioneering studies that have reported the use of hemodynamic and anatomical endpoints for assessing the AVF functionality. We performed a literature search using PubMed, Medline, and Google Scholar for studies written in English from 1995 to 2014. We used the following search terms: arteriovenous fistula; surveillance strategies; stenosis; flow rate; pressure; coronary flow reserve; fractional flow reserve; pressure drop coefficient; resistance index, in combination with the exploded term "diagnosis". The references were included based on their relevance and contribution to address the challenges and future directions in the diagnostic field of stenosis linked to AVF.

AVF MATURATION, FUNCTIONALITY, AND DYSFUNCTION

According to the guidelines of NKF-KDOQI[11,12], the venous segment of a matured AVF should follow the rules of sixes: a blood flow > 600 mL/min, a diameter > 6 mm, a depth of around 6 mm, and at least 6 cm of a straight segment for cannulation. Normally, a minimum of 28 d (d: days) should be allowed for AVF maturation before performing the first needling; however, this time could be extended if the AVF fails to mature. Once an AVF is used for cannulation, it should be able to provide a minimum flow rate of 350-450 mL/min during 3-5 h of dialysis without recirculation, a characteristic that defines a functional AVF. A dysfunctional AVF is, however, defined as an access that is not able to provide the minimum flow during dialysis and is clinically identified by variations in thrill/bruit, difficult cannulation, recirculation, excessive bleeding from the venopuncture sites and

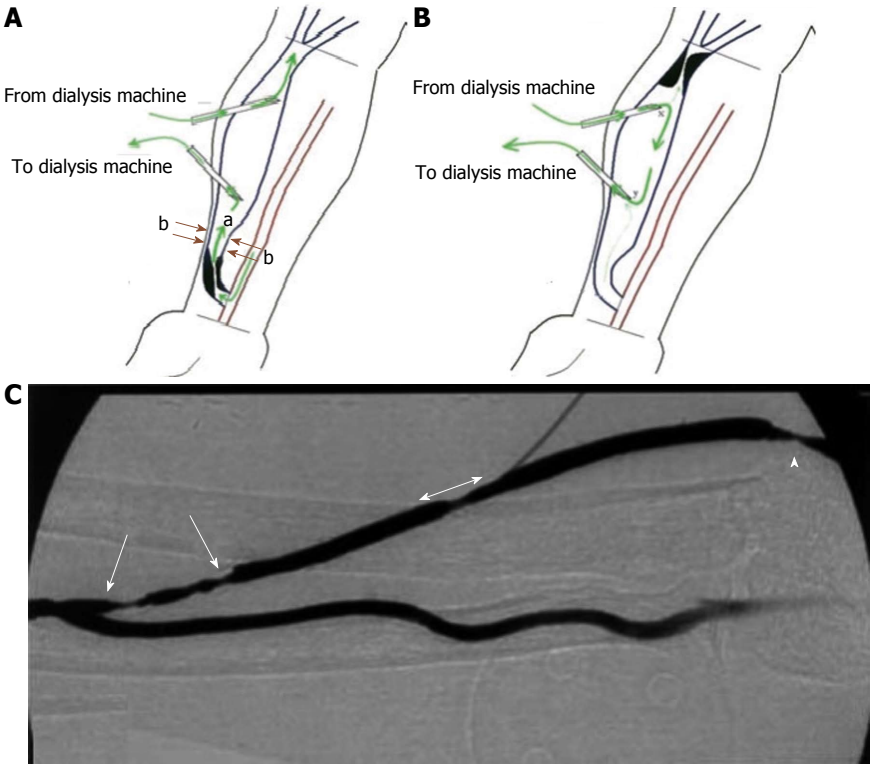

Figure 1 Schematic of the locations of (A) inflow stenosis, and (B) outflow lesion with respect to the anastomosis and cannulation sites[27]. The (green) arrows in A and B represent the direction of blood flow within the arteriovenous fistulas (AVF) and the dialysis needles; B: Depicts a recirculation condition under which due to significant outflow stenosis the blood flow from dialysis machine returns back to dialyzer; C: An angiographic picture of an AVF with multiple inflow stenoses (single head arrows) and outflow stenosis (double head arrow and the arrow head). Reprinted from Asif *et al*[25] and Fahrtash *et al*[27], with permission.

ultimately thrombosis. Thrombosis, the main cause of access failure, is usually preceded by the development of an underlying stenosis. Consequently, the detection of stenosis in AVFs before thrombosis could offer a strategy to improve AVF survival by early intervention.

Stenosis in vascular access

Stenosis in vascular access can be categorized into two main groups of inflow and outflow stenoses[9,25]. An inflow stenosis is a lesion that occurs around the anastomosis and proximal to the venous needle, while an outflow stenosis is located further from anastomosis and distal to the venous needle. The stenosis location has been shown to be dependent on the type of access. Radio-cephalic AVFs are more prone to inflow stenosis, whereas outflow stenosis is more likely to occur in the brachio-cephalic AVFs. In contrast to AVFs, the arteriovenous grafts (AVGs) mostly develop outflow stenosis[26]. Figure 1A[27] and 1B[27] show the schematics of an inflow and outflow stenosis with respect to the anastomosis and cannulation sites, respectively, while Figure 1C[25] shows an angiographic picture of an AVF with multiple inflow and outflow lesions. All the clinical monitoring and surveillance programs have been designed to predict the development of a significant stenosis early enough to allow preemptive corrections of AVFs. Despite the importance of stenosis severity, its definition is still controversial[27,28]. A significant stenosis has been defined as a local reduction of > 50% in luminal diameter as compared to the adjacent normal vessel. This definition is inherently biased and is dependent on the location of the reference cross-section in the adjacent normal vessel (Figure 2[27]). Also, imaging techniques such as Doppler ultrasound only provides a 2D illustration of a 3D lesion, while other imaging modalities such as computed tomography (CT)-scan or magnetic resonance imaging (MRI) are expensive and not readily accessible. Despite these drawbacks, the stenosis severity still serves as the most important endpoint to direct the clinical decision making for the timing of further interventions.

MONITORING AND SURVEILLANCE PROGRAMS

Monitoring strategies mainly include physical examination (PE) and other clinical evidences of access dysfunction for stenosis detection, while surveillance programs were intended to supplement clinical monitoring by measuring variations in blood flow rate and venous access pressure. The PE, backbone of all screening programs, is a readily available and cost-effective tool to detect inflow and outflow stenosis in AVFs. PE has proved to be an accurate predictor of venous stenosis, and several studies have concluded that PE should be the part of all screening programs[29]. The only drawback of PE is the need for

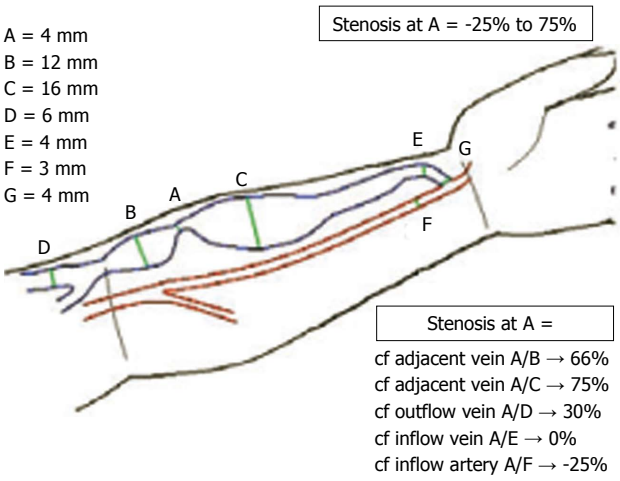

Figure 2 Dependency of stenosis severity to the location of reference cross sec-tion[27]. In this figure stenosis is located at A. Depending on the location of the reference cross-section, one can calculate a stenosis severity ranging from -25% to 75%. The -25% diameter changes represents a case in which the minimum diameter in the arteriovenous fistulas circuit has happened elsewhere than cross-section A. Reprinted from Fahrtash *et al*[27], with permission.

trained and experienced dialysis personals, leading to variability in decision making.

FUNCTIONAL (HEMODYNAMIC) ENDPOINTS

A developing stenosis eventually reduces blood flow and alters pressure profiles in the vascular access. Effects of stenosis on hemodynamic (blood flow and pressure) profiles are dependent on the type of vascular access (AVF or AVG) and the location of stenosis (inflow, outflow, or both). Therefore, monitoring the changes in flow and pressure can provide useful functional information on the severity of the underlying stenosis. NKF-KDOQI guidelines have recommended that both AVGs and AVFs undergo routine surveillance for blood flow and venous access pressure measurements.

Flow surveillance

The blood flow (Q_a) measurement is currently the gold standard of all surveillance programs. The Q_a measurement has been shown to be fairly reproducible both within and between the dialysis sessions. Blood flow rate can be measured by either indirect techniques such as ultrasound dilution or direct methods such as Doppler ultrasound, or MRI. The latter can provide valuable information about the location and severity of stenosis; however, it has the disadvantage of being more expensive and time consuming. Figure 3[8,9] shows a sample velocity pulse obtained from Doppler ultrasound as well as an example of detected stenosis using such technique. Although Q_a has been widely used as the most reliable surveillance strategy, there are numerous different Q_a thresholds for clinical decision makings in AVFs[30]. A wide range of Q_a from 300 mL/min to 900 mL/min[31-35] has been reported in different studies as the threshold for intervention. Under current KDOQI guidelines, an AVF should be referred for fistulogram when Q_a < 400-500 mL/min.

Polkinghorne *et al*[15] looked at the effectiveness of the KDOQI guideline for Q_a (< 500 mL/min) to detect a significant stenosis in 137 patients with AVF. These patients were randomly assigned to a control group receiving standard-of-care clinical treatment and Q_a surveillance group that received the same treatment as the control group plus monthly Q_a measurement. Under the normal treatment, an AVF was referred to fistulography if any of the following occurred: (1) raised venous dynamic pressure; (2) reduced blood pump flow; or (3) excessive bleeding from venopuncture site. They showed that the likelihood of stenosis detection in the Q_a surveillance group was two times but insignificant in relation to the control group, with a trend for a significant stenosis to be detected earlier. However, they also showed that over reliance on only blood flow threshold < 500 mL/min could misdiagnose some cases with positive sign of stenosis under standard-of-care treatment and angiography. They concluded that this misdiagnosis could be due to lack of understanding of the relationship between the blood flow in the vascular access and a developing stenosis.

Tessitore *et al*[33] tested different surveillance techniques such as PE, venous access pressure ratio, recirculation, and Q_a on a random population of 119 matured AVFs to find the ability and accuracy of these methods in detecting a significant stenosis. In addition to the surveillance methods, all patients underwent angiography to identify the grade of stenosis in the AVFs. Almost 50% of the AVFs had a significant stenosis either upstream of venous needle (inflow stenosis) or downstream of venous needle (outflow stenosis) or at both sites. A combination of PE and Q_a < 650 mL/min was able to provide a moderate-to-excellent tool to detect inflow stenosis with sensitivity of 85% and specificity of 89%. However, Q_a was not determined to be an adequate predictor of outflow stenosis. Therefore, they concluded that accuracy of Q_a to detect a significant stenosis is strongly dependent on the location of lesion.

Moreover, a randomized study[36] on 58 patients showed that preemptive intervention for AVFs with a Q_a > 500 mL/min results in 3-fold reduction in thrombosis and loss of vascular access as compared to the KDOQI guideline (Q_a < 400-500 mL/min) that is more suitable to detect a hemodynamically significant stenosis. Therefore, the current Q_a surveillance for AVF needs modification for improved detection of a significant stenosis well before it adversely affects the functional or hemodynamic condition.

Pressure surveillance

Besarab *et al*[37,38] proposed the use of venous access pressure to predict the stenosis severity in AVGs. Figure 4[38] shows the schematic for the measurement of venous access pressure using pressure transducers at the

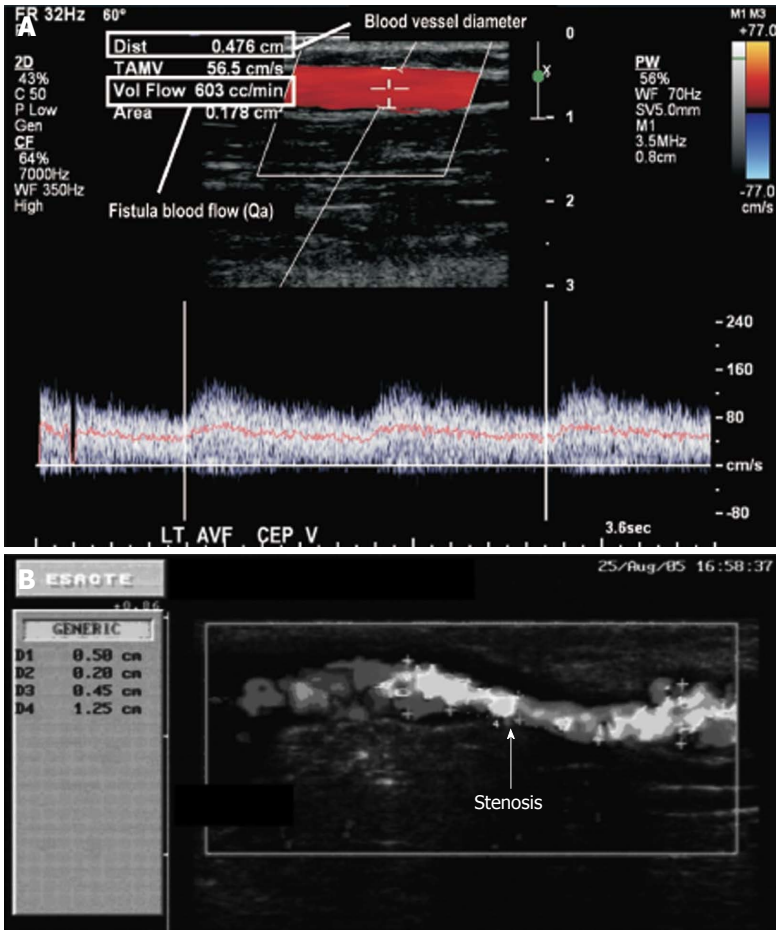

Figure 3 (A) Velocity pulse from Doppler ultrasound, and (B) detection of stenosis and estimating its grade using Doppler ultrasound[8,9]. AVF: Arteriovenous fistula. Reprinted from Campos *et al*[8] and Feddersen *et al*[9], with permission.

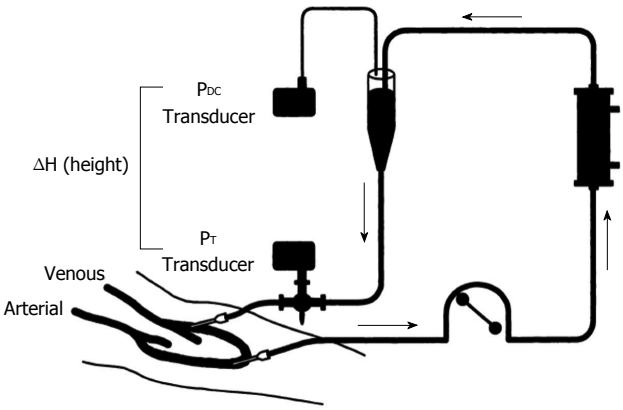

Figure 4 Schematic for measurement of venous access pressure in an arteriovenous graft using pressure transducers located at the venous needle and drip chamber[38]. Reprinted from Besarab *et al*[38], with permission.

venous needle and also at the drip chamber of dialysis circuit. Further with the development of this technique, the ratio of venous access pressure (VAP) to the mean arterial pressure (MAP) was used for detecting the stenosis severity (VAPR = VAP/MAP). A VAPR > 0.55 was associated with a clinically significant stenosis. In a prospective study, Besarab *et al*[39] monitored the variation of VAPR on 832 patients among whom 80% of accesses were AVG. The VAPR > 0.55 was found to be an excellent criterion for angiographic referral and intervention of a clinical stenosis in AVGs. It should be noted that VAPR was primarily developed to detect outflow stenosis in AVGs and by design was unable to detect an inflow stenosis[10]. It should be noted that AVGs are more prone to develop an outflow stenosis than an inflow lesion. In case of an inflow stenosis pressure drops in the access and consequently, VAPR remains below the cut-off level. Thus VAPR has been unsuccessful to detect the inflow lesions in AVGs.

Although, the VAPR is a promising parameter in AVGs[13,18,39,40], it has failed to show much advantage in assessing the functionality of AVFs[8,9,15]. This assertion originates from the fact that there is a fundamental difference between AVFs and AVGs. The AVG is essentially a single tube that connects the artery to vein, and thus, all the blood that enters the arterial anastomosis has to exit from the venous anastomosis. Therefore, any abnormal elevation in the pressure can be associated with the formation of stenosis mainly in the outflow segment. In contrast, the VAPR may show lesser variation while a stenosis is developing in an AVF because of the collateral pathways (accessory or collateral veins) that provide an alternative route to bypass the significant stenosis. Consequently, the flow in

the upstream venous segment of AVFs may not change even in the presence of downstream significant stenosis. In other words, the VAPR may undergo minimal changes as the flow can occur due to presence of downstream collateral channels.

The effect of stenosis on the flow and pressure fields is dependent on the location of stenosis. In general, an outflow stenosis causes an increase in the venous access pressure, while the access flow decreases over time. This is particularly more evident in an AVG than an AVF. In such scenario, an AVF can maintain a relatively high flow rate with almost unchanged pressure levels due to the development of collaterals. In the case of an inflow stenosis venous access pressure either remains stable or can decrease with the reduction in access flow under adverse remodeling. Therefore, pressure monitoring alone may not be able to detect such inflow stenosis, while it can be detected by sequential flow measurements and PE.

ANATOMICAL ENDPOINTS

Once an access is diagnosed for a significant stenosis either based on the standard-of-care clinical treatment or any of the functional surveillance strategies, a fistulogram is acquired to determine the grade and location of stenosis. However, as discussed earlier, there are a few criticisms to the current measurement protocol of the stenosis severity based on a fistulogram. These include: (1) fistulogram provides only a 2D illustration of 3D vessel; and (2) stenosis definition is biased to the location of the reference cross-section in the adjacent normal vessel for which the diameter varies a lot.

For example in a recent study, Fahrtash *et al*[27] criticized the current definition of stenosis severity and showed that, stenosis can have a wide range of severity from -25% to 75% reduction in luminal diameter based on the location of reference cross-section (Figure 2). Therefore, they hypothesized that a significant stenosis can be determined based on the absolute minimum diameter in the AVFs. They divided 170 radio-cephalic AVFs into two groups: dysfunctional (n = 93) and functional (n = 77) AVFs. The absolute minimum diameters of two groups were measured using grayscale and color ultrasound. They found that a diameter of 2.7 mm can be a good cutoff value to distinguish a functional radio-cephalic AVF from a dysfunctional one with 90% sensitivity and 80% specificity. Thus, it was concluded that a minimum diameter can be a more accurate measure to decide on the dysfunctionality of an AVF. However, this study was limited to only radio-cephalic AVFs and thus, more studies are needed to determine the critical minimum diameter for other types of AVFs.

Other studies[19,41-43] have primarily introduced the diameter as a pre-operative factor to predict if an AVF will mature. Current guidelines suggest a minimum diameter of 2 mm for successful AVF creation at wrist, but agreement on minimal diameter for other sites is lacking[19]. Lauvao *et al*[42] evaluated 158 patients undergoing initial dialysis access creation with native AVF. Three types of AVFs were created in these subjects including posterior radiocephalic AVF (n = 24), wrist radiocephalic AVF (n = 72), and brachiocephalic AVF (n = 62). Using multivariate logistic regression analysis, a vein diameter > 4 mm was found to be the only independent predictor of AVF maturation. However, a previous study by Wong *et al*[44] on 46 patients with initial radio-cephalic fistula suggested a diameter > 1.6 mm as the predictor of successful maturation of AVF. Therefore, despite its significance, there is still not a uniform agreement on the minimum diameter of AVF that can assist the clinical decision makings.

OTHER FUNCTIONAL ENDPOINTS

In addition to flow, pressure, and anatomical endpoints, wall shear stress has also been shown to have a strong correlation with functionality of AVFs. A multifold increase in blood flow rate after the AVF placement results in an extensive raise in the wall shear stress (WSS) levels acting on the luminal surface of the fistula. In order to accommodate for the marked increase in the hemodynamic stresses, the arterial and venous segments of the AVFs undergo structural changes such as vasodilation and wall hypertrophy[45-48]. These compensatory responses, also known as remodeling, attempt to regain the baseline levels of hemodynamic stresses (pre-surgery condition) in the vessels. Arterial remodeling in AVFs is mainly characterized by dilation and intima-media hypertrophy (outward hypertrophic remodeling)[49]. However, this adoptive remodeling in the venous segment can be interrupted by aggressive formation of neointimal hyperplasia, which can result in an undesired hypertrophy (thickening of the venous wall in the inward direction) and later venous stenosis, the major cause of failure in the AVFs. Therefore, monitoring the WSS levels can provide useful information on the functionality status of the AVFs.

Rajabi-Jaghargh *et al*[50] studied the linkage between the longitudinal changes in WSS and the luminal dilation for the AVFs in a pig model. Changes in the WSS levels within the AVFs were evaluated from the computational fluid dynamics models of the fistulas that were developed based on the acquired CT-scan and Doppler ultrasound data of the pigs at 2 d, 7 d, and 28 d post surgery time points. It was found that the slope of changes of WSS over time [τ' = ($WSS_{28\ d\ or\ 7\ d}$ - $WSS_{7\ d\ or\ 2\ d}$)/(time difference)] can be used to assess the functionality status in AVFs. The τ' for the AVFs with favorable remodeling (FR), as shown in Figure 5A[50], was negative between all the successive time-points representing a consistent decrease in the WSS levels over time. In contrast, for the AVFs with adverse remodeling (AR), Figure 5A, the τ' at all the successive time-points were positive showing that WSS levels were

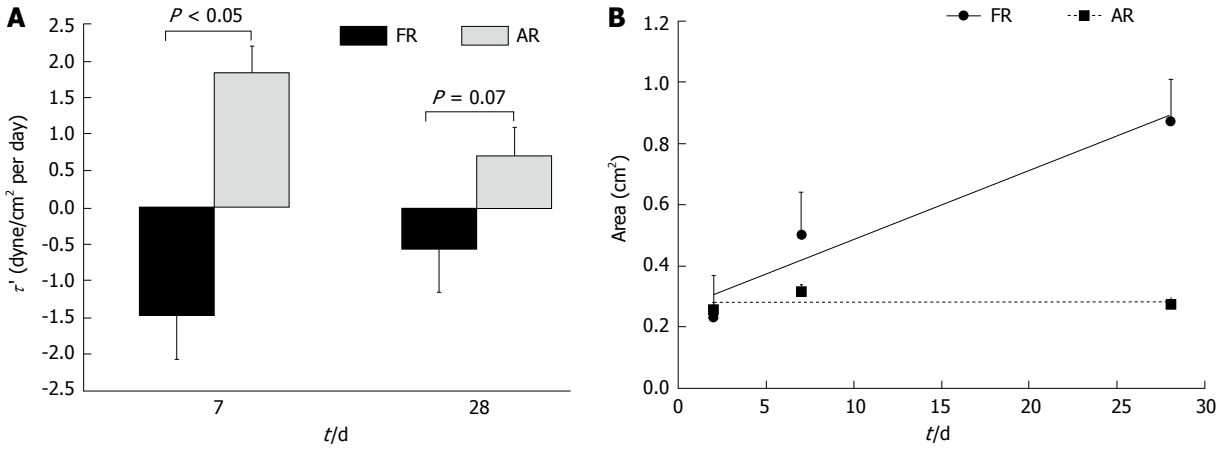

Figure 5 Variation in (A) temporal gradient of wall shear stress and (B) temporal gradient of luminal area of the venous segment for arteriovenous fistulas with favorable remodeling and adverse remodeling over time[50]. FR: Favorable remodeling; AR: Adverse remodeling. The *t*/d in the x axis stands for time (days). Reprinted from Rajabi-Jagahrgh *et al*[50], with permission.

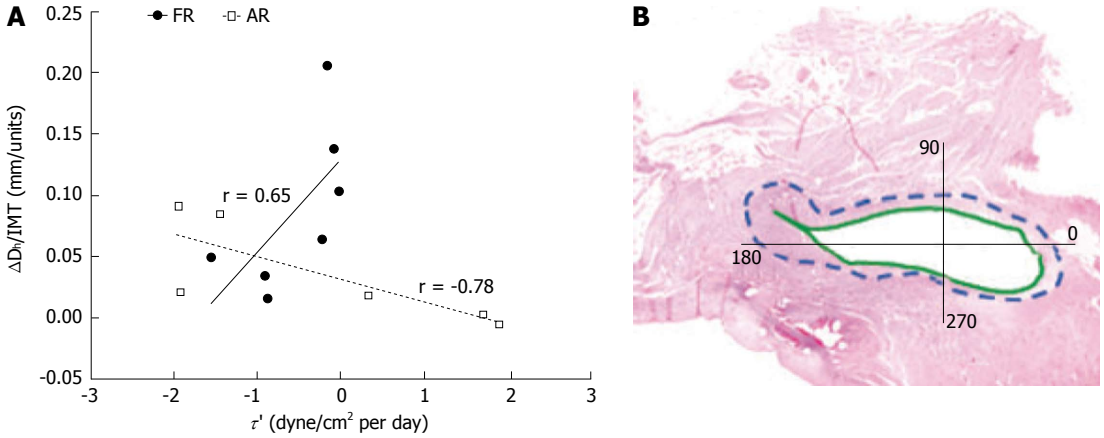

Figure 6 (A) Variation in morphological changes of the venous segment with respect to corresponding temporal gradient of wall shear stress (τ') for arteriovenous fistulas with favorable remodeling and adverse remodeling[51]. Morphological changes were quantified by calculating the ratio of differences in luminal diameter of venous segment over time (ΔD_h) to the corresponding amount of intima-media thickening (IMT). The IMT was calculated from (B) histology analysis using hematoxylin and eosin (H and E). The IMT was calculated as the average of intima-media thicknesses at four quadrants of each H and E staining slide. FR: Favorable remodeling; AR: Adverse remodeling; AVFs: Arteriovenous fistulas. Reprinted from Rajabi-Jagahrgh *et al*[51], with permission.

increasing over time for this group. These opposite patterns of WSS over time were accompanied with distinct remodeling behaviors within the two groups. The luminal area of the venous segment for the FR increased over time (Figure 5B), while for the AR the luminal area remained unchanged or reduced.

Also, in another study[51] by the same group, they showed that the temporal changes in shear stress can be correlated with morphological changes in the venous segment (Figure 6A). The morphological changes were quantified with the ratio of differences in diameter between successive time points (ΔD_h) by the amount of intima media thickness (IMT). The IMT was obtained from histology analysis (Figure 6B). The τ' showed distinct correlation between the AVFs with FR as compared to the ones with AR (Figure 6A). The positive τ' in the AR group was associated with the largest amount of IMT and lowest or negative ΔD_h, which also revealed stenosis formation in the venous segment. In contrast, the negative τ' was shown to be associated with relatively larger ΔD_h and larger IMT. This showed that the IMT in the AVFs with FR was in the outward direction as compared to the inward hypertrophy in AR group. Therefore, it was concluded that the increase in WSS of the venous segment of an AVF over time can be associated with adverse remodeling and reduced functionality, while the decrease in WSS over time can be considered a sign of favorable remodeling.

Although WSS has a key role on remodeling behavior and functionality of AVFs, the complexities and limitations associated with the accurate calculation of WSS under clinical settings have made it an undesirable clinical endpoint for surveillance strategies. Therefore, the main focus of this review is to introduce new diagnostic tools that can be readily available under current clinical settings such as flow, pressure, and anatomical endpoints. These endpoints, especially flow and pressure, have also been the main focus of surveillance strategies. However, as mentioned earlier the ability of current surveillance strategies to predict the functionality

status of AVFs has been controversial. This limitation can be associated with the fact that Q_a is based on only flow measurements, while the VAPR is a pressure based parameter. However, it may be noted that both flow and pressure change under a developing stenosis. Therefore, relying on parameters that are either based on pressure or flow can result in inaccurate and less than optimal decision making outcomes. Consequently, there is a need for new functional diagnostic endpoints that can combine both effects of pressure and flow.

FUNCTIONAL DIAGNOSTIC PARAMETERS FOR CARDIOVASCULAR AND RENAL STENOSIS

Adequacy of diagnostic tools that are based on either pressure or flow has been one of the major challenges for assessing the functional severity of stenosis in vasculatures such as coronary and renal arteries[52,53]. In this section, the limitations of current hemodynamic (pressure or flow) based endpoints for detecting the cardiovascular and renal stenoses will be discussed. Also, the combined functional (pressure and flow) endpoints for better detection of stenosis in vasculatures will be introduced.

Existing cardiovascular and renal diagnostic endpoints

Currently, fractional flow reserve (FFR; a pressure ratio) and coronary flow reserve (CFR; a flow ratio) are the two gold standards to assess the functional severity of a stenosis in coronary arteries[54]. FFR is the ratio of mean pressure distal to a stenosis to the mean proximal pressure under hyperemic condition. CFR is the ratio of blood flow rate to a diseased vessel under hyperemic condition to the corresponding basal (non-hyperemic or resting) flow. The values of FFR and CFR decrease as the stenosis severity increases. However, both FFR and CFR are affected not only by the stenosis severity but also by distal microvascular flow resistance that can increase under left ventricle hypertrophy, chronic or acute ischemia, diabetes mellitus, and other disease conditions[55-57]. If microvascular resistance is abnormal (high), then CFR decreases while FFR tends to increase. Under such scenario, FFR may be incorrectly above the cut-off (0.75-0.8) range despite the existence of a significant stenosis[58,59]. This may lead to inaccurate diagnosis which can result in either delay or missing of intervention procedure. Similar limitations exist for current KDOQI surveillance strategies to detect an outflow stenosis. Also, in the presence of collateral channels both CFR and FFR increase which result in uncertainty in diagnostics[60]. This is also similar to the scenario of an outflow stenosis with developed collateral channels in AVFs. These limitations have been associated with the inherent inaccuracies of current diagnostic parameters to detect stenosis severity that are based on either pressure or flow measurements. Such gap has resulted in recent attempts to introduce better diagnostic tools that can combine the effects of pressure and flow[61-64]. The new diagnostic endpoints rely on fluid dynamics principals that are based on non-linear relationship between pressure and flow in the presence of a developing stenosis.

Pressure-flow relationship in stenosed vessels

Based on fluid mechanics fundamentals, pressure drop for an incompressible (blood) flow inside a vessel is a function of frictional forces (viscous losses) and losses due to momentum changes. The latter can be induced by variation in luminal diameter (or area) of the vessel (*i.e.*, as a result of a stenosis), or presence of bends, bifurcations, anastomosis, and, *etc*. The viscous forces are linear function of flow, while the momentum changes have quadratic (non-linear) relationship with flow. In general, the pressure drop-flow relationship can be written as below:

$$\Delta p = Av + Bv^2 \quad (1)$$

where A and B represent the coefficients of viscous losses, and losses due to momentum changes, respectively. Equation 1 can be also written in a more general form as below:

$$\Delta p = kv^n \quad (2)$$

where n can vary between 1 and 2. The exponent in this relationship is of specific importance. For a fully developed laminar flow in a straight vessel, viscous losses are the main component of pressure drop. For such flows, n is nearly equal to 1. However, if the momentum of flow changes due to change in luminal diameter (*i.e.*, as a result of a stenosis), or due to existence of bends, bifurcations, or anastomosis, then the exponent of Δp-v relationship will become greater than 1. As the exponent becomes closer to 2, the contribution of momentum changes to pressure drop become more pronounced.

Using analytical formulations, Rajabi-Jaghargh *et al*[65] have shown that at early stages of stenosis, viscous losses are the most dominant component of pressure drop and n stays closer to 1. However, as the stenosis severity increases from 64% to 90% area stenosis the contribution of momentum changes to pressure drop becomes more pronounced and n will be > 1.5. Figure 7[65] shows the contribution of viscous losses and losses due to momentum changes to the total pressure drop in a stenosed artery with the increase in the percentage area stenosis. The n is equal to 1.5 at the point where the viscous losses and momentum changes contribute equally to the total pressure drop. For n < 1.5, the contribution of viscous losses are more, while losses due to momentum changes are more pronounced for n > 1.5. In case of an AVF, anastomosis segment imposes a local pressure loss due to momentum changes (blood flow acceleration in the bend), and thus Δp-v relationship is expected to have an exponent > 1. Although the Δp-v relationship does not directly help us to evaluate the functionality of an AVF, analyzing the exponent of Δp-v

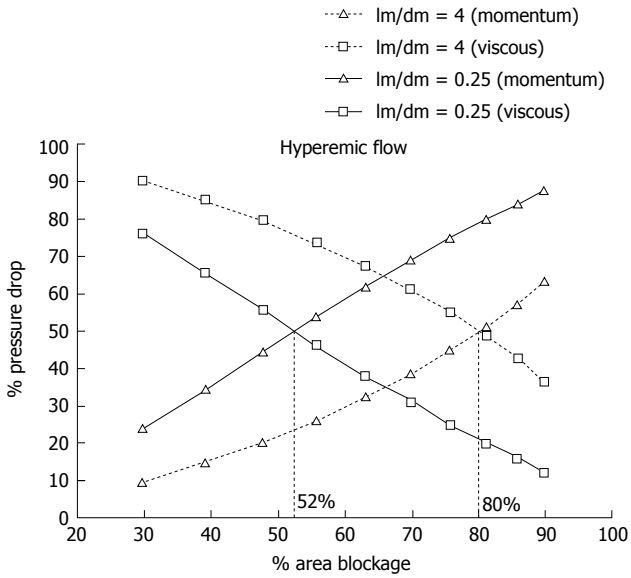

Figure 7 Contribution of viscous losses and losses due to momentum changes to the total pressure drop in a stenosed coronary artery with the increase in the percentage area stenosis[65]. Here, the pressure drop values at different stenosis severity were ob-tained under the corresponding hyperemic flow for coronary arteries. Also, lm and dm represent the length of stenosis throat and diameter of throat, respectively. Reprinted from ref. [65], with permission.

relationship can identify the contribution of viscous or momentum losses to the total pressure drop and the underlying geometrical variations (diameter or area change) in the AVF.

COMBINED FUNCTIONAL DIAGNOSTIC ENDPOINTS

Based on the pressure-flow relationship in stenosed vessels, the new functional diagnostic parameters can be defined as (1) resistance index, which represents the linear scaling of pressure drop with the velocity at the proximal artery ($R = \Delta p/v$); and (2) pressure drop coefficient, which is a pressure drop normalized by the dynamic pressure at the proximal artery ($C_p = \Delta p/(0.5\rho v^2)$, where ρ is blood density). The resistance index would be more helpful to predict a developing stenosis in early stages where *n* is closer to 1, while the pressure drop coefficient becomes more important as the stenosis becomes more severe over time in which case *n* is closer to 2. Both R and C_p have been shown to be better predictors of the functional severity of coronary stenosis under the limiting scenarios (*i.e.*, micro-vascular disease and collateral channels) as compared to FFR and CFR, the current gold standards[66-69].

Combined functional diagnostic endpoints in AVFs

The new functional diagnostic endpoints (resistance index, *R*, and pressure drop coefficient, C_p) have been recently[70] used in a pilot study on a pig model. It was shown that these parameters are capable of detecting the very early signs of a developing stenosis in AVFs. In this study, six AVFs were created between the femoral arteries and veins of 3 pigs, each pig having two AVFs on either limb. The variation in flow rates and geometries of the AVFs were studied at three post-surgery time points (2 d, 7 d, and 28 d) over about one month using Doppler ultrasound and CT-scan techniques. Also, computational fluid dynamics were used to calculate the pressure and velocity profiles for all the AVFs at every time points. Time averaged pressure difference between the proximal artery and outflow vein in conjunction with the average velocity at proximal artery were used to calculate C_p and *R* in AVFs. During the first week, all AVFs attained favorable remodeling[50,51] characterized by dilation, significant increase in flow rate, unchanged pressure drop, reduction in severity of local stenosis, and some amount of thickening due to the development of intimal hyperplasia. These changes were associated with the reduction in C_p and R levels over the first week (Figure 8).

In contrast, from 7 d to 28 d some of the AVFs showed significant dilation, while others experienced minimal changes in the mean diameter. During this period, the amount of thickening was also doubled in all the AVFs. Therefore, the minimal dilation and high amount of IMT from 7 d to 28 d for some AVFs resulted in adverse remodeling characterized by inward hypertrophy in those AVFs. On the other hand, the AVFs with significant dilation and venous wall thickening underwent positive remodeling with outward hypertrophy. Over this time period (7 d-28 d), the increase in average diameter and flow rate were minimal as compared to the first week, while the pressure drop increased for 50% of its baseline value. Also, the severity of the local stenosis, measured by area reduction, in AVFs (Figure 8A[70]) increased to 41.7% ± 8.3% which was below the clinically significant level (= 75% area stenosis). Corresponding to these changes, C_p and *R* (Figure 8B[70]) increased considerably over this time period. It was concluded that assessing the AVF functionality based on only diameter or flow rate could be misleading because they may not reveal complete information regarding the developing stenosis. However, C_p and *R* significantly increased over this time period and showed better delineation of stenosis severity. Thus, C_p and *R* could better assess the functionality of an AVF. However, this study was limited to relatively small number of data points and thus, studies with larger population and longer duration are needed to better determine advantages of the new combined pressure-flow diagnostic endpoints over the current gold standards.

Bedside measurement of new functional diagnostic endpoints

It should be noted that all the pressure-flow parameters that are needed to calculate C_p or *R* can be measured under current clinical settings. Velocity at the proximal artery can be measured through Doppler ultrasound probes and the pressure drop can be measured from the pressure readings at the cannulation sites during

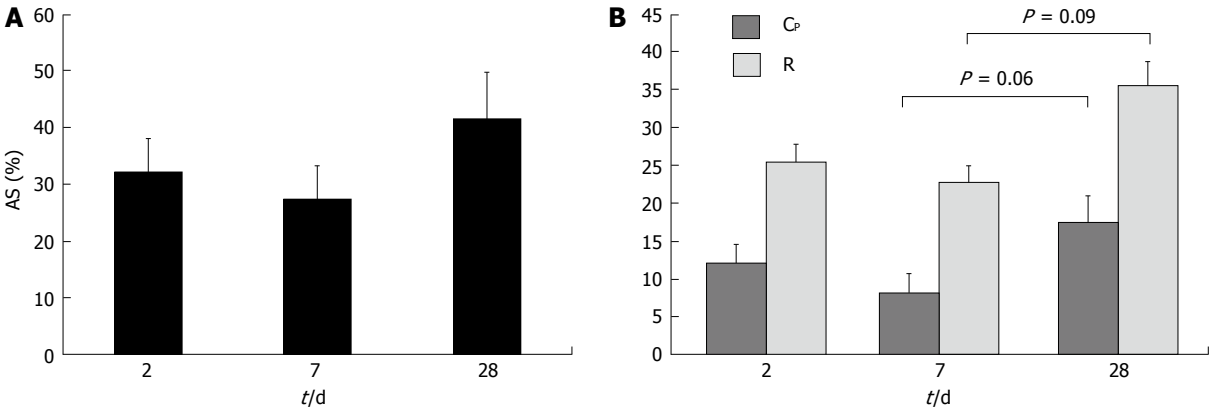

Figure 8 Variation of (A) percentage area stenosis and (B) pressure drop coef-ficient and resistance index over time for arteriovenous fistulas created in a pig model[70]. AS: Area stenosis; C_p: Pressure drop coefficient; R: Resistance index. The *t*/d in the x axis stands for time (days). © 2014, Copyright the Authors. Artificial Organs, © 2014 International Center for Artificial Organs and Transplantation and Wiley Periodicals, Inc.

the dialysis treatment. In the case of an inflow stenosis both *R* and C_p increases as pressure drop increases and flow rate decreases. Thus, both *R* and C_p are capable of detecting a developing inflow stenosis. If stenosis occurs downstream of the venous cannula (outflow stenosis) in AVFs, the pressure readings can show either lower or unchanged values of pressure drop over time, which does not reflect the presence of downstream stenosis. This is because of the development of collateral channels at the outflow stenosis area in AVFs. This shortcoming has been the major criticism to the current pressure surveillance program in which the AVF patency is assessed based on only pressure ratios at the outflow venous cannula and proximal artery. In such scenarios, if the venous flow rate decreases, both C_p and *R* begin to increase. It is noteworthy that irrespective of Δp status (numerator), as C_p has inverse quadratic relation with flow, any reduction in flow shows non-linear and pronounced increase in C_p as compared to *R*. Therefore, *R* and C_p are also capable of detecting an outflow stenosis. Thus, combining the flow and pressure data in the functional diagnostic endpoints could improve the ability of these parameters in assessing the patency of AVFs.

Limitations

The new functional diagnostic endpoints have not been tested for patient population. Thus, multi-central randomized studies on human subjects are needed to evaluate the potential advantages of the proposed diagnostic parameters for longitudinal assessment of AVF functionality.

CONCLUSION

According to KDOQI guidelines, a blood flow (Q_a) < 400-500 mL/min and a ratio of venous access pressure to main arterial pressure (VAPR) > 0.55 are associated with the existence of a significant stenosis which needs immediate interventional care. However, an AVF can maintain a relatively high blood flow rate and a normal VAPR despite the presence of a significant stenosis. The Q_a has shown to be a strong predictor of an inflow stenosis, whereas VAPR has shown to be a poor predictor of stenotic lesions in AVFs. These shortcomings have been mainly attributed to the fact that under a developing stenosis both pressure and flow profiles change, and thus, relying on only one of these parameters to detect a significant stenosis could be inadequate. Similar shortcomings also have been reported in detection of coronary and renal stenosis based on diagnostic endpoints that are either based on flow or pressure. In the context of coronary or renal stenosis, it has been shown that the diagnostic endpoints that combine the effects of pressure and flow can better predict the functional severity of a stenosis. This similarity inspired us to bridge between the advances in diagnostic field of coronary and renal stenosis with the diagnosis of stenotic lesions in AVFs. The new functional diagnostic endpoints are based on fundamental fluid dynamics concepts and are primarily presented in two major forms including: (1) resistance index (a ratio of pressure drop by flow); and (2) pressure drop coefficient (the pressure drop normalized by the dynamic pressure). We believe that these endpoints are capable of better distinguishing changes in the hemodynamic variations (pressure and flow) and thus, could be promising diagnostic tools to detect the functional severity of stenosis in AVFs.

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REVIEW

Aging and uremia: Is there cellular and molecular crossover?

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Abstract

Many observers have noted that the morphological changes that occur in chronic kidney disease (CKD) patients resemble those seen in the geriatric population, with strikingly similar morbidity and mortality profiles and rates of frailty in the two groups, and shared characteristics at a pathophysiological level especially in respect to the changes seen in their vascular and immune systems. However, whilst much has been documented about the shared physical characteristics of aging and uremia, the molecular and cellular similarities between the two have received less attention. In order to bridge this perceived gap we have reviewed published research concerning the common molecular processes seen in aging subjects and CKD patients, with specific attention to altered proteostasis, mitochondrial dysfunction, post-translational protein modification, and senescence and telomere attrition. We have also sought to illustrate how the cell death and survival pathways apoptosis, necroptosis and autophagy are closely interrelated, and how an understanding of these overlapping pathways is helpful in order to appreciate the shared molecular basis behind the pathophysiology of aging and uremia. This analysis revealed many common molecular characteristics and showed similar patterns of cellular dysfunction. We conclude that the accelerated aging seen in patients with CKD is underpinned at the molecular level, and that a greater understanding of these molecular processes might eventually lead to new much needed therapeutic strategies of benefit to patients with renal disease.

Key words: Aging; Uremia; Apoptosis; Autophagy; Senescence; Telomeres; Mitochondria; Post-translational protein modification; Klotho

Core tip: This review presents evidence that suggests that the morphological similarities between uremia and physiological aging are underpinned by similarities at a cellular and molecular level. Several of the classical cellular features of aging such as mitochondrial dysfunction and altered proteostasis have been observed in the cells and tissues of uremic humans and animals, and in *in vitro* models of uremia. There are also many shared features between aging and uremia in terms of

cell death and survival pathways. These commonalities may present new targets for the future management of patients with chronic kidney disease.

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INTRODUCTION

Observation alone suggests that patients with end stage kidney disease (ESKD) are biologically older than their unaffected peers. As a group, ESKD patients have a morbidity and mortality profile similar to that of the geriatric population, and the pathophysiology of the uremic syndrome has interesting parallels with the aging process. Based on these thoughts it has been posited that kidney failure results in accelerated, pathological aging[1]. Indeed there are striking analogies between the effects of aging and uremia on the structure and function of the heart and vasculature, with similar changes seen in pulse contour, pulse wave velocity, and impedance, and similar structural abnormalities with wall thickening, decreased elastin, and increased collagen content[2].

Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death[3]. Dialysis dependent patients of any age have an increased risk of mortality when compared to those with a functioning transplant and healthy controls of the same age[4], and are more susceptible to disease, particularly that of the cardiovascular system: a 25-34-year-old dialysis patient has a relative risk of cardiovascular mortality similar to that of a > 75-year-old in the general population[5]. Furthermore, the prognosis for chronic kidney disease (CKD) patients is still extremely poor and has not improved greatly despite many treatment advances: CKD patients receiving dialysis aged 50 and under are likely to live 30 years less than age-matched people without CKD[5]. Whilst survival rates have slightly improved they have not kept pace with the rises seen in the normal population without CKD, with the result that relative survival in age-specific patients with CKD actually decreased between 1977 and 2007[6]. There is thus a need to identify if CKD is inducing an aging-like cellular and molecular dysfunction, and if so whether any novel potential therapy might be derived from an increased understanding of the pathways that are induced by both CKD and aging.

ESKD confers a greatly increased risk of infectious morbidity and mortality, whilst simultaneously being a chronic inflammatory state, a pattern of immune dysfunction also associated with aging[7]. These abnormalities also seem to be reflected at a cellular level, with preferential loss of cells belonging to the lymphoid cell lineage, and inflammation and expansion of proinflammatory immune cells[8].

There is a high prevalence of the frailty syndrome amongst dialysis patients, a phenotype partly defined by weight loss, muscle weakness, and fatigue, which is associated with adverse outcomes in geriatric patients[9]. In the original study that developed this definition, 6.9% of participants ⩾ 65-year-old were classified as frail; in a more recent study of dialysis patients 44% of those under 40-year-old were found to be frail[10]. Cognitive impairment is also highly prevalent in the dialysis-dependent population and occurs in comparatively young patients[1,11].

Whilst much has already been written about the intriguing similarities that appear to exist between the aging process and CKD[1,8,12,13], comparatively little work has been undertaken looking at the cellular and molecular hallmarks of aging in the context of the known evidence concerning uremia-induced cellular and molecular pathways. Therefore in this review, in order to try and fill this perceived gap in the literature, we have first briefly outlined what the main cell death pathways are and by what means these processes interact with each other, followed by an analysis of published research concerning the mechanisms of aging and uremia-induced cell death and their common molecular pathways and cellular characteristics. Lastly we provide an assessment of how this knowledge may lead to benefits in both nephrology and gerontology.

CELL DEATH AND SURVIVAL PATHWAYS

An outline of cell death

Since the first descriptions of apoptotic cell death appeared more than 40 years ago[14] the study of cell death has become a substantial and important area. The main cell death pathways have been reviewed exhaustively in the literature and it is not the aim of this review to repeat this information. What is pertinent here is how much our understanding of cell death has changed and evolved in recent years. This is because cell death and survival pathways are now being assessed more as molecular processes and less as a series of morphological characteristics. One of the most fundamental changes is that each death pathway is no longer considered in isolation and there is an appreciation that cell death can no longer be considered as a choice between apoptotic, autophagic or necrotic death. Pathways once thought of as discreet have been found to be closely interconnected with others whilst some pathways have needed to be recategorized. In addition several completely novel pathways have been described. An example of reclassification is that necrosis is now subdivided into two distinct forms, one being programmed necrosis that

is usually termed necroptosis or regulated necrosis, and accidental or non-regulated necrosis which is more in line with the original concept of necrosis. Another example of recent developments is that apoptosis has now been split into four different classes whilst a total of 13 functional classes of regulated cell death have been described[15]. So whilst this review is focusing on the most established and described death and survival pathways they must not be considered as being complete. Lastly, the role of autophagy in cell death has been recently challenged[16,17] whilst its role in cell survival[18] asserted.

Uremia induced apoptosis

Although apoptosis and uremia have been studied extensively both separately and together, a clear picture of how uremia induces apoptosis has yet to be established. Instead a large number of studies using experimental models and human subjects have shown that uremia is associated with apoptosis in a wide range of cells and tissues such as skeletal muscle[19,20], myocardium[21], platelets[22,23], monocytes[24], neutrophils[25], lymphocytes[26], leukocytes[27] and vascular endothelial cells[28]. The kidney has also been shown as a target for apoptosis in uremia with both podocytes[29] and proximal tubular cells identified as having increased apoptotic cell death[30]. Furthermore, it has become known that it certain circumstances dialysis itself can be an activator of apoptosis[20,26]. It is unclear if the apoptosis seen in the kidney is the cause or the effect of CKD. However, it does seem probable that acute kidney injury (AKI) induced apoptosis can subsequently lead to the activation of interstitial fibroblasts *via* transforming growth factor beta (TGF-β) resulting in CKD[31,32]. In fact expression of TGF-β has been found to be elevated in nearly all human and experimental forms of CKD[33] and demonstrated to be directly associated with age in healthy human subjects[34].

Uremia induced necroptosis

Uremia induced necroptosis (or programmed necrosis) has yet to feature prominently in the literature although this is possibly due, at least in part to previous cell death descriptions not being classified correctly according to current definitions (see aging induced apoptosis below).

Aging induced apoptosis and necroptosis

The induction of apoptosis in aging in most tissues awaits clarification. However, in skeletal muscle at least there is clear evidence that muscle mass decreases with age[35-37] with apoptosis being known to be elevated in the skeletal muscle of aged subjects[38-41]. It has been suggested that aging increases cell death by caspase independent mechanisms. There is also some evidence that terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining is greater the kidneys of aged in mice[42] but TUNEL staining has been shown not to be specific for apoptosis[43]. It seems plausible that at least some of the examples for age induced apoptosis in the literature instead reflect increases in necroptosis.

Apoptosis and necroptosis crosstalk

It is now appreciated how significantly involved the apoptosis machinery is in other cell death and survival pathways. Many of the described apoptotic death receptors such as tumor necrosis factor receptor 1 and FAS are now also known to be able to induce necroptotic cell death[44,45]. Caspase-8, a key component of receptor mediated apoptosis is now thought to regulate the activation of necroptosis[45]. Inhibitor of apoptosis (IAP) are endogenous caspase inhibitors and therefore play a role in controlling apoptosis. When IAP levels are reduced this leads to caspases being activated which results in apoptotic cell death. Another IAP, X-Chromosome-linked IAP has been shown to be reduced in the muscle of CKD mice and *in vitro* in muscle cells treated with serum obtained from CKD mice[46].

The activation of autophagy is known to breakdown IAPs and lead subsequently to the induction of necroptosis. Furthermore, in conditions where IAPs are suppressed or absent and caspase activity is inhibited can lead to the activation of necroptosis *via* receptor-interacting protein1 (RIP1) and its downstream kinase (RIPK1)[47]. It has been postulated that RIP1 together with RIP3, cIAP, Caspase-8 and cFlip act as essential components of the ripoptosome, a signalling platform that can switch modes between apoptotic and necroptotic cell death[48]. Recent work indicates that it is RIPK3 activity that determines whether cells die by necroptosis, or in its absence, by caspase-8 mediated apoptosis[49] whilst another group have suggested that necroptosis can be induced in the absence of RIPK1 and without the formation of a functioning ripoptosome[50], the complex considered essential for necroptosis to occur.

Autophagy

Autophagy is the dynamic, multistep cellular process wherein portions of cytoplasm, including organelles, are sequestered into double-membrane vesicles (termed autophagosomes) and delivered to lysosomes where they are degraded, with eventual recycling of the resultant macromolecules[51]. By removing excessive and aberrant organelles and proteins, autophagy contributes to cellular homeostasis and protein quality control, and functions as a source of energy for the cell[52]. Autophagy is up-regulated and has a protective function in the face of cellular stressors such as starvation[53] and ischemia[54].

Autophagy and apoptosis crosstalk

It is perhaps not surprising that autophagy and apoptosis exhibit crosstalk as both pathways play such significant roles in development, homeostasis and pathology[55]. Evidence of this crosstalk has been plentiful[56-60] and indicates that the pathways can interact in an additive or antagonistic fashion and that the molecular machinery

for both can combine *via* p27[56], p38[57], p53[58] and beclin-1[59,60]. It is likely that these overlapping pathways are involved in uremia and aging induced dysfunction. For example in autophagy-deficient mice the onset of ischemia/reperfusion injury resulted in greater proximal tubular apoptotic injury with significantly elevations in serum urea and creatinine compared to wild type animals. This indicates that autophagy maintains proximal tubular homeostasis and protects against ischemic injury[61]. In another study using a dietary adenine-induced chronic renal failure model a high phosphate diet was found to increase apoptosis in vascular smooth muscle cells (VSMC) and that this rise could be reduced by autophagy inhibition. However, reducing autophagy was associated with an increase in calcium deposition in VSMC. The study concluded that autophagy might be an endogenous protective mechanism against phosphate-induced vascular calcification[62].

Autophagy and necroptosis

In addition to necroptosis crosstalking with apoptosis *via* IAP (see apoptosis and necroptosis crosstalk) there is also evidence of autophagy and necroptosis crosstalk in a similar fashion. Using a novel chalcone derivative as an anti-cancer agent it was found that Jun N-terminal kinases-mediated autophagy was able to cause IAP degradation followed by necroptosis[63]. It seems likely therefore that there is a therapeutic potential for autophagy to be exploited by anticancer agents to provoke cancer cell death. However, it should be noted that the molecular interactions between the two processes is still largely unknown and indeed there is evidence that autophagy activation can block necroptosis in several cell lines[64,65].

Autophagy in aging

Beyond its function at a cellular and organ level, autophagy has been heavily implicated in the aging process and the determination of life span. Normal and pathological aging are associated with failing proteostasis and reduced autophagic activity[3], and genetic inhibition of autophagy produces degenerative changes in mammalian tissue resembling those seen in aging. Caloric restriction, which has been shown to promote longevity in model organisms, stimulates autophagy, as do some pharmacological interventions and genetic manipulations that increase life span in model organisms, and inhibiting autophagy attenuates this effect[66].

Autophagy in uremia

Much work has been published describing the role of autophagy in the pathophysiology of AKI and CKD, but very little has been published looking at the effects of uremia on autophagy in other tissues. Chen *et al*[67] assessed autophagy activation in leukocytes isolated from peripheral blood samples, which had been taken from stage 5 CKD patients and healthy controls after overnight fasting and 2 h after breakfast. Overnight fasting induced conversion of microtubule-associated protein light chain 3 (LC3) Ⅰ to Ⅱ (as detected by western blot as increased quantities of the latter, and signifying autophagosome formation) in healthy subjects. mRNA levels of autophagy-related gene 5 (*Atg5*) and beclin-1 also increased in fasted healthy subjects but not in CKD patients. Interestingly there was no difference between CKD patients receiving or not receiving hemodialysis. Furthermore, a negative association was found between LC3Ⅱ and left atrium size, Atg5 transcription and left ventricular end-diastolic diameter, and beclin-1 transcription and mitral inflow E- and A-wave sizes. The authors conclude that autophagic activation is impaired in CKD patients and is not reversed with hemodialysis, and that this impairment is related to cardiac abnormalities.

Siedlecki *et al*[68] assessed the effect of rapamycin administration in a murine model of normotensive uremic cardiomyopathy. Treatment of surgically induced renal injury mice with rapamycin blocked the development of cardiac hypertrophy and fibrosis when compared with vehicle-treated animals. The experimenters suggest that this protective effect is mediated by the extracellular signal-regulated kinase and mammalian target of rapamycin (mTOR) pathways. They do not speculate on the possible involvement of autophagy, but rapamycin is known to stimulate autophagy *via* mTOR, and has been shown to have anti-aging effects in mammals[69]. The authors raise the interesting question of whether renal transplant recipients taking rapamycin as an immunosuppressant exhibit reversal of uremia-induced cardiac changes beyond that associated with successful transplantation.

In summary, the principle cell death and survival molecular pathways consisting of apoptosis, necroptosis and autophagy are strongly interrelated and crossover at many points. Whilst our current knowledge on how these interacting pathways are controlled and regulated is far from complete our appreciation of how similar many of the molecular signalling induced by uremia and aging appears to be growing pathways.

SHARED CELLULAR CHARACTERISTICS OF AGING AND UREMIA

Cell senescence, telomere shortening and stem cell exhaustion

Cellular senescence can be defined as stable arrest of the cell cycle coupled to classic phenotypic changes[70]. This was originally described by Hayflick *et al*[71] in serially passaged human fibroblasts, which undergo a certain number of divisions before entering a senescent phase (the "Hayflick limit"). This phenomenon was subsequently shown to be due to telomere shortening[72], but can be triggered by non-telomeric aging-associated stimuli such as DNA damage and excessive mitogenic signaling[3].

Senescent cells accumulate in aged organisms, although senescence *per se* does not cause aging,

having a protective effect by preventing the propagation and causing the removal of damaged and potentially oncogenic cells from tissues. A failure to clear senescent cells and replace these with new ones may, however lead to their accumulation[3]. Senescent cells are known to possess large amounts of proinflammatory cytokines and matrix metalloproteinases (the "senescence-associated secretory phenotype") which may in themselves contribute to aging[73].

Senescent cells have a flattened and enlarged morphology, and express a different set of genes such as p16, p21, p53, and retinoblastoma protein (pRb)[74]. Senescence-associated β-galactosidase (SA-β-gal) is a frequently used biomarker of cell senescence *in vivo* and *in vitro*[75].

Jimenez *et al*[76] looked at markers of senescence in circulating immune cells in uremic pre-dialysis, hemodialysis-dependent and transplanted patients. Abnormal telomere shortening was seen in a subpopulation of lymphocytes in pre-dialysis patients. In hemodialysis patients who dialyzed with cellulosic membranes, a subset of mononuclear cells demonstrated telomere shortening and exhibited increased levels of intracytoplasmic proinflammatory cytokines, which were released in response to substimulatory doses of lipopolysaccharide and bacterial DNA *in vitro*. The authors postulate that these senescent mononuclear cells both result from and contribute to chronic inflammation in such patients. A subpopulation of lymphocytes with shortened telomeres was also found in transplant patients with near normal renal function. It was suggested that these resulted from chronic activation due to major histocompatibility complex incompatibility and immunosuppressive therapy.

Tsirpanlis *et al*[77] measured the activity of telomerase (the enzyme that preserves telomere length and structure and thus prevents senescence[78]) in peripheral blood mononuclear cells in hemodialysis-dependent patients and non-renal failure subjects. Telomerase activity was reduced in hemodialysis patients compared to healthy controls, and was lower in long-term than in short-term dialysis patients. These findings indicate that defence against senescence is reduced in this cell type and associated with chronicity in hemodialysis patients.

Several groups have looked at the role of senescence in the endothelial dysfunction associated with cardiovascular disease in uremia. Adijiang *et al*[79] administered indoxyl sulphate, a uremic toxin, to hypertensive and normotensive rats, and examined their aorta for histological and immunohistochemical evidence of senescence. The indoxyl sulphate-treated animals showed significantly increased aortic calcification and wall thickness, and significantly increased expression of SA-β-gal, p16, p21, p53 and pRb in cells embedded in the calcification area. The same group went on to demonstrate that indoxyl sulphate stimulated senescence of cultured human aortic smooth muscle cells *via* an oxidative stress mechanism[74].

Carracedo *et al*[80] evaluated the effects of uremia on low-density lipoprotein (LDL) carbamylation and the effect of carbamylated LDL (cLDL) and oxidized LDL on the number, function, and genomic stability of endothelial progenitor cells (EPCs) obtained from healthy volunteers. EPCs were exposed to cLDL generated after incubation of native LDL (nLDL) with uremic serum from patients with CKD stages 2-4. Compared with cLDL, nLDL induced an increase in oxidative stress, depolarization and senescence in EPCs, and a decrease in EPC proliferation and angiogenesis. The authors hypothesize that cLDL triggers genomic damage in EPCs resulting in premature senescence, and that this contributes to atherosclerotic disease in uremia.

Klinkhammer *et al*[81] demonstrated that bone marrow mesenchymal stem cells (MSCs) isolated from uremic rats (both surgically induced and adenine diet) showed signs of premature senescence, and failed to accelerate healing of glomerular lesions when injected into the left renal artery of rats with acute anti-Thy1.1-nephritis when compared to MSCs obtained from control rats. The authors conclude that CKD leads to a sustained loss of *in vitro* and *in vivo* functionality in MSCs, possibly due to premature senescence. Stem cell exhaustion and the resultant decline in tissue regenerative potential has been noted as one of the hallmarks of aging[3].

In summary, aging and uremia share many important cellular characteristics such as increases in cell senescence, telomere shortening and exhaustion of stem cells. This provides further evidence that supports the contention that uremia can be considered as a form of accelerated aging[1].

Klotho

The *klotho* gene was originally identified as being involved in the suppression of aging in transgenic mouse studies[82]. Defective klotho expression resulted in mice having a premature aging phenotype, which had striking similarities to that of CKD patients, including reduced life span, arteriosclerosis, hyperphosphataemia and high concentrations of plasma fibroblast growth factor-23 {FGF23, a bone derived hormone that promotes renal phosphate excretion and reduces serum levels of 1,25-dihydroxyvitamin D3 [1,25-(OH)2VD3][83]}. This observation, coupled with the fact that, although found in multiple tissues, klotho expression is highest in the kidney (predominantly in the distal convoluted tubules[84]), suggested that CKD might be a state of klotho deficiency, and this might contribute to the accelerated aging phenotype of uremia[85].

Through alternative splicing klotho exists in membrane-anchored and soluble, secreted forms, the latter being found in mammalian cerebrospinal fluid, blood and urine[84]. These forms have distinct functions. Membrane klotho forms a complex with FGF receptors and functions as a co-receptor for FGF23. Soluble klotho functions

as an endocrine factor, and has a role in a number of processes including modulation of ion transport[86] and counteraction of the renin-angiotensin system[87]. Klotho suppresses 1α-hydroxylase in the kidney to regulate calcium metabolism[88], and participates in in the regulation of parathyroid hormone synthesis in the parathyroid gland by FGF23[84,89].

Both physiological aging and CKD are associated with reduced klotho levels. Lower renal klotho protein expression has been shown in aging rodents compared to young ones[90], and plasma klotho concentrations were found to be two-fold higher in normal children than in adults[91]. Renal klotho RNA has been shown to be reduced in CKD kidneys[92], as have urinary klotho levels[85]. Klotho concentrations in plasma, urine and kidney were found to be decreased in parallel in a rodent CKD model[85].

Klotho may influence cell death and survival pathways *via* its anti-senescence and oxidation effects. Liu *et al*[93] analysed various tissues and organs from klotho$^{-/-}$ mice and demonstrated a decrease in stem cell number and an increase in progenitor cell senescence. Tissues from klotho-deficient animals showed evidence of increased Wnt signalling. *In vivo* and *in vitro* Wnt exposure triggered by the absence of klotho accelerated cellular senescence. The authors conclude that klotho might act as a secreted Wnt antagonist and that a decrease in klotho concentration leads to an increase in Wnt signalling and this may play a role in aging.

de Oliveira *et al*[94] generated a klotho-knockdown human fibroblast, in which premature senescence was seen alongside an increase in p21 expression. p53 knockdown in klotho attenuated cells restored normal growth and replicative potential. These results suggest that klotho regulates cell senescence by suppressing the p53/p21 pathway. Ikushima *et al*[95] demonstrated that purified recombinant klotho protein could attenuate apoptosis and senescence in human umbilical vein endothelial cells. The same group went on to show that this occurred *via* mitogen-activated kinase and extracellular signal-related kinase pathways[96].

Klotho may exert an anti-aging effect by suppressing the inflammatory effect of substances secreted by senescent cells. Liu *et al*[97] have shown that cellular klotho interacts with retinoic acid-inducible gene-Ⅰ (RIG-Ⅰ) and that this interaction inhibits the RIG-Ⅰ induced expression of interleukin 6 (IL-6) and IL-8 both *in vivo* and *in vitro*.

Thus the deficiency in klotho seen in uremia and aging might underpin the enhanced cell senescence, apoptosis and stem cell depletion common to both states[81]. Given that tissue klotho expression is greatest in the kidneys a common mechanism is perhaps to be expected. Indeed recent data indicate that kidney tissue klotho expression greatly effects systemic concentrations and they concluded that the kidney is the prime mediator of klotho function[98]. Therefore klotho, a recognised anti-aging factor, is under the control of the kidney and thus lends further support to there being a molecular basis for the observed shared phenotype between uremia and aging.

Post-translational protein modification

Spontaneous post-translational protein modifications result from the non-enzymatic attachment of reactive molecules to protein functional groups. This process occurs in healthy individuals with aging, but is increased in certain disease states. Alterations to protein structure may result in functional changes, which can be pathogenetic[99]. Carbamylation is one form of post-translational protein modification specifically associated with CKD and uremia. Cyanate, a dissociation product of urea, binds to proteins and free amino acids, resulting in abnormal cellular responses that may contribute to inflammation and atherosclerosis. As carbamylation results from a direct product of uremia it may serve as a quantitative biomarker of time-averaged urea concentrations in addition to its potential use in risk assessment[99].

One of the most widely studied and publicised forms of post-translational protein modification is glycation. Advanced glycation end products (AGEs) are formed by the non-enzymatic modification of tissue proteins by physiologic sugars. AGEs accumulate in tissues as a function of increased production (*e.g.*, in diabetes mellitus), decreased renal removal of AGE precursors (*e.g.*, in advanced CKD) and time (as occurs in physiological aging)[100]. Covalent cross-linking occurs in affected proteins, leading to increased stiffness of the protein matrix, thus impeding function, and increased resistance to proteolytic removal, thus affecting tissue remodeling[101]. This contributes, for instance, to the histological and functional changes seen in diabetic glomerulosclerosis and atherosclerosis[102]. AGE accumulation also stimulates cytokine and reactive oxygen species (ROS) production through AGE-specific receptors, modifies intracellular proteins[100], and has been shown to promote senescence[103] and apoptosis[104] in the cells of affected tissues, contributing to cell death and tissue dysfunction.

Significantly elevated serum levels of AGEs are present in ESKD, with no differences between patients with and without diabetes[105], and uremic patients are known to be exposed to high levels of oxidative stress[106]. Taki *et al*[107] demonstrated that plasma levels of pentosidine, an AGE, was correlated and independently associated with coronary artery calcification score in hemodialysis patients. Pentosidine formation is accelerated by oxidative stress[108], and in this study was correlated with indoxyl sulphate. The authors thus conclude that indoxyl sulphate may enhance oxidative stress, which in turn enhances AGE generation.

Increased oxidative stress and AGE generation are known to play a role in the pathophysiology of aging[100], and both of these events are present in patients with CKD[105,106] and therefore represent two further potential crossovers between uremia and the aging process.

Table 1 Events common to aging and uremia covered by this review

Aging	Uremia
TGF-β ↑	TGF-β ↑
Autophagy ↓	Autophagy ↓
Apoptosis ↑ (muscle)	Apoptosis ↑
Senescence ↑	Senescence ↑
Telomere shortening ↑	Telomere shortening ↑
Stem cell exhaustion ↑	Stem cell exhaustion ↑
Klotho ↓	Klotho ↓
AGEs ↑	AGEs ↑
Mitochondrial dysfunction ↑	Mitochondrial dysfunction ↑

TGF-β: Transforming growth factor beta; AGEs: Advanced glycation end products.

Mitochondrial dysfunction

According to the mitochondrial free radical theory of aging, progressive, age-related mitochondrial dysfunction results in increased production of ROS, which causes further mitochondrial deterioration and cellular damage[109]. Recent data have questioned the idea that ROS have an entirely deleterious effect in aging, suggesting that they represent a stress-induced survival signal which acts to activate homeostatic responses to cellular stress and damage. As these accumulate with aging ROS eventually pass a threshold and aggravate the damage[110].

Dysfunctional mitochondria can contribute to aging independently of ROS[3]. Damaged mitochondria have an increased tendency to permeabilize in response to stress, leading to apoptotic cell death[111] and inflammation[112]. Aging associated mitochondrial dysfunction arises *via* several mechanisms[3]. For example, mitochondrial decline occurs as a consequence of telomere attrition in telomerase-deficient mice with subsequent p53-mediated repression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PCC1a) and PGC-1b[113], and can be partially reversed in wild-type mice by telomerase activation[114]. Sirtuins, a group of nicotinamide adenine dinucleotide-dependent protein deacetylases[115], also play a role in controlling mitochondrial function. Silent information regulator two protein 1 modulates mitochondrial biogenesis *via* the transcriptional co-activator PGC-1a[116] and the removal of damaged mitochondria by autophagy[117]. SIRT3 targets many enzymes involved in energy metabolism[118], and may directly control ROS production by deacetylating manganese superoxide dismutase, a mitochondrial antioxidant enzyme[119].

Mutations and deletions in mitochondrial DNA are known to accumulate with aging[3]. One of the most common and abundant mitochondrial DNA mutations is a 4977 base pair deletion between nucleotide positions 8470 to 13,477 (mtDNA4977)[120], which is known to accumulate in a variety of human tissues with age and has been demonstrated to be associated with several neurodegenerative diseases (including Alzheimer's) and atherosclerosis[121,122]. Defective quality control by mitophagy (organelle-specific autophagy that targets abnormal or worn out mitochondria for degradation) leads to reduced clearance and turnover of ineffective and toxic mitochondria[123]. The net result of these processes is that there is a reduction in the formation of healthy mitochondria, an increased incidence of mitochondrial damage, and a failure to clear and recycle abnormal organelles, with consequently increasing bioinefficiency, inflammation and cell death with aging.

Patients with advanced uremia are recognised to have low body temperatures, reduced stamina and low basal energy expenditure, suggesting a hypometabolic state[124]. Thompson *et al*[125] examined the forearm muscles of patients with ESKD using ^{31}P-magnetic resonance spectroscopy. They noted increased phosphocreatine depletion and increased glycolytic ATP production during exercise, suggesting mitochondrial dysfunction due to either limitation of oxygen supply, reduced mitochondrial content or an intrinsic mitochondrial defect. Exercise-related abnormalities remained despite anemia correction with erythropoietin[125].

Lim *et al*[126] demonstrated a high frequency of mtDNA4977 in the skeletal muscle of chronically uremic patients, and that this correlated with enhanced oxidative damage to DNA, lipids and proteins of mitochondria compared to healthy controls. Liu *et al*[127] found that the incidence and proportion of mtDNA4977 in hair follicles was significantly higher amongst hemodialysis patients compared to age matched controls. Therefore mitochondrial abnormalities, contributing and consequent to high levels of oxidative stress in uremia, are strongly suspected to play a role in the causation of pathological aging in CKD, acting as a nexus for several processes, including defective bioenergetics, telomere attrition, DNA mutations, autophagy, inflammation and cell death. Mitochondrial abnormalities therefore represent a further crossover point between aging and the uremia.

DISCUSSION

In this review we have sought to draw the reader's attention not just to the morphological similarities between advanced aging and uremia, but also to their shared characteristics at a cellular and molecular level (see Table 1). Experimental evidence has been provided to suggest common involvement of established cell death and survival pathways (apoptosis, necrosis, necroptosis and autophagy), and the presence of several of the recognised cellular and molecular features of the aging process in patients with ESRD and in experimental models of uremia. These include mitochondrial dysfunction, damage to genetic material, telomere shortening, impaired proteostasis, cell senescence, stem cell loss, oxidative stress, AGE accumulation, and klotho deficiency. Based on this evidence it could be posited that the physical resemblance between advanced age and uremia is underpinned by shared cellular and molecular

"abnormalities". These observations also reinforce the idea of the "uremic syndrome", in which dysfunctions in multiple body systems arise due to a pervasive defect at a cellular level.

Information gathered by research into aging pathways and "anti-aging therapies" might inform interventions to avoid, slow the progression of or even reverse some of the pathological changes seen in uremia. Given that these pathways are seen throughout most tissues and cell types it is also possible that a single intervention might treat several pathologies. However, the aging process remains incompletely understood in healthy individuals, and those pathways that are known are complex and heavily interconnected. Disentangling these in the uremic syndrome, in which multiple co-existing and interdependent metabolic abnormalities arise, will be a challenge. Additionally, many of these pathways have known (and possibly unknown) protective mechanisms (against malignant transformation, for example), thus blocking them may have unwanted and deleterious effects. What could be more immediately practicable would be employing some of the therapies known to be effective in improving the health of elderly patients, such as exercise.

The concept of accelerated aging in uremia is an intriguing and complex one that may yield important therapeutic targets and strategies to improve health outcomes in patients with CKD. Much work, however, remains to be done in understanding its cellular and molecular basis before any potential benefits can be realised.

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REVIEW

Complement activation in progressive renal disease

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Abstract

Chronic kidney disease (CKD) is common and the cause of significant morbidity and mortality. The replacement of functioning nephrons by fibrosis is characteristic of progressive disease. The pathways that lead to fibrosis are not fully understood, although chronic non-resolving inflammation in the kidney is likely to drive the fibrotic response that occurs. In patients with progressive CKD there is histological evidence of inflammation in the interstitium and strategies that reduce inflammation reduce renal injury in pre-clinical models of CKD. The complement system is an integral part of the innate immune system but also augments adaptive immune responses. Complement activation is known to occur in many diverse renal diseases, including glomerulonephritis, thrombotic microangiopathies and transplant rejection. In this review we discuss current evidence that complement activation contributes to progression of CKD, how complement could cause renal inflammation and whether complement inhibition would slow progression of renal disease.

Key words: Complement; Innate immune system; Chronic kidney disease; Transplantation; Proteinuria; Fibrosis

Core tip: Complement activation occurs in progressive chronic kidney disease and may contribute to the chronic inflammation that is characteristically found in the kidney. It is therefore possible that inhibiting complement activation would reduce inflammation, lead to reduced fibrosis and preservation of renal function.

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INTRODUCTION

Chronic kidney disease (CKD) is recognised worldwide as a major public health problem[1]. In 2007 the United Kingdom age-standardised prevalence of CKD stages 3-5 was 8.5% (10.6% in females and 5.8% in males)[2] and similar prevalences have been described in other countries. In 2012 in the United Kingdom, the number of new patients requiring renal replacement therapy was 6891, equating to 108 patients per million population, with diabetes and glomerulonephritis being the two most common diagnoses in incident dialysis patients. Although not all patients with CKD will progress to renal failure, all stages of CKD are associated with increased

morbidity and mortality[1].

Tubulointerstitial inflammation and fibrosis is a major factor in the progressive loss of renal function in most kidney diseases[3]. The process is complex due to the number of interacting pathways which ultimately result in the replacement of functioning nephrons with scar tissue. Cellular stress and injury induces an inflammatory and pro-fibrogenic response involving growth factors[4-6] and pro-inflammatory cytokines as well as activation of the renin-angiotensin system. This leads to a chronic inflammatory cell infiltrate, increasing numbers of activated fibroblasts (myofibroblasts) and excessive matrix deposition. There is evidence from preclinical models that the immune system is important in the development of renal fibrosis[7,8]. A component of the innate immune system that may be important in driving renal inflammation is the complement system, which can directly affect cell function and also influence the adaptive immune response.

COMPLEMENT SYSTEM

The complement system is a biochemical cascade made up of approximately 30 serum and membrane-bound proteins and represents a major part of the innate immune system. Complement was identified in the late 19th century by German scientist Paul Ehrlich as a heat-labile blood serum component with non-specific antimicrobial activity that "complements" other immune functions. As part of the innate immune system, the complement system responds rapidly to defend the host against a variety of invading microorganisms[9]. The complement system can also participate during the inductive phase of the acquired immune response by contributing to the recognition and presentation of non-self antigen, triggering antigen presenting cell activation, maturation and proliferation[10,11].

The primary location for biosynthesis of complement is the liver. Although Erlich and Morgenroth suggested the liver as the main source of complement production in 1900, it was only confirmed in 1976 by Alper *et al*[12] who described recipient to donor C3 allotype conversion after liver transplantation. This is also supported by studies of hepatocyte cell function[13]. In addition, evidence for extrahepatic synthesis of complement increased and it is now known that extrahepatic complement synthesis contributes approximately 10% of circulating C3. The alternative sites for complement production include epithelial cells, fibroblasts, lymphocytes and macrophages derived from different organs, including the kidney[14]. In the kidney, local complement production has been shown to occur at different sites along the nephron and may be further enhanced by the presence of cytokines and infiltrating immune cells during acute inflammation[15-17].

ACTIVATION OF THE COMPLEMENT SYSTEM

Activation of the complement cascade is triggered by one of three distinct pathways: the classical pathway, the alternative pathway and the mannose-binding lectin (MBL) pathway (Figure 1). All three pathways converge to cleave complement component C3, which subsequently initiates activation of the terminal complement pathway and formation of the membrane attack complex (MAC). The classical pathway is initiated by the activation of the C1 complex when C1q binds the Fc region of IgG or IgM. There is sequential cleavage of activation of C4 and C2, leading to the assembly of the classical pathway C3 convertase.

Activation of the alternative pathway is dependent on the spontaneous low level hydrolysis of the internal thioester bond of C3 to C3(H_2O). C3(H_2O) resembles C3b and can bind to factor B (FB). FB is activated by factor D forming the alternative pathway C3 convertase. The alternative pathway also amplifies the classical and lectin-binding pathways and is therefore critical for the full activity of complement. The third complement activation pathway, the lectin-binding pathway, is homologous to the classical pathway except that it is activated by the binding of a lectin to carbohydrates on microbial surfaces. The C3 convertase cleaves C3 resulting in assembly of the C5 convertase and sequential binding of C6, 7, 8 and 9 to form C5b-9, the membrane attack complex.

The main purpose of complement activation is to remove invading pathogenic organisms such as bacteria. This is achieved directly through the formation of the MAC or indirectly by opsonisation and stimulation of phagocytosis. Products of C3 and C4 activation on the surface of pathogens are recognised by the complement receptors CR1 and CR3 present on macrophages and neutrophils leading to phagocytosis of the opsonised target. Complement activation results in production of the small, biologically active anaphylatoxins, C3a and C5a. These readily diffusible complement components have a variety of functions, including chemotaxis and release of histamine from mast cells, mediated through binding to specific receptors[18]. These receptors, and also CRs1-4 (Table 1), are present on many immune cells and provide links between complement and the adaptive immune system[19,20].

The complement system contains proteins, both membrane bound and fluid phase, which regulate activation to prevent damage to host cells. They act by promoting decay of the convertase complexes, act as cofactors for the enzymatic degradation of the active proteins and by preventing the assembly of the MAC. The importance of these regulators is seen when their function is impaired, resulting in excessive complement activation and tissue injury.

COMPLEMENT ACTIVATION IN RENAL DISEASE

Complement activation is known to occur in immune mediated glomerular diseases (lupus nephritis, membranous nephropathy and post-infectious glomerulo-

Table 1 Properties of complement receptors

Receptor	Alternative name(s)	Location	Specificity	Role
CR1	CD35	Macrophages Neutrophils B-cells Some T-cells Renal epithelium	C3b C4b	Binding of opsonised immune complexes for transport to phagocytes
CR2	CD21	B-cells Some T-cells Dendritic cells Epithelia	C3d	Link between innate and acquired immune response on B-cells Presentation of immune complexes to B-cells
CR3	CD11b/18	Macrophages Natural killer cells Neutrophils	iC3b	Cellular-extracellular matrix linkage Promotes phagocytosis of opsonised complexes
CR4	CD11c/18	Macrophages Neutrophils	iC3b C3dg	Receptor for iC3b-opsonised particles
C3aR	-	Renal epithelium Macrophages	C3a	Mediation of inflammation
C5aR	CD88	Neutrophils	C5a	
C1qR	CD93	Leukocytes Platelets Monocytes Neutrophils	C1q	Up-regulation of phagocytic capacity Protein chaperone

Adapted from Morgan *et al*[94] 1999.

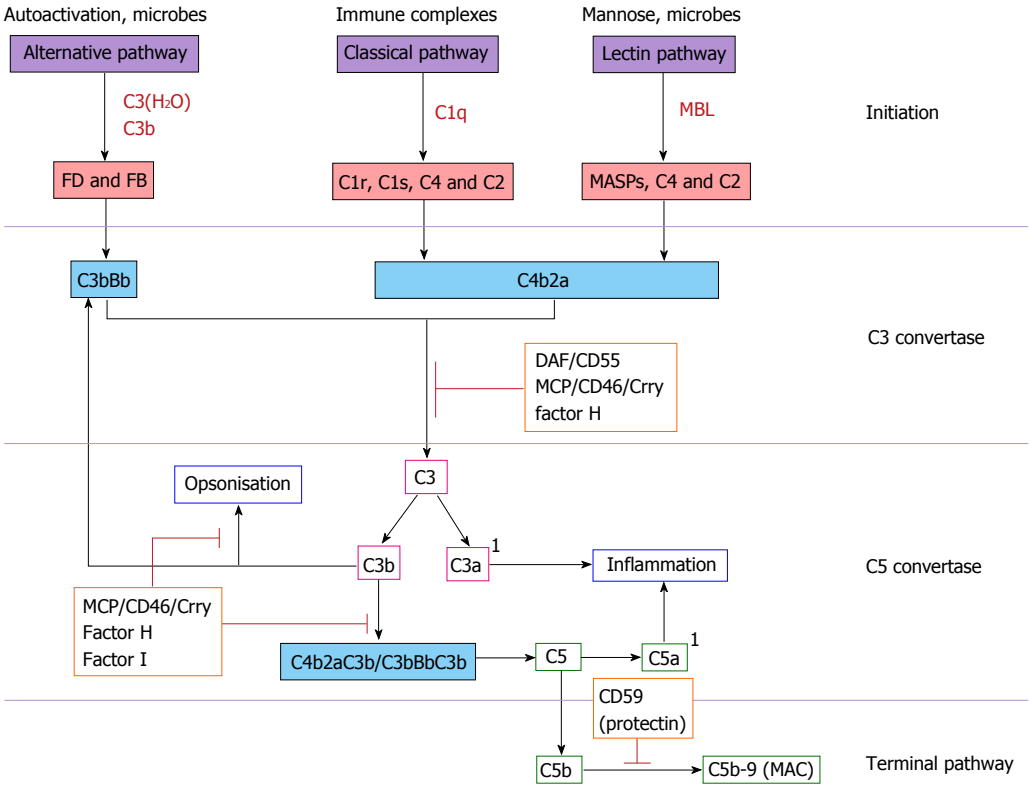

Figure 1 Complement activation pathways. Complement activation is triggered *via* activation of either the alternative, classical or lectin pathways, all three of which converge to cleave central component C3. Briefly, activation of the alternative pathway occurs following the spontaneous hydrolysis of C3 to C3(H_2O). C3(H_2O) binds factor B (FB) to form C3bB which is then cleaved by FD leaving the C3bBb complex. C3bBb is stabilised by properdin to form the alternative pathway C3 convertase. C3 is subsequently cleaved to C3a and C3b to form the C5 convertase C3BbC3b. Activation of the classical complement pathway occurs when immunoglobulin-bound antigens bind to and activate the C1 complex (consisting of C1qr2s2). Activated C1qr2s2 cleaves C4 to C4a and C4b. C4b becomes membrane-bound and binds to pro-enzyme C2, which is then cleaved to C2a and C2b fragments by C1s. C2a remains bound to C4b, forming the classical C3 convertase C4b2a. C3 is cleaved to C3a and C3b to form the C5 convertase C4b2aC3b. The lectin complement pathway is homologous to the classical pathway, with the exception that it is activated by the binding of lectin to microbial cell surface carbohydrates (mannose). Surface-bound lectin activates MBL-associated serine proteases (MASPs), which directly activate C3 and directly cleave C2 and C4. Activation of the terminal complement pathway occurs when the alternative and classical C5 convertases C3bBbC3b or C4b2aC3b cleave C5 in to C5a and C5b. C5b binds to C6 and C7, forming C5b67, which associates with an adjacent membrane. C5b67 then binds to C8 and multiple C9 molecules forming the transmembrane pore C5b-9, also known as the MAC. [1]Denotes the anaphylatoxins C3a and C5a. Orange boxes highlight regulatory complement proteins. MBL: Mannose-binding lectin; MAC: Membrane attack complex; DAF: Decay accelerating factor; MCP: Membrane cofactor protein.

nephritis), atypical haemolytic uraemic syndrome and during antibody mediated rejection. However, what is less clear is whether complement activation contributes to the non-disease specific inflammation, tissue injury and fibrosis that are characteristic of progressive nephropathies.

COMPLEMENT ACTIVATION IN CLINICAL PROTEINURIC DISEASE

The association between proteinuria, tubulointerstitial fibrosis and declining renal function is well established, however, the mechanism by which proteinuric glomerular disease casuses interstitial injury is uncertain. Complement proteins will be filtered when glomerular permselectivity is impaired and enter the tubular compartment. Complement activation products can be found in the urine of patients with a wide variety of proteinuric diseases; diabetic nephropathy, membranous nephropathy, IgA nephropathy and focal segmental glomerulosclerosis (FSGS)[21]. In some cases this may be due to spill over of complement activated in the glomerulus, however, complement activation products can be found in diseases where glomerular complement activation is not a major feature, for example diabetic nephropathy and FSGS[21,22]. This implies that complement is activated within the tubular compartment.

The tubular epithelium activates complement on its apical surface[23,24], which occurs primarily *via* the alternative pathway[25,26]. There are several explanations for this. It may be related to urinary pH[21] or ammonia production from stressed epithelial cells[27] directly activating C3. There may be enzymes with convertase-like activity in the apical brush border of the proximal tubule which is also known to be relatively deficient in complement regulatory proteins[28]. Properdin, which stabilises the alternative pathway convertase binds to the glycosaminoglycans on the apical surface of tubular epithelium[26]. Factor H also binds but at a different site[29,30], suggesting a balance between complement activation and inhibition which may be disturbed in proteinuria as albumin reduces Factor H binding[31]. Whatever the explanation, proteinuria provides a source of complement proteins to a host cell surface which is unable to control activation.

Demonstrating that complement activation is the cause of tissue injury in clinical proteinuric disease is difficult. Mosolits *et al*[32], studying patients with proteinuria, found a spatial and quantitative relationship between renal MAC deposition and inflammatory cell infiltrate and tubulointerstitial expansion. Urinary MAC concentrations are increased in proteinuric renal diseases. The concentration can relate to disease activity[33] although this is not always the case[34,35] and is further complicated when there is glomerular immune complex deposition.

COMPLEMENT ACTIVATION IN PRECLINICAL MODELS OF PROTEINURIA

Pre-clinical models have significantly contributed to our understanding of the role of complement in proteinuric disease. Aminoglycosides (puromycin and adriamycin) disrupt glomerular epithelial function leading to glomerulosclerosis, proteinuria and tubulointerstitial fibrosis in rodents. Induction of disease in complement deficient mice has shown that complement activation contributes to glomerulosclerosis and tubulointerstitial fibrosis and that activation occurs *via* the alternative pathway[36,37]. These studies have also demonstrated the important function of complement regulatory proteins in controlling complement activation, as their deficiency exacerbates injury[36]. Evidence from studies in both mice and rats suggests that both MAC[38] and the anaphylatoxins may be responsible for the damage that occurs[36,39].

Other pre-clinical models have also been used to investigate how complement influences the development of tubulointerstitial injury including a model of mesangioproliferative glomerulonephritis[40] and the remnant kidney model[41,42]. Disease severity is reduced if an intact complement system is absent. These models also allow testing of the potential for therapeutic targeting of complement. There is strong evidence that either complement depletion[43] or inhibition[40] can reduce the severity of proteinuria-related tubulointerstitial disease. There is the potential to target therapeutic complement inhibition at the tubular epithelium. A recombinant protein with an antibody portion directed at the tubular brush border linked to a complement inhibitor reduced interstitial injury and preserved renal function in rats with puromycin nephrosis[44].

COMPLEMENT ACTIVATION IN NON-PROTEINURIC KIDNEY DISEASE

Less is known about the role of complement in non-proteinuric renal disease. Unilateral ureteric obstruction (UUO), induced by ligation of one ureter, is the most commonly used preclinical model of progressive renal disease. The injury is characterised by the gradual development of interstitial inflammation and fibrosis, macrophage, T cell and fibroblast infiltration and eventual loss of functioning nephrons, closely resembling the pathology of chronic renal disease observed in patients. The first study to address the role of complement in UUO used rats deficient in C6 which are unable to assemble MAC. No difference was seen in disease severity in normal or C6 deficient animals, indicating no role for MAC in disease development[45].

Boor *et al*[46] found that interstitial fibrosis and macrophage infiltration were significantly reduced in C5 deficient ($C5^{-/-}$) mice after five days of UUO. Similarly,

significant reductions in fibronectin, vimentin, platelet-derived growth factor (PDGF)-B and PDGF-D mRNA were observed in $C5^{-/-}$ mice. A protective effect was also observed when UUO mice were treated with a C5aR antagonist. In a more recent study of UUO in C3 deficient mice there was a significant reduction in interstitial fibrosis and tubular atrophy in the absence of complement activation[47]. It is clear from the above animal studies that activation of C3 and C5 contribute to the development of progressive renal fibrosis during experimental obstructive nephropathy, however, the mechanism by which this occurs remains largely uncharacterised. There are no corresponding clinical studies to support these pre-clinical observations.

COMPLEMENT FUNCTION IN DIABETIC NEPHROPATHY

Although diabetic nephropathy is initially glomerular, progression of disease is associated with both glomerulosclerosis and tubulointerstitial fibrosis. There is evidence that complement may be involved in susceptibility to and progression of diabetic nephropathy. The serum concentration of mannose-binding lectin is variable and determined by polymorphisms in the gene promoter and coding sequence. Higher MBL concentrations early after the onset of type 1 diabetes may predict the development of nephropathy many years later[48]. Serum MBL concentrations are higher in type 1 diabetics with albuminuria and overt nephropathy[49,50] although this does not appear to relate to genotype. The effect of MBL on the development of diabetic nephropathy has been studied in MBL deficient mice with streptozotocin induced diabetes. The absence of MBL retards the development of glomerular disease and albuminuria[51], although not in all mouse strains[52].

MAC can be detected in the glomeruli of the patients with diabetes. Deposition may be increased by the glycosylation and loss of function of CD59, an inhibitor of MAC assembly[53]. In diabetic disease there is differential regulation of complement genes in both the glomerulus and tubular compartment[54], suggesting a possible role for complement proteins synthesised within the kidney. In addition, complement activation may be involved in the coronary and renal vascular disease associated with diabetes[53,55] and again the MBL pathway may be involved[56].

POLYCYSTIC KIDNEY DISEASE

In patients with inherited cystic kidney disease cysts develop within the nephron disrupting normal renal structure and function. It is evident that inflammatory and fibrotic changes occur in renal tissue surrounding the cyst and this may in part be responsible for the loss of renal function that occurs. In mouse models of cystic kidney disease there is increased expression of complement genes in cyst epithelium, particularly C3, and also evidence of complement activation[57,58]. To investigate a role for complement in cystogenesis $Cys1^{cpk/cpk}$ polycystic kidney disease mice were crossed with C3 deficient mice which lack the main effector functions of complement[58]. Mice deficient in C3 developed fewer cysts and had reduced renal volume, suggesting that complement, possibly by modifying inflammation, has a role in cyst development. This is supported by studies of complement inhibition in two different animal models of cystic disease[59]. Complement inhibition reduced kidney volume, cyst number and reduced the inflammatory infiltrate in tissue adjacent to cysts. Critically complement inhibition also preserved renal function.

Proteomic analysis of cyst fluid from patients with autosomal dominant polycystic kidney disease (ADPKD) identified complement proteins within the cysts[60]. Increased concentrations of complement proteins, including C3, Factor B and C9, can also be found in the urine and by immunostaining along the cyst epithelium, suggesting activation of the alternative pathway[59,61]. Song *et al*[62] described the pattern of gene expression in ADPKD kidneys. Complement genes were consistently up regulated suggesting that a proportion of the complement proteins in the cyst fluid may be derived from local synthesis.

COMPLEMENT AND PROGRESSIVE LOSS OF TRANSPLANT FUNCTION

Complement is important at many stages during the course of a kidney transplant: complement polymorphisms may alter outcome after transplantation[63], complement gene expression is increased in pre-implantation biopsies and this can predict outcome[64], complement has a role in the development of ischaemia reperfusion injury[65] and augments the alloimmune response[66]. All of these factors may impact upon long-term transplant outcome, however it is the role of complement in antibody mediated rejection (AMR) that has generated most interest recently.

Antibody binding to graft endothelium will activate complement, initially *via* the classical pathway. Complement can then alter endothelial function to enhance thrombosis and leukocyte chemotaxis. When this occurs acutely, most frequently seen in pre-sensitised or ABO incompatible transplants, it can lead to rapid graft loss. The most compelling evidence that complement is important in acute AMR is the reported experience of complement inhibition with monoclonal anti-C5 in the treatment[67,68] or prevention of AMR[69].

The role of complement in chronic AMR, increasingly recognised as a cause of graft failure, is less clear. When the classical pathway is activated C4 covalently binds the endothelium. It is degraded to limit activation leaving a biologically inert fragment, C4d, attached to the endothelium. This can persist in the glomerulus and peri-tubular capillaries and act as a biomarker of

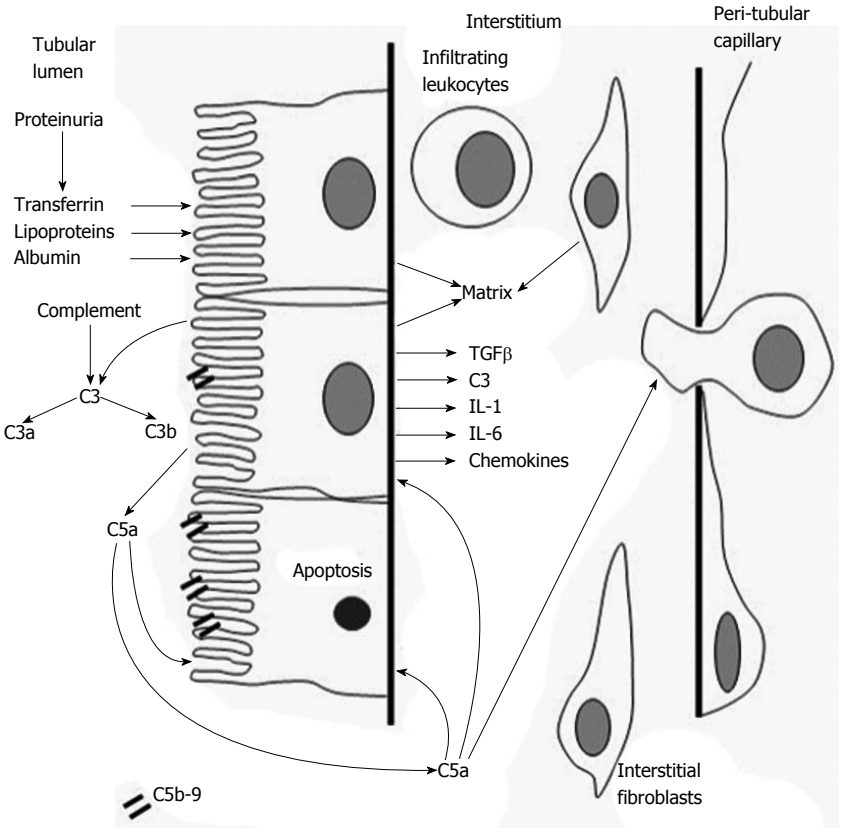

Figure 2 Complement-mediated renal injury. Complement proteins can access the tubulointerstitial compartment from the tubular lumen, the circulation or due to local synthesis. Activation can injure and activate the tubular epithelium inducing the synthesis of pro-inflammatory and pro-fibrotic cytokines. This results in an increase in interstitial inflammatory cells and fibroblasts, finally resulting in the deposition of excess matrix proteins. TGFβ: Transforming growth factor beta; IL-1: Interleukin 1.

antibody binding to endothelium. Detection of C4d in a transplant biopsy is now routinely used in the diagnosis of AMR and when present predicts a poor long-term outcome. Antibody can directly affect endothelial function[70] and whether complement further contributes to injury is less clear. There is currently a trial ongoing (NCT01327573) to assess the efficacy of anti-C5 treatment in treating chronic AMR which will answer this question.

EFFECT OF COMPLEMENT ON RENAL CELL FUNCTION

Complement activation, through generation of anaphylotoxin and sub-lytic concentrations of MAC can alter cell function. This may be the mechanism by which complement influences the progression of renal disease (Figure 2). Complement activation by the alternative pathway and deposition of sub-lytic concentrations of MAC on tubular epithelial cells stimulates the synthesis and release of pro-inflammatory cytokines (including tumor necrosis factor α and interleukin 6)[71], reactive oxygen species[25] and increases the synthesis of matrix proteins[72]. Complement can also induce expression of major histocompatibility antigens on tubular cells, allowing these cells to drive T cell proliferative responses, potentially augmenting allo and autoimmunity[73].

Renal epithelial can also respond to the anaphylatoxins, expressing both C3a receptor (C3aR)[74] and C5aR[75]. Tubular cells exposed to C3a, possibly synthesised from the tubular cells themselves[76], increase collagen synthesis[74] and adopt a more mesenchymal phenotype[39]. Similar effects can be seen in tubular epithelial cells exposed to C5a. The effect of C3a and C5a on tubular cells may in part be indirect due to increased activation of transforming growth factor beta and other growth factors that can be induced by the anaphylatoxins[77]. In an attempt to mimic more physiological conditions, the tubular cell line HK2 was grown on a microfluidic device allowing flow across its apical membrane. When the medium contained normal human serum or C3a the cells underwent mesenchymal transition and migrated into the supporting basement membrane[78].

LOCAL RENAL COMPLEMENT SYNTHESIS

The first study to demonstrate that human renal proximal tubular epithelial cells synthesised and secreted complement component C3 *in vitro* was published by Brooimans *et al*[15] more than 20 years ago. It is now

evident that resident renal cells, including tubular[79-82] and glomerular epithelial cells[16,83], mesangial cells[16,84,85] and endothelial cells[17] can synthesise many, if not all complement proteins[86]. Complement production is stimulated by pro-inflammatory cytokines and gene expression is increased in biopsies form patients with inflammatory glomerulonephritis[87], acute rejection[88] and chronic kidney disease[89]. Making use of polymorphisms in C3 it is possible to quantify the amount of protein produced from a transplanted kidney when the donor and recipient are mismatched for these polymorphisms. Renal derived C3 can contribute up to 10% of circulating C3 when the transplant is undergoing acute rejection[90].

Several pre-clinical studies have highlighted the importance of intrarenal synthesis of complement as an important mediator of local tissue. Renal complement production is increased in models of renal disease[91]. A strategy of renal syngenic or allogeneic transplantation in knockout mice can create a mouse which has a deficiency only in local complement synthesis. Application of this strategy has demonstrated a role for local C3 synthesis in transplant rejection[66], ischaemic reperfusion injury[92] and tubulointerstitial injury in proteinuric disease[93].

CONCLUSION

Chronic, non-resolving inflammation drives fibrosis in the kidney leading to a progressive loss of renal function. Complement activation occurs in the kidney during the progression of a broad range of renal diseases and could contribute to the inflammatory environment in which fibrosis occurs. There is increasing from pre-clinical models that complement activation may be linked with fibrosis, with some evidence for this from clinical studies. However, further work is required to define the role of the complement system in clinical disease progression.

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REVIEW

Immune profiling and cancer post transplantation

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Abstract

Half of all long-term (> 10 year) australian kidney transplant recipients (KTR) will develop squamous cell carcinoma (SCC) or solid organ cancer (SOC), making cancer the leading cause of death with a functioning graft. At least 30% of KTR with a history of SCC or SOC will develop a subsequent SCC or SOC lesion. Pharmacological immunosuppression is a major contributor of the increased risk of cancer for KTR, with the cancer lesions themselves further adding to systemic immunosuppression and could explain, in part, these phenomena. Immune profiling includes; measuring immunosuppressive drug levels and pharmacokinetics, enumerating leucocytes and leucocyte subsets as well as testing leucocyte function in either an antigen specific or non-specific manner. Outputs can vary from assay to assay according to methods used. In this review we define the rationale behind post-transplant immune monitoring assays and focus on assays that associate and/or have the ability to predict cancer and rejection in the KTR. We find that immune monitoring can identify those KTR of developing multiple SCC lesions and provide evidence they may benefit from pharmacological immunosuppressive drug dose reductions. In these KTR risk of rejection needs to be assessed to determine if reduction of immunosuppression will not harm the graft.

Key words: Immune-profiling; Immunosuppression; Kidney; Malignancy; Transplantation

Core tip: Kidney transplant recipients (KTR) with cancer have different leukocyte compartmentalisations and immune cell functions than KTR with no cancer. These differences can be used to determine KTR at risk of developing cancer and identify those who do not mount a reaction to their graft. Indicating there is a group of KTR that may benefit from pharmacological immunosuppressive drug dose reductions.

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INTRODUCTION

Kidney Transplant Recipients (KTR) have a 3 to 12-fold increased risk of developing Non-Lymphoid or solid organ cancers (SOC) when compared to the general population[1-4]. Cancers in KTR have poorer prognoses for a given stage/grade than the general population, which leads to higher mortality[5-9]. In Australia, it is observed that 20% of KTR will develop SOC within 15 years post transplantation (the median graft survival). Over a 5 year period (2007-2011) 267 KTR (or 31%) of all KTR died with a functioning graft (ANZDATA, 2012).

Additionally, KTR have a 60 to 250-fold increased risk of developing a Non-Melanoma Skin Cancer (NMSC), which includes; squamous cell carcinoma (SCC), basal cell carcinoma, Kaposi's sarcoma, Merkel cell carcinoma, and adnexal tumours[1,7,10]. SCC is the most common cancer in KTR with 50% of KTR who are 15 years post transplantation developing an SCC[11]. The disease progression of SCC is much more aggressive than the general population and is exemplified by the development of multiple SCC lesions and metastatic potential, phenomena that rarely occur in the immune competent[5,6,12].

The cumulative risk of subsequent SCC tumours is 30%-32%, 60%-62% and 75%-80% over 1, 3 and 5 years after first tumour, respectively[13]. Compounded, this equates to approximately 10% of KTR having > 5 tumours within 5 years of their first tumour, with some individual KTR reaching 40 primary SCC tumours during recipient life[14]. A single SCC lesion is a risk factor for subsequent SCC development with 60%-80% of KTR with one or more tumours developing another tumour within 1-3 years[15]. SCC tumour characteristics that are risk factors of metastatic SCC and include: size[16], depth[16,17], thickness[17], diameter[18] and poor differentiation[17]. Depth > 2.8 mm has a three-fold greater risk of metastasizing in KTR than the general population[19].

Further evidence of tumour aggression is the invasive potential of SCC in KTR, with more perineural and lymphatic invasion that the general population[20]. Metastatic incidence increases by 5%-8% with every SCC tumour accrued in KTR[14]. Due to SCC lesions mainly located in ultra violet (UV) exposed areas, *e.g.*, the neck, face and scalp there is a possibility of invasion into subcutaneous cranial nerves in the perineural space, leading to extensive surgery and perhaps death[21]. Reports observed an incident mortality of 1%-18%[22,23]. Observational studies have showed a 37% incidence of SCC metastasizing[18] which leads to the median KTR survival after diagnosis being only 2 years[24]. Furthermore, it has been observed that a previous SCC is a risk factor for multiple SCC and even development of SOC[11,13,19]. This is probably due to the exposure of pro-carcinogenic agents as well as the compounding effects of cancer induced, and pharmacological administrated, immunosuppression.

Therefore there are various risk factors and clinical parameters that influence the development of post-transplant cancer. The next section will introduce some of these factors and the rationale behind why they are factors of risk.

IMMUNOSUPPRESSION TYPE

There are limited and conflicting data on the use of different types on immunosuppressive drugs and the associated cancer risks. The conflict mainly due to the multiple confounding factors associated to cancer, immunosuppressive drugs in particular have the dual capacity to suppress both anti-graft and anti-cancer immunity. The immunosuppressive drug types introduced in this section include; azathioprine (AZA), mycophenolate mofetil (MMF), calcineurin inhibitors (CNI), steroids and mammalian target of rapamycin inhibitors (mTORi). These immunosuppressants are rarely used in monotherapies and are therefore hard to compare one another; instead modes of action and evidence for cancer development are presented.

AZA

AZA is catabolised to 6-mercaptopurine, which directly affects the synthesis of purines and has the ability to incorporate into DNA[25,26]. Lymphocytes rely heavily on *de novo* purine synthesis making AZA an effective immunosuppressant. AZA was originally used as an anti-cancer therapy however some cancers intrinsically have, or gain, purine scavenging and are, or become, resistant to AZA treatment[27]. When incorporated, the metabolite and the DNA form a complex that can block DNA repair, is photosensitive and produces reactive oxygen species (ROS) under UV exposure[25,27]. These work synergistically to affect DNA repair which form lesions[26,27]. One case-controlled study identified that AZA increased risk of developing SCC by 5-fold. However, in the same study calcineurin inhibitors (CNI) and steroids were also identified as risk factors[28].

MYCOPHENOLATE

MMF is a pro-drug of mycophenolic acid (MPA), which directly affects purine synthesis and is classified as an anti-proliferative drug[29]. The reaction of MPA is reversible and does not interfere with the DNA structure as AZA does[29]. One study showed a decrease photosensitivity when a cohort was randomised onto a MMF from AZA suppression regimen[30]. In another study comparing MMF to AZA usage in organ transplant recipients showed that the MMF group had a 27% adjusted risk reduction[31]. Conversely, a 3 group randomised control trial of 133 KTR; 45 KTR randomised to AZA treatment, 44 KTR randomised to 3 g daily of MMF and 44 KTR randomised to 3 g daily of MMF with no differences in cancer incidences between all three groups[32].

CALCINEURIN INHIBITORS

Cyclosporine A (CsA) forms a complex with cyclophilin which inhibits calcineurin, making CsA and CNI[33]. Calcineurin de-phosphorylates nuclear factor of activated T cells (NFAT), which translocates to the nucleus. It is in the nucleus where NFAT activates pro-inflammatory cytokines such as interleukin 2 (IL-2)[34]. Therefore CsA indirectly affects pro-inflammatory cytokine IL-2 transcription. An isotype of cyclophilin is expressed in the mitochondria which releases apoptotic signals under oxidative stress. CsA blocks this signal transduction and allows cells to by-pass apoptosis when under oxidative stress, including ROS and UV-damage, contributing to carcinogenesis[35,36]. Other tumorigenic side effects of CsA are direct or in-direct suppression of p53, production of transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF)[37-39].

When investigating this in the clinic, a retrospective analysis of 1000 KTR showed that KTR on CsA based regimens had greater cumulative incidence of tumours than those on an AZA based regimens[40]. In another retrospective study any regimen with CsA had an Odd Ratio of approximately 4.5[41]. Inversely, A CsA based mono-therapy was shown to be less carcinogenic than a MMF and prednisone dual-therapy[42,43]. Another CNI, tacrolimus (TAC), inhibits calcineurin by forming a complex with FK506-binding protein 12 (FKBP12) and outcompetes calmodulin therefore still inhibiting IL-2 transcription. TAC does not target cyclophilin, so avoids all interference with the mitochondria that CsA has. In a retrospective study of 609 liver transplant patients, TAC had a higher incidence rate for *de novo* cancers than CsA[44]. However in most database analyses, TAC-based immunosuppressive regimens have either no significant difference or a reduced risk of cancer incidence and/or risk over CsA-based immunosuppression regimens[45-48].

CORTICOSTEROIDS

Corticosteriods are mainly utilised for treatment of auto-immunity, inflammatory disorders and transplantation rejection. Corticosteriods function by inhibiting transcription of IL-1, IL-2, IL-6, interferon (IFN)-γ and tumor necrosis factor (TNF)-α and transcription factors such as nuclear factor-κB[49-54]. Inhibition of these Th1 cytokines promotes a Th2 response, which provides another indirect immunosuppressive function[55]. Corticosteroids induce TGF-β and can increase the incidence of Kaposi's sarcoma cell proliferation[56,57].

MAMMALIAN TARGET OF RAPAMYCIN INHIBITORS

Both Sirolimus (SIR) and Everolimus (EVO), like TAC, bind to FKBP12. However the formed complex inhibits mTOR's *via* mTORC 1 subunit (Raptor) binding and are considered mTORi. mTORi can also be classified as anti-proliferatives as they induce apoptosis *via* p53 dependent and independent pathways. This and mTORi's ability to prevent IL-2 signalling cause it to have both anti-cancer and anti-rejection properties. Additionally, mTORi affect protein synthesis, including VEGF which inhibits metastatic potential in murine models[58,59]. SIR has been used to treat patients with renal cell carcinoma (RCC) and EVO has shown to benefit patients with metastatic RCC who do not response to mainstream treatment[60-62]. Sirolimus Conversion from CNI based regimens, is beneficial in Kaposi sarcoma and SCC involution[63-66] However it can often lead to increased adverse reactions and increases in rejection episodes if performed too early post-transplant[67,68].

ANTI-THYMOCYTE GLOBULIN INDUCTION THERAPY

Anti-thymocyte globulin (ATG) is either horse- or rabbit-derived antibodies directed against human T cells, given as an induction therapy of transplant recipients. The T cells that reconstitute have a regulatory phenotype and return much faster than other T cells[69]. There is an association with prolonged CD4 lymphopenia and ATG as well as CD4 lymphopenia and cancer[70]. Without knowing cause and effect it is speculative to say that ATG is associated with cancer.

Despite the various functions of immunosuppressive types each playing a role with cancer in KTR, overall immunosuppressive load or immunosuppressive dose can also have detrimental effects and promote cancer development.

IMMUNOSUPPRESSION DOSE

There is an association between immunosuppression dose and cancer incidence. KTR have 3-fold increased cancer risk compared to dialysis patients, in a retrospective registry based study[71]. Furthermore, heart transplant patients have higher levels of immunosuppression than KTR and also have corresponding increases in cancer (100% compared to 88% 5 year incidence, respectively[14]). Additionally, KTR randomised to a low dose CsA base regimen had reduced incidence of cancer following reduction, with the caveat that they had higher rejection rates[72].

IMMUNOSUPPRESSION DURATION

Maintained immunosuppression increases the risk of cancer over time which is evident in the steady increase in KTR that accrue cancer in the years post-transplant. Australian KTR SCC incidence is 20%, 50% and 80% at 5, 15 and 30 years post transplantation respectively[11,73]. Included in the duration of immunosuppression would be the age and aging of the KTR.

AGE AND GENDER

Age is a risk factor of cancer development, independent of immunosuppression duration[74]. This is exemplified in a retrospective study that showed both Age and male gender were risk factors[41]. When comparing KTR to the general population in an aged matched cohort of median age 39 years old, there was a 12-fold increased risk of developing non-skin cancers[4]. Age and gender can influence other parameters of cancer risk. This is particularly the case in Australia were certain, culturally male-orientated, jobs may involve higher exposure to UV radiation.

ULTRA-VIOLET RADIATION

It is evident that UV exposure increases the risk of skin cancer, including NMSC, by the observations recorded by clinicians of the locations of tumours. Cumulative sun exposure, including outdoor occupation, latitudinal residence and even childhood burning events all increase risk of post-transplantation cancer development[75-77]. These increases in carcinogenesis are in part to the aforementioned AZA-UV interactions but mainly *via* direct UV-related mutagenesis. Due to the structure of DNA, it absorbs of UV-A (315-400 nm) and UV-B light (280-315 nm), in doing so the DNA itself forms cyclobutane pyrimidine dimers in two adjacent pyrimidines of the same DNA strand, which alters the structure of DNA and restricts transcription[78,79]. A single point mutation can lead to transcriptional arrest[79]. A study found that invasive SCC contained mutations of the tumour suppressor gene P53[80]. An important conclusion from this study is that P53 mutation could have happened in childhood, as most UV exposure happens in childhood[81].

In addition to direct DNA mutagenesis, UV exposure can also have local and systemic effects on the immune system. It is thought that the local effect involves antigen presenting cells (APC)'s, including resident keratinocytes and Langerhans cells[82,83]. Whereas the systemic immunosuppression may come from splenic cells, migrated Langerhans cells, dendritic cells. Increased expression of IL-4, IL-10, prostaglandin E2, IL-1α and TNF-α with polarisation of immunity to a Th2 response also plays a role in systemic immunosuppression[83-85]. In combination with this, co-stimulation is effected on both APC and T cells[86]. Other cell types that are affected by UV irradiation are innate immune cells and suppressor cells[87-91]. Regulatory T cells (Tregs) that are induced by UV express lymph node homing molecule CD62L and may provide systemic immunosuppression[87,88].

The DNA damage and immune suppression of UV can be reversed by IL-12 dependent induction of nucleotide excision repair protein[92]. Also immunity can be restored by the administration of IL-12[93], activating APC's, increasing IFN-γ and thus balancing Th1-Th2 polarisation[93,94].

Other clinical parameters are associated with cancer risk that are also orientated by human behaviour, apart from UV exposure, are communicable diseases such as oncogenic viral infections that remain latent in the immune competent.

VIRAL INFECTION

Human papillomavirus (HPV) is a group of more than 150 viruses with some types associating with anogenital, oropharyngeal and skin cancers[95,96]. It has been speculated that HPV infection may prevent UV light-induced apoptosis[97]. Between 65% and 90% of SCC lesions from transplant recipients are positive for HPV DNA[98].

Epstein barr virus (EBV) is associated with: sino-nasal angiocentric T-cell lymphoma, Hodgkin lymphoma and nasopharyngeal carcinoma[95]. There are data that EBV associates with mononucleosis, Burkitt lymphoma and post-transplant lymphoproliferative disorder in KTR[99,100].

Chronic Cytomegalovirus virus (CMV) infection can cause graft rejection, but with malignancy however it does have indirect associations with cancer[99]. A prospective study followed 63 KTR and retrospectively included 131 KTR, with convincing data that CMV positive KTR with increased $\gamma\delta$ T cell proportions, the $V\delta2^{neg}$ sub-population in particular, had decreased cancer incidence[101]. This case-control study compared 18 short-term KTR (median 3 years post Tx), who developed 12 skin and 6 solid tumours over the prospective period and compared to 45 KTR who did not develop cancer. The skin nor solid organ tumour types were not disclosed.

IMMUNE PHENOTYPING

The association with cellular markers and cancer has been previously studied. The identification of immune cell populations and sub-populations in patient blood is called immune phenotyping. Measurement of CD4 T cells in 150 KTR revealed that KTR with skin cancer had 330 $CD4^+$ cells/μL of blood in comparison to KTR with no cancer who had 565 $CD4^+$ cells/μL ($P < 0.01$). Additionally KTR with cancer had non-significant increases in CD8 and CD19 lymphocytes[102]. Another study involving 250 KTR over a 10 year period showed a mean of $CD4^+$ lymphocytes of < 600 $CD4^+$ T cells/μL for those with cancer and > 700 $CD4^+$ T cells/μL for those with no cancer, however there was no useful threshold found using receiver operator curve (ROC) analysis[103]. Additionally, $CD8^+$ T cells and $CD19^+$ B cells were also investigated in the same study; there was no difference between KTR with SCC when compared to KTR without SCC[104]. It was noted however, that immune phenotype was more pronounced in KTR with SOC compared to KTR with SCC: CD4 count: 234 cells/μL *vs* 543 cells/μL, $P < 0.001$; CD8: 328 cells/μL *vs* 640 cells/μL $P = 0.100$; CD19: 19 cells/μL *vs* 52 cells/μL, $P < 0.001$[104]. All these

studies showed an association with CD4 lymphopenia and cancer, however the majority of the cohorts underwent ATG induction therapy. However they did not define $CD4^+$ subsets or other lymphocytes that may be affected by cancer.

While these studies provide some evidence that cancer may influence the peripheral immune cells, there was no investigation into sub-types of these cells, primarily because multi-parameter flow was not common place. Recently, it was reported that high numbers of $CD4^+$ Regulatory T cells (Tregs, *i.e.*, $CD4^+FOXP3^+CD127^{Lo}CD25^{Hi}$) and low numbers of Natural Killer (NK cells, *i.e.*, $CD56^+CD16^+$), in peripheral blood associated with and predicted recurrent SCC in KTR[105]. This study also showed an increase in $CD8^+CD28^-$. These CD8 T cells co-localise with Tregs within cancer tissue and have been shown to be suppressive from patients with cancer, and therefore abbreviated to $CD8^+$ Tsupps[106]. Furthermore, there was a decrease in $CD8^+CD45RA^-CD62L^+$ CD8 central memory T cells ($CD8^+$ Tcm), which has been shown to decrease in KTR using the corticoid steroid prednisolone, despite cancer status[105]. This indicates that immunosuppression may affect immune phenotype and warrants investigation.

Operationally tolerant organ transplant recipients have increases in Regulatory T cells, B cells (particular naïve B cells), Vδ1 γδ T cells and decreases in $CD3^+$ proportions (B:T ratio), NK cells, Vδ2 γδ T cells within their peripheral blood[107]. Transplant patients have increased Regulatory T cells, B cells (memory B cells), $CD8^+$ γδ T cells and $CD8^+$ $CD27^-CD28^-$ T cells and decreases in CD4 counts, NK cells and $CD8^+$ Tcm[105,108].

REGULATORY T CELLS (TREGS)

Immune suppressor cell existence has been debated from the early 1970's through to the mid 1990's[109-112]. The pivotal paper adoptively transferred $CD4^+CD25^+$ T cells in CD25 depleted mice, which mitigated the autoimmune diseases that manifested[112]. However, CD25 is also expressed on activated lymphocytes with only the highest proportion being suppressive *in vitro via* competitive absorption of IL-2[112-115]. The discovery and transfection of the transcription factor forkhead box protein 3 (FOXP3) into naïve T cells helped identify FOXP3 and its function as the master regulatory gene[116,117] and CD127 inversed expression to FOXP3 expression has given Tregs the current phenotype $CD4^+FOXP3^+CD25^{hi}CD127^{lo}$[114].

Tregs are required in a healthy immune system to maintain self-tolerance and immune homoeostasis during immune reactions, pregnancy and disease. Uncontrolled immune reactions and organ failure result when mutations in FOXP3 occur, as observed in the scurfy mouse models and similarly Immunodysregulation, Polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome observed in humans[118-120]. Both IPEX and X-linked Autoimmunity-Allergic Dysregulation syndrome cause multi-organ failure due to mass lymphocyte proliferation of self-reactive effector cells [119].

$CD4^+$ TREG SUBSETS

The $CD4^+$ Treg in the periphery, defined by $FOXP3^+CD25^{hi}CD127^{lo}$, contain two subsets: those that originate from the thymus, known as natural Tregs (nTregs), and those that are induced in the periphery, known as induced Tregs[121]. The Ikaros family transcription factor, Helios is expressed in 100% of all $CD4^+FOXP3^+$ thymocytes of mice and approximately 70% of Tregs in the periphery of both mice and humans[122]. Though the premise that Helios only defines nTreg is currently under debate, nonetheless, it may provide evidence of *in vivo* activated Tregs[101,123]. Despite the debate it seems that KTR with cancer have similar Helios expression than KTR without cancer[108].

TREG MODES OF ACTION

Treg apoptosis induction requires cell contact with co-stimulatory molecule Cytotoxic T cell Late Antigen-4, Fas/Fas ligand interaction and release of Perforin and Granzyme B[124-126]. Indirectly, Tregs can down-regulate B7 Co-stimulation molecules CD80/CD86 on APC[127]. In addition, prostaglandin E2 (PGE2) excreted by Tregs, mediates expression of indoleamine 2,3-dioxygenase in APCs causing tryptophan starvation and leading to impaired lymphocyte proliferation[128]. Another form of suppression is the formation of localised adenosine by cleaving phosphate groups from ATP, ADP and AMP by ecto-NTPDase-1 (CD39) and ecto-5'-nucleotidase (CD73) cell surface enzymes[129]. Expression of CD39 and CD73 has been shown on murine and human Tregs[129]. Human Tregs also may work in concert with other CD73 expressing cells to elicit a regulatory response. Adenosine has been shown to act *via* Adenosine receptors (A1, A2a, A2b and/or A3), with A2a receptor being the dominate receptor on effector cells[130,131]. The adenosine formed by the hydrolysis of ATP can regulate lymphocyte proliferation in autoimmune disease, transplantation and cancers[132-134]. Additionally, it has been shown that adenosine and PGE2 in Tregs co-operate when regulating immune responses[133]. Other regulatory cells are $CD4^+$ helpers that have suppressive function are classified by the ability to secret of IL-10 (Tr1) and TGF-β (Th3) which they are also induced by, respectively.

TREGS IN VIRAL INFECTIONS

EBV antigen specific Tregs, mainly IL-10 secreting Tr1 and recruited nTregs, can inhibit the EBV-specific immunity permissive in tumour progression[100,135]. Thus reduction in Tregs may be beneficial in treatment of chronic viruses. Interestingly, Treg depletion in a herpes simplex virus (HSV) mouse model decreased paralysis onset, indicating that Tregs have an early role in protective immunity to HSV infection, similarly observed

in Lymphocytic Choriomenigitis virus mouse model, shown in the same study[136].

TREGS AND TRANSPLANTATION

In regards to transplantation, when isolated $CD4^+CD25^-$ cells are administered to BLABc *nu*/*nu* mice grafted with C57BL/6 skin there is a swifter rejection rate than administering untouched lymphocytes of the same source[112]. This indicates $CD4^+CD25^-$ T cell subpopulation has greater cytotoxicity when absent from $CD4^+CD25^+$ T cells and that $CD4^+CD25^+$ T cells are possible inducers of tolerance.

In KTR, Tregs can differ in accordance with the situation of the patient. Two different studies on clinically tolerant, chronic rejection, stable, minimally suppressed KTR and healthy controls, showed tolerant KTR and minimally suppressed KTR had similar $CD4^{+-}$ $CD25^+FOXP3^+$ and $CD4^+CD25^{hi}$ cells with similar FOXP3 transcription levels when compared to the healthy controls[137,138] and that chronically rejecting KTR had lower $CD4^+CD25^{hi}$ cells with low FOXP3 transcripts, indicating that Tregs may be protective or involved with tolerance[137,138]. An additional study supported this in liver transplant recipients which showed increased FOXP3 mRNA expression in $CD4^+CD25^{hi}$ T cells of tolerant patients compared to patients who had rejection episodes after cessation of immunosuppression[139]. Thus induction of Tregs for suppression of allograft cellular rejection episodes[140] and possible induction of tolerance[141] seem like an attractive substitute to immunosuppression. However Tregs that co-express CD25 and CD39 have been denoted as a memory subtype of Treg (mTreg) and are associated with cellular rejection episodes[142] in KTR. Increases in Tregs are also associated with cancer in the general population[143] and KTR[105].

TREGS IN CANCER AND IMMUNE SURVEILLANCE

It has been shown that the percentage of $CD4^+CD25^{high}$-$FOXP3^+$ Tregs and Tr1 cells are increased in Head and Neck Squamous Cell Carcinoma (HNSCC) patients in comparison to healthy controls[144,145]. Ectonucleotidase activity contributed by CD39 and CD73 is also increased on Tregs in this cohort[133]. CD39 has been shown to down-regulate IL-17 production, decreasing Th-17 cell linage. This particular Treg subtype, in the same study, has been shown to be down-regulated in autoimmune Multiple Sclerosis[132]. It has been shown that high levels of Treg in HNSCC patients from the general population associate with a poor prognosis[146-148].

Cancers and Tregs not only have commonalities between each other but they also promote each other. TGF-β and IL-10 secretions from tumours activate Th3 and Tr1 regulatory cells respectively, consequently regulating surrounding cancer cytotoxic lymphocytes[145]. Also tumour cells recruit Tregs with a series of chemokines such as C-X-C Ligand 12 and C-C motif 20 and 22 (CCL20/22)[100]. CD39 and CD73 have been shown to be expressed on Tr1 and tumour cells alike[129,149]. Cancer progresses by the tumours' ability to secrete these soluble factors into its microenvironment. PGE2 is a product of Cyclooxygenase 2 (COX-2) and is involved in aiding immune escape. COX-2 is expressed on Tr1 and over-expressed on cancer cells[145,150,151].

In a post-transplant cancer setting, it has been shown that Tregs ($CD4^+FOXP3^+CD25^{hi}CD127^{lo}$) in blood from KTR with a history of SCC can predict the risk of developing a subsequent SCC lesion[105]. Another study has shown that Tregs alone can predict cancer onset and associate to the severity of the cancer developed[108]. In this same study Hope *et al*[108] shows prospectively that Tregs increase in KTR when the cancer becomes apparent and then decreases post-resection of tumour tissue.

NK CELLS IN CANCER AND IMMUNE SURVEILLANCE

Carroll *et al*[152] revealed that NK cells, which have cytolytic ability to kill cancerous and pre-cancerous cells, are decreased in KTR with cancer. NK cells are a part of the innate immune system that identify abnormal cells and supply the signals to undergo apoptosis thus "killing" abnormal cells. The identification process involves Major Histo-incompatibility Complex (MHC) class I down regulation, which some viruses and cancerous cells adopted to avoid the adaptive immune system[153]. It is an important step in metastatic cells to successfully invade the host[154]. Once the cell has been identified the NK cell only activates if there is an imbalance of CD94: NKG2A and the killer-cell immunoglobulin-like receptors (KIR) family. Once activated internal granules locate to the synapse that is created between the NK cell and target cell[152]. During the effector stage the granules are released out of the NK cell and into the synapse and onto the target cell. These proteins include Perforin, granzyme A and B. It is these proteins that play their role in the killer phase of NK cells[155]. Perforin creates pores in the membrane that granzyme B can enter and activate the caspase kinase pathway and cause the target cell to undergo apoptosis[155]. This cytotoxic ability to kill cancer cells can be inhibited by Tregs but also cancer cells themselves[156,157]. This NK-Treg interaction is a TGF-β and cell-cell contact mechanism of down-regulation NKG2D and induction of apoptosis, respectively[158,159]. This leads to decreased NK cell numbers and function in the peripheral blood of cancer patients that have elevated TGF-β[160,161]. There are two other types of NK cells: those that express CD1-d restricted T cell receptor, NK T cells and those that lack Fc receptor CD16 and over express CD56, $CD56^{bright}$ NK cells[162-164]. Both these cells can interact with the adaptive immune system and enhance

anti-tumour ability by direct and indirect mechanism respectively[162,164].

CD8 SUBSETS IN CANCER AND IMMUNE SURVEILLANCE

Another cell type with anti-tumour properties is $CD8^+$ cytotoxic T lymphocytes (CTL). $CD8^+$ CTL are in the effector arm of the adaptive immune system. CTLs use the ability to lyse tumour cells using Fas-Fas ligand as well as perforin-IFN-γ granules similar to NK cells[165]. It has been shown that antigen specific CTL are defective in cancer patients and that removal of Tregs can restore cytolytic function[166-168].

CD4 and CD8 T cells follow an immunogenic pathway to immune senescence. T cells exiting the thymus are naïve since they express both CD27 and CD28 co-stimulation molecules and home to the lymphoid organs[169,170]. When antigen is presented they become CTL, clear the threat, and the majority apoptose with the minority homing to lymphoid organs as central memory T cells or extra-lympoid sites as effector memory T cells[169,170]. Upon subsequent exposures the cells become exhausted and lose expression of co-stimulation molecules and are termed T effector memory CD45RA+ or TemRA cells[169,171]. These cells are loosely phenotyped as $CD8^+CD28^-$ and shown to be regulatory in cancer patients and may associate with poor prognosis[106]. Tumours themselves may induce this loss of CD28[106,172] and they are also expanded in patients with CMV infection[173]. It has been shown that Memory T cells and (NK cells have anti-tumorigenic properties and that Tregs regulate both of these lymphocyte subsets[158,174]. Thus, an excess of Tregs is associated with poor prognosis in cancer and is thought to aid cancer cells evade this immune surveillance.

IMMUNE CELL FUNCTIONS

Kidney transplant recipients (KTR) with cancer have increased numbers and proportions of Regulatory T cells (Tregs) and decreased numbers and proportions of NK cells[105,108]. However, the immune system's effectiveness cannot be gauged by cell numbers and proportions alone; this chapter investigates the immune function of KTR with cancer.

It has been shown that Tregs isolated from tumour tissue and the peripheral blood of KTR with cancer have higher suppressive function than Tregs from the blood of normal donors[145,175,176]. Importantly, the stage and grade of HNSCC are associated with greater numbers and greater suppression capacity of the Tregs on a cell-per-cell basis than healthy controls[177] and, as such, also associate with poor cancer prognosis in the general population[176].

In the Transplant population it is known that CNI regimens are associated with reduced numbers and proportions of Tregs and how mTORi maintain these Treg parameters[178,179]. Furthermore, Tregs numbers and proportions are increased by mTORi usage in KTR with no cancer and CNI usage decreases Tregs in KTR with cancer. A proposed mechanism is CNI's ability to reduce Nuclear Factor of Activated T cells (NFAT), decreasing production of IL-2 which is vital for function and homeostasis, in mice[180]. Molecular interactions between NFAT and FOXP3 show that NFAT acts as a molecular switch between immune stimulator and immune regulator, thus down regulation decreases FOXP3 expression and FOXP3's ability to form these regulatory complexes[178,181]. Additionally, FOXP3 mRNA transcription was decreased in CNI treated peripheral blood mononuclear cells compared to Rapamycin in an allo-stimulated mixed lymphocyte reaction[182]. There is also an inverse correlation to CNI level and Treg function[183].

Tregs promote cancer survival whereas NK cells have anti-cancer abilities. The function or dysfunction of NK cells plays an important role in the apoptosis of pre-cancer and cancerous cells. Patients with genetically (*MCM4* or *GATA2* mutations) related NK cell deficiencies in either number or function, have increased risk of infections, in particular: Herpes viruses, HPV, CMV and EBV (reviewed elsewhere[184]).

NK cells are large granular lymphocytes that lack the CD3 T cell complex. They function by identifying and spontaneously causing apoptosis in cancerous and infected cells without prior antigen presentation[152,185]. The identification process requires abnormal cells to display stress signals such as down-regulation of "self" surface proteins: Major Histo-incompatibility Complex (MHC) class Ⅰ and regulatory KIR[154,155,186]. The down regulation of MHC-Ⅰ, reduces the effectiveness of cytotoxic CD8+ T cells and adaptive immune responses but makes the cells more sensitive to NK and innate immune responses[187]. Once an NK cell identifies this down-regulation, it binds and activates, expressing a type Ⅱ transmembrane glycoprotein CD69 and other surface markers of activation[188]. Internal granules locate to the immune synapse that is created between the NK cell and the target cell and the effector molecules (perforin, TNF-α, granzymes and interferons) are released into the synapse and onto the target cell. Upon degranulation, Lysosome-Associated Membrane Protein 1 (CD107a) is exposed on the surface of the NK cell[155]. The released perforin creates pores in the target cell membrane through which granzyme B can enter the target cell and initiate apoptosis *via* the caspase kinase pathway. Therefore there are several ways to measure NK cell activity including: CD69 up-regulation in the activation stage, CD107a in the effector stage, release of cytokines (perforin, granzyme B, IFN-γ) in the killing stage, and total cytolysis of the target cells.

Cancer cells have greater metabolic demands than normal cells[189], utilising glycolysis and lactate pathways, *via* Lactate Dehydrogenase (LDH), causing an 18-fold increase in glucose utilisation, even under aerobic conditions[190]. This LDH can be measured as a cytotoxic assay (first described in 1988[191]). Additionally, in *in*

vitro assays, NK cells undergo apoptosis when they are exhausted from their last kill. Recently, it has been shown that the loss of NK cells from an *in vitro* assay with a set number of NK cells, can relate to the amount of target cells killed. This loss has been termed "target induced NK cell loss" (TINKL). These two assays have been chosen for clinical application. LDH is a single platform, self-contained, non-radioactive, sensitive assay that can be used in any laboratory. TINKL is a flow-based assay that can be readily implemented in clinical flow laboratories.

It is widely accepted that NK cell function is decreased in cancer patients however it is not reported if KTR with cancer have further reduced NK cell function. The effect immunosuppression has on NK cells have been investigated both *in vitro* and *in vivo*[192,193]. Immunosuppressive drugs: AZA, MMF, CNI, and prednisolone all have individual effects. These effects depend on the how the NK cells are stimulated and how NK function is measured. One particular study showed only a decrease in NK function in short term KTR compared to healthy controls, which was not observed in long term KTR[192]. Both IFN-γ and CD107a expression have been shown to decrease when NK cells were co-cultured in the presence of clinically relevant concentrations of a variety of immunosuppressive drugs[193].

TREATMENT OPTIONS FOR KTR WITH CANCER

The aforementioned assays give clinicians the ability to objectively identify patients that may develop premetastatic cancer with relatively high sensitivity and specificity. However they do not inform clinicians if KTR will benefit from cancer prevention therapy.

A randomised control trial randomised pre-transplant KTR to a standard level CNI regimen and a CNI sparing regimen[72], thus investigating the benefit of reduced immunosuppression as primary cancer prevention. However, those with reduced CNI had increases in rejection episodes[72]. Other studies investigated converting CNI based regimens to mTORi based regimens as secondary prevention therapy, as mTORi are used as anti-cancer therapies[194,195]. There was a benefit, however not all conversions were successful (30%) and an additional 30% did not tolerate the mTORi side effects[14,196,197]. Furthermore, immune phenotype has revealed that those who maintain high levels of Tregs after mTORi conversion (> 20 Tregs/μL) do not benefit from conversion and may benefit from immunosuppressive drug reduction. To perform immunosuppressive drug reduction as secondary cancer prevention, risk of graft rejection will need to be measurable.

Pre-transplant anti-Human Leukocyte Antigen (HLA) and IFN-γ ELISPOT associate post-transplant with antibody and cellular mediated rejection episodes[198-200]. Monitoring HLA molecules and Donor Specific Antibodies (DSA) routinely has decreased antibody mediated rejection episodes dramatically[201,202]. IFN-γ ELISPOT has been used to predict 6-mo graft function and rejection episodes[200]. Additionally it has been used pre-transplant to categorise patients into CNI or mTORi maintenance therapy[203]. These studies are limited in clinical application as donor specific cells were used to stimulate the mixed lymphocyte reactions, requiring use of precious or non-existent deceased donor material. This restricts the utility of ELISPOT to live recipient/donor pairs. An IFN-γ ELISPOT assay has been developed that utilises a variety of unrelated HLA disparate material to measure total allo-response and is termed "Panel of Reactive T cells"[204]. This assay has been shown to have potential to determine post-transplant risk of rejection when measured pre-transplant. However there are no current studies utilising IFN-γ post-transplant as a form of rejection prediction in long-term KTR.

The IFN-γ ELISPOT may be extended to guide immunosuppression reductions[205,206]. There are a few studies utilising a viral peptide stimulated IFN-γ ELISPOT to discriminate KTR who may benefit from reduced immunosuppressive drugs as a form of treatment[205,206]. KTR with unresolved BK pathogenesis also had a non-significant decrease in EBV peptide and phytohaemagglutinin mitogenic IFN-γ ELISPOT responses[205,207]. This may share a link with development of malignancy as they are both considered manifestations of over-immunosuppression.

When KTR have a cancerous lesion, surgical resection is the recommended treatment. There are no randomised control trials investigating the effect of tumour resection and minimal evidence of benefit in KTR when reducing immunosuppression. However, treatment in the general population is associated with a decrease in Tregs. Failure of Tregs to fall after tumour excision, chemo or immunotherapy is due to incomplete resection or predicted relapse of disease[208,209].

When switching or reducing immunosuppression, adequate precautions must be used. Currently there are no assays that reliably determine cancer risk although there is an immune phenotype that can predict time to next tumour in KTR with a history of SCC[105]. CNI avoidance or reduction results in increases of rejection; one way to potentially avoid these rejection episodes is to identify those KTR with cancer who have evidence of a potential alloresponse and exclude them from dose reduction. In order to reduce immunosuppression safely, both the cellular and humoral alloresponses need to be assessed.

PRE-TREATMENT ALLORESPONSE MEASURES

Assessment of allo-responses would be needed to assess risk of rejection episodes for it to be possible to reduce immunosuppression. Currently cytokines and HLA antibodies can be measured by Enzyme Linked Immuno SPOT (ELISPOT) and Luminex technologies respectively[198,210]. Inflammatory cytokines such as IFN-γ are secreted by Th1 effector T cells and are a predictor of

acute rejection and infection[200,204]. A National Institute of Health funded Clinical Trials in Organ Transplant consortium approved ELISPOT has been able to detect 6-mo post-transplant acute rejection in pre-transplant patients[211,212]. Additionally a similar assay has been used to run CNI avoidance maintenance therapy with a 3-fold reduction in acute rejection as shown in literature[203]. The humoral aspect of the immune system is already routinely assessed in most transplant programmes by solid phase alloantibody detection systems[202]. HLA DSA are clinically relevant and observed DSA presence has informed clinicians to alter immunosuppression regime of patients[199,201]. However both these techniques have not been measured in long-term kidney transplant recipients with a history of cancer.

CONCLUSION

Long-term immunosuppression increases the risk of cancer development. The dose of immunosuppression can be increased by closely monitoring graft function and survival. In this review we present that there are several emerging immune monitoring tools that are available to potentially help reduce immunosuppression. Future studies may be undertaken to determine if these assays can help identify those at risk of cancer development and if reduction of immunosuppression is of benefit.

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REVIEW

Biomarkers in chronic kidney disease, from kidney function to kidney damage

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Abstract

Chronic kidney disease (CKD) typically evolves over many years, with a long latent period when the disease is clinically silent and therefore diagnosis, evaluation and treatment is based mainly on biomarkers that assess kidney function. Glomerular filtration rate (GFR) remains the ideal marker of kidney function. Unfortunately measuring GFR is time consuming and therefore GFR is usually estimated from equations that take into account endogenous filtration markers like serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the currently used markers. The aim of this review is to discuss the utility of the GFR estimating equations and biomarkers in CKD and the different clinical settings where these should be applied. The CKD-Epidemiology Collaboration equation performs better than the modification of diet in renal disease equation, especially at GFR above 60 mL/min per 1.73 m^2. Equations combining CysC and SCr perform better than the equations using either CysC or SCr alone and are recommended in situations where CKD needs to be confirmed. Combining creatinine, CysC and urine albumin to creatinine ratio improves risk stratification for kidney disease progression and mortality. Kidney injury molecule and neutrophil gelatinase-associated lipocalin are considered reasonable biomarkers in urine and plasma to determine severity and prognosis of CKD.

Key words: Chronic kidney disease; Estimated glomerular filtration rate; Kidney damage; New biomarkers; MicroRNA

Core tip: Until more accurate equations are developed the chronic kidney disease (CKD) epidemiology collaboration appears to be superior to other glomerular filtration rate (GFR) estimating equations. In circumstances where CKD requires confirmation estimated GFR based on the combined creatinine-cystatin C equation is recommended. The recent advances in molecular biology have resulted in promising biomarkers for CKD detection and prognosis; however more research is needed before applying them into clinical practice.

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INTRODUCTION

Chronic kidney disease (CKD) has become a public-health problem. The definition of CKD was introduced by de National Kidney Foundation (NFK/KDOQI) in 2002 and latter adopted by the international group Kidney Disease Improving Global Outcomes (KDIGO) in 2004. The definition of CKD requires a decrease in kidney function with a glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m^2 and/or kidney damage for 3 mo or more. Kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g)[1].

One important aspect about of classification of CKD is that it can usually be detected with non invasive testing. CKD classification is relevant as it has been associated with outcomes such as kidney disease progression, cardiovascular disease and all cause mortality. It is also important as it can allow therapeutic interventions in earlier stages to slow disease progression reduce complications related to decreased estimated GFR (eGFR), cardiovascular (CVD) risk and improve quality of life and survival[2-4]. GFR is the most important marker of kidney function. Unfortunately GFR cannot be easily measured in most clinical or research settings (see below), and therefore estimating equations are based on filtration markers such as serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the commonly used markers of kidney disease. The aim of this review is to summarize the most recent findings of most biomarkers in CKD and its implications in clinical practice.

KIDNEY FUNCTION MEASUREMENT

Kidney function estimation was commonly made using SCr concentration, blood urea nitrogen (BUN) level and urine analysis[5]. However accumulating evidence has demonstrated that these biomarkers are not optimal to detect kidney disease in early stages[6-9]. The KDIGO recommends that CKD be diagnosed, classified, and staged by GFR[10]. In clinical practice GFR is crucial for diagnosis, management, drug dosing and prognosis, in addition to its utility for research and public health[11-13]. GFR is the volume of fluid filtered from the glomerular capillaries into the Bowman's capsule per unit time[14,15]. GFR values are associated with age, sex and body surface and are 120 and 130 mL/min per 1.73 m^2 in young men and women, respectively (GFR declines with age)[16-18].

mGFR

Establishing the true GFR is difficult because the filtration process simultaneously takes place in millions of glomeruli and filtrate composition and volume change when passing through the kidney. GFR is measured (mGFR) indirectly as the clearance of filtration markers that are eliminated by the kidney only by glomerular filtration. Clearance can be measured as either plasma or urinary methods that record the clearance of endogenous or exogenous substances by the kidney[11]. As such, an ideal substance is one that is freely filtered at the glomeruli and neither secreted nor reabsorbed by the renal tubules[15,18]. Inulin is an exogenous filtration marker derived from a fructose polymer and is a physiologically inert substance and is considered an ideal substance for mGFR[19,20]. Although inulin clearance is considered the gold-standard method for mGFR[20,21], the need for continuous infusion, multiple blood samples and urine collection, make it cumbersome and expensive to measure and has led to research of alternative methods with other biomarkers[10,21-24].

Other methods for mGFR have also been validated. Soveri *et al*[24] reported that kidney excretion of 51Cr-EDTA or iothalamate, and plasma removal of 51Cr-EDTA or iohexol, using inulin clearance as reference, were sufficiently accurate (P30 > 80%) methods to measure GFR[24]. Among these iohexol is the most recent biomarker for mGFR, it is a non-ionic and non radioactive contrast agent, its molecular weight is 821 Da, has a small extra renal clearance and could be measured only as plasma clearance without the need of urine collections[25]. Some of its other advantages are low expense, wide availability, stability in biologic fluids, and rare adverse reactions when given in a small dose (5 mL of 300 mg/mL iodine)[26,27]. In addition, iohexol does not require a continuous Ⅳ infusion and can be given as an intravenous bolus injection. It can be measured by several different techniques, the most used is the high-performance liquid chromatography (HPLC). However, HPLC requires a great deal of effort which limits its usefulness in the clinical setting[28]. Capillary electrophoresis (CE) a technique in which electrophoretic separations are performed in capillary tubes and is easier and faster than HPLC[29]. Shihabi *et al*[30] demonstrated that the iohexol determination by CE correlates well with HPLC.

However all these methods still require the need of continuous infusion or bolus administration of the marker (subcutaneous or intravenous) and like inulin, their complexity limits their application in clinical practice and epidemiological studies, mostly for the length of time that the procedure entails.

Routinely, GFR is usually estimated from prediction equations which are based on endogenous serum markers like creatinine or CysC in addition to demographic variables such as age, sex and race[13,16,31]. Measured GFR is reserved for situations where eGFR may be inaccurate such as patients in non-steady state, or individuals that

possess different characteristics compared to those where the estimating equation was created such as old age, loss of muscle mass (malnutrition, amputation, paraplegia) obesity, chronic illness or in situations where precise GFR is important, like kidney definition[12,32-34].

GFR estimation

Given the limitations of creatinine as a marker of kidney function, implementation of prediction equations has been widely used to eGFR from endogenous filtration markers without the need of clearance calculation[32]. As mentioned above, SCr and CysC are the most commonly used endogenous filtration markers for eGFR.

Creatinine: SCr derives from creatine degradation with a weight of 113 Da[35]. It is freely filtered but is not reabsorbed or metabolized however a significant percentage of creatinine in the urine derives from proximal tubular secretion[16,36]. One of the requirements for utilizing estimating equations based on SCr is stable kidney function. In addition, non-GFR determinats, such as variation in production associated to dietary intake, or changes in muscle mass, variation in tubular secretion and extrarenal creatinine excretion (associated with advanced kidney disease) need to be accounted when utilizing creatinine[13,32,37,38].

Another important factor that limits the accuracy of equations is the variability in SCr measurement[39]. In a study that examined frozen samples from 554 participants, where creatinine was measured with different assays, the SCr changed on average 0.23 mg/dL. This difference can result in substantial variations in GFR estimation when the SCr concentration is relatively normal[40]. The recognition that small variations in SCr translates in significant changes in kidney function has prompted to standardize creatinine determinations throughout clinical laboratories. In 2006 a standard method was introduced as a reference and was used in combination with the isotope-dilution mass spectrometry method in order to achieve better consensus among methods[41,42].

CysC: CysC has come to light as another marker of kidney function during the past decade. However, its clinical use worldwide remains limited compared with that of SCr[43]. CysC is a non-glycosylated protein produced by all nucleated cells. CysC is freely filtered, reabsorbed and completely metabolized in tubular cells and therefore is not subjected to tubular secretion[44,45]. Compared to creatinine, CysC has a more stable rate of production with less intra variability; however CysC serum levels are also influenced by non GFR determinants, such as uncontrolled thyroid disease, corticosteroid use, age, sex, ethnicity, smoking and adipose tissue[46-48]. In a recent meta analyses, the reciprocal value of CysC was more closely related to GFR (correlation coefficient 0.82 *vs* 0.74) and higher area under de curve (0.93 *vs* 0.84)[49].

In addition, CysC predicts outcomes and the association is stronger than SCr. Shlipak *et al*[50] reported CysC level to have an important association with mortality across the GFR range, including individuals with GFR between 60 and 90 mL/min per 1.73 m^2, grouped as "preclinical kidney disease"[50]. These findings have been reproduced in other studies in older adults where CysC has been shown to be a better predictor of adverse cardiovascular and non cardiovascular outcomes compared to to SCr[51-56]. Potential explanations for these findings may be accounted by the fact that compared to SCr, CysC is not influenced by muscle mass and reflect a better marker of GFR in this population[53]. In addition, these findings have also been reproduced in the general population and CysC estimated GFR has consistently provided a stronger association with outcomes than equations based on SCr eGFR[57].

Estimating equations

Since Effersoe in 1957 developed the first equation to estimate GFR[58], more de 20 equations have been developed. Most of the equations incorporate demographic and clinical variables[39]. The most commonly used equations include Cockroft Gault (CG)[59], 4-modification of diet in renal disease (MDRD)[60,61], 2009 CKDEPI[62] and more recently the equation that combines creatinine and CysC[63]. Since the standardization of creatinine, the CG equation is barely used in clinical practice[39].

CG: The CG formula was created almost thirty years ago in order to estimate creatinine clearance. It was developed in a population of white men and therefore the equation does not take into consideration sex, race and body surface area. Until recently, CG equation was solely utilized for drug dosing however the equation has been recently compared to the widely used equations with similar findings[59,64].

MDRD equation: The MDRD equation was developed in 1999 from a study including 1628 mostly white and non diabetic patients with CKD stages 3 and 4. The original equation included 6-variables and was further abbreviated in year 2000 to a four variable equation that included age, sex, ethnicity, and SCr[60]. In 2006 it was adapted to be used with standardized creatinine[61]. The four variable equation demonstrated to have similar performance compared to the six variable equation[65]. Although the MDRD has demonstrated to have high accuracy for individuals with CKD, the equation underestimates GFR in healthy individuals resulting in false positive diagnosis of CKD in this population[66].

CKD-epidemiology collaboration equation: The CKD epidemiology collaboration (CKD-EPI) was developed in 2009 and resulted from a study that included 8250 participants and was validated in similar cohort of 3900 subjects. Compared to the MDRD coh–ort, the CKD-EPI had higher GFR (68 mL/min per 1.73 m^2 *vs* 40 mL/min per 1.73 m^2), younger

age, included diabetics, blacks and kidney transplant recipients[39,62,67]. Linear regression was employed to estimate the logarithm of measured GFR from standardized SCr concentrations, gender, race, and age. The main objective for the CKD-EPI was to develop an equation that was superior to the MDRD, especially amongst those subjects with GFR > 60 mL/min per 1.73 m^2. Indeed, the same variables were used in CKD-EPI and MDRD equations but CKD-EPI performed better in those with GFR > 60 mL/min per 1.73 m^2. In subjects with GFR > 60 mL/min per 1.73 m^2 the P30% was 88.3% (86.9%-89.7%) and 84.7% (83%-86.3%) for CKD-EPI and MDRD, respectively, while in subjects with GFR < 60 mL/min per 1.73 m^2 the P30% for CKD-EPI was 79.9% (78.1%-81.7%) and for MDRD was 77.2% (75.5%-79%). Furthermore the CKD prevalence was estimated using the CKD-EPI and MDRD Study equations among 16032 adults from the NHANES cohort. Median eGFR by CKD-EPI was almost 10 mL/min per 1.73 m^2 higher than by MDRD. As a result, the CKD-EPI resulted in a significantly lower estimated CKD prevalence than the MDRD equation in the g (11.6% *vs* 13.1%, respectively)[62].

CysC and combined CysC and creatinine equations: In order to overcome the imprecision of creatinine estimating equations, Stevens *et al*[48], developed three eGFR equations for CysC (using CysC alone, CysC with demographic factors, and CysC with SCr and demographic factors) and compared them with mGFR iothalamate and 51-EDTA in 3418 patients. The equation that included CysC with SCr yielded the most accurate GFR estimates (P30 of 89%)[48]. Segarra *et al*[68] found that CysC-based GFR equations performed better than the CKD-EPI equation in a study of 3114 hospitalized patients because creatinine generation is dependent on the presence of muscle mass and malnourishment[68]. Similarly CysC-based GFR was superior than the CKD-EPI equation in certain subgroups of patients in which SCr level may be insensitive to capture reduced kidney function such as patients with chronic liver disease, frail elders, AIDS and malignancy[69-74].

Inker *et al*[63] developed a new GFR estimating equation that was based on CysC alone or in combination with creatinine in a cohort of 5000 subjects and was further validated in a cohort of 1119 subjects with measured GFR. The authors developed two new equations involving CysC (2012 CKD-EPI cys, and 2012 CKD-EPI Cys-cr) and compared them to the 2009 CKD-EPI equation. Bias was not different between the three equations however precision and accuracy was improved with the combined CysC-cr equation. Also in subjects whose eGFRcr was of 45-59 mL/min per 1.73 m^2, the combined equation reclassified correctly 17% to a no CKD category (GFR > 60 mL/min per 1.73 m^2). The authors concluded that the combined equation performed better than equations based on either CysC or SCr and should be used in those subjects where CKD needs to be confirmed[63].

Ongoing studies include the eGFR-C study which is a prospective longitudinal cohort study of 1300 adults with stage 3 CKD that will be followed for 3 years with reference iohexol mGFR. The objective of the study is to evaluate the performance of GFR-estimating equations, including the new equations that incorporate CysC in addition to albuminuria, in order to monitor GFR progression in this populations. Data will be analyzed to assess the impact of race, proteinuria and diabetes on equation performance[75].

Equations, their performance and their implications

When we evaluate the performance of an equation we should take into account bias, precision, and accuracy. Bias has been defined as a median difference between the measured and estimating GFR, precision this is the repeatability or reproducibility of the measurement and accuracy is defined as percentage of eGFR within 30% of measured GFR. Accuracy is probably the best single measure for comparing equations because it incorporates bias and precision. The 2002 KDOQI guidelines concluded that an eGFR within 30% of an mGFR was satisfactory for clinical interpretation, and as a performance metric for accuracy, the guidelines recommended that > 90% of participants in the validation population have eGFR within 30% of the measured GFR (P30 > 90%)[76]. Although accuracy in GFR assessment has significantly improved and bias was decreased with the CKD-EPI equation, precision has not substantially improved. This imprecision is due to random error secondary to variation in non-GFR determinants and GFR measurement error, whilst bias reflects differences between the development and validation populations in measurement methods for GFR, assays for filtration markers, or the relationship of the surrogates to the non-GFR determinants of the filtration marker[13].

In one study conducted by Michels *et al*[77] that included 271 patients with a mean SCr of 1.2 mg/dL, the CG, MDRD, and CKD-EPI equations were compared with mGFR using the I-iothalamate filtration marker (median mGFR 78.2 mL/min per 1.73 m^2), to assess the agreement between equations and examine whether the agreement was influenced by other known variables such as age, weight, body mass index and level of GFR. In general this study concluded that the CKD-EPI equation gives the overall best GFR estimation however the performance was close to MDRD[77].

One of the largest studies where MDRD and CKD-EPI were compared with the aim to assess performance was performed in a population of 12898 individuals from North America, Europe and Australia. The P30 ranged from 59%-95% and was higher for the CKD-EPI than for the MDRD equation in most studies, bias varied according to level of eGFR, was smaller for the CKD-EPI than for the MDRD equation at higher eGFR, but larger at lower eGFR. Table 1 shows the performance comparison of the equations in these populations. Authors from this study concluded that equations did

Table 1 Performance comparison of creatinine-based estimated glomerular filtration rate in North America/Europe/Australia

Ref.	Country	Patients, *n*	mGFR (value mL/min × 1.73 m^2, SD)	eGFR (equation)	Results		
					[1]Bias (95%CI) mL/min × 1.73 m^2	[2]Precision (95%CI)	[3]P30 (95%CI), %
Murata *et al*[180]	United States	5238	I-Iothalamate, urine (55.9, SD 29.7)	MDRD	-4.1	ND	77.6
				CKD-EPI	-0.7		78.4
Levey *et al*[62]	United States	3896	I-Iothalamate, urine and others (68, SD 36)	MDRD	-5.5 (-5.0 to -5.9)	0.274 (0.265-0.283)[4]	80.6 (79.5-82.0)
				CKD-EPI	-2.5 (-2.1 to -2.9)	0.250 (0.241-0.259)[4]	84.1 (83.0-85.3)
Lane *et al*[181]	United States	425	I-Iothalamate, urine (50, IQR 29 to 69)	MDRD	-1.0	15.0[5]	75
				CKD-EPI	-1.7	13.8[5]	80
Michels *et al*[77]	The Netherlands	271	I-Iothalamate, urine (78.2, SD 33)	MDRD	14.6 mL/min	19.9[6]	81.2
				CKD-EPI	12.3 mL/min	12.1[6]	84.5
Tent *et al*[182]	The Netherlands	253 before donation, 253 after donation	I-Iothalamate, urine (115, SD 20) and (73, SD 13)	MDRD	-22 mL/min (20-25)	20 (14-26)[5]	73 (68-79)
				CKD-EPI	-14 mL/min (11-16)	18 (14-22)[5]	89 (85-93)
				MDRD	-15 mL/min (14-16)	12 (9-15)[5]	71 (65-76)
				CKD-EPI	-11 mL/min (9-11)	12 (10-16)[5]	89 (85-93)
Kukla *et al*[183]	United States	107 on steroid-free early post tranplantation 81 on steroid-free at 1 yr	I-Iothalamate, urine (55.5, SD 17) and (56.8, SD 17.7)	MDRD	8.23	17.9[4]	71.7
				CKD-EPI	13.30	21.1[4]	58.5
				MDRD	2.40	15.8[4]	75.0
				CKD-EPI	6.91	17.3[4]	66.7
White *et al*[184]	Canada	207	Tc-DTPA, plasma (58, SD 22)	MDRD	-7.4	14.4[5]	79 (73-84)
				CKD-EPI	-5.2	15.7[5]	84 (78- 88)
Pöge *et al*[185]	Germany	170	Tc-DTPA, plasma (39.6, IQR 11.8 to 82.9)	MDRD	4.49	10.0[6]	71.8
				CKD-EPI	8.07	10.9[6]	64.1
Jones[186]	Australia	169	Tc-DTPA, plasma (75, IQR 5 to 150)	MDRD	-3[7]	ND	81
				CKD-EPI	-1.5[7]		86
Cirillo *et al*[187]	Italy	356	Inulina, plasma (71.5, SD 36.3)	MDRD	-5.2	14.9[6]	87.4
				CKD-EPI	-0.9	13.2[6]	88.2
Eriksen *et al*[188]	Norway	1621	Iohexol, plasma (91.7, SD 14.4)	MDRD	1.3 (0.4-2.1)	18.2 (17.2-19.5)[5]	93 (91-94)
				CKD-EPI	2.9 (2.2-3.5)	15.4 (14.5-16.3)[5]	95 (94-96)
Redal-Baigorri *et al*[189]	Denmark	185	Cr-EDTA, plasma (85.1, SD 20.3)	MDRD	0.81 (IQR, -1.56 to 3.19)	16.49[6]	88.6
				CKD-EPI	1.16 (IQR, -0.76 to 3.09)	13.37[6]	89.7

[1]Computed as estimated GFR minus measured GFR. Positive numbers indicate overestimation and negative numbers indicate underestimation of measured GFR. Smaller absolute values indicate lesser bias; [2]Lower values indicate greater precision; [3]Higher values indicate greater accuracy. Among the 3 studies (14, 18, 19) that reported alternative measures of accuracy, results were consistent with P30 in all. In addition to P30, references 14, 18, and 19 reported P10; Reference 14 also reported P20; [4]Evaluated as the root mean square error for the regression of estimated GFR on measured GFR; [5]Evaluated as the IQR for the differences between estimated and measured GFR; [6]Evaluated as the SD of the differences between estimated and measured GFR; [7]Converted to raw scale by multiplying percentage of bias by measured GFR. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; mGFR: Measure glomerular filtration rate; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; MDRD: Modification of diet in renal disease; ND: Not documented; P30: Percentage of estimated GFR values within 30% of measured GFR; Tc-DTPA: Technetium-diethylene-triamine-pentaacetate; Cr-EDTA: Chromium-ethylenediamine-tetraacetic-acid. Adapted from Earley *et al*[39].

not perform as well in regions outside North America, Europe, and Australia. In Asia and Africa, equations were less accurate (P30 ranged from 29%-94%). Equation performance can be improved by deriving local "race/ethnicity" coefficients; however, the new equations are more accurate in the Caucasian populations. The coefficients also do not seem to be generalizable beyond the local population presumably reflecting differences in SCr generation due to racial, ethnic, and regional variations in muscle mass and diet, and use of non standardized SCr[39].

Thus far the new equation CKD-EPI Cys-cr has been evaluated in diverse populations. The berlin initiative study (BIS) included 610 older adults with a mean SCr level

of 1.0 mg/dL, and mean CysC level of 1.15 mg/L. The study intended to assess the performance of the CKD-EPI Cys-cr equations compared to the mGFR by iohexol. A major finding of this study was that CysC had a stronger association with mGFR than creatinine and the best GFR estimation was derived from a combined Cys-cr equation (named BIS-2)[78]. The combined CKD-EPI Cys-cr equation performed well in Japanese and Chinese individuals[79-81]. One recent study compared the CKD-EPI Cys-cr and other four approved equations in a cohort of 788 adult Chinese patients and a Tc_DPTA mGFR of 76 mL/min per 1.73 m^2. Compared to other equations, the CKD-EPI Cys-cr had less bias, (-4.11 mL/min per 1.73 m^2) and higher accuracy (P30% of 77.03%)[80]. In a population of almost 700 kidney transplant recipients the performance of the CKD-EPI Cys-cr was superior showing less bias and better accuracy compared with 2009 CKD-EPI, using inulin mGFR as reference[82].

In addition, it is important to mention that the performance of the equations is affected not only by demographic and clinical factors but by the reference method considered as the gold standard to measure GFR in different populations[83-85].

From the epidemiological standpoint, CKD prevalence was assessed in diverse populations comparing the MDRD and CKE-EPI equation[62]. For example, the Atherosclerosis Risk in Communities Study reclassified 43.5% to a higher eGFR category compared with CKD stage 3 for MDRD[86]. The AusDiab (Australian Diabetes, Obesity and Lifestyle) study reclassified 266 participants identified as having CKD with MDRD to no CKD with CKD-EPI, decreasing the prevalence of CKD in adults > 25 year 1.9% in Australia[87]. The kidney early evaluation program included 116321 individuals where 17.5% and 2.7% were reclassified to higher or lower eGFR categories, respectively, when compared with MDRD[88].

Reclassifying subjects to a higher GFR has demonstrated to translate in a lower risk for outcomes. In a recent meta-analysis, the CKD-EPI and MDRD equations were compared with respect to CKD stage and risk prediction in a 1.1 million adults from distinct cohorts followed over seven years. Outcomes included mortality, cardiovascular mortality, and kidney failure. In this study CKD-EPI reclassified to a higher and lower estimated GFR category 24.4% and 0.6% respectively, compared with the MDRD, and when the CKD-EPI equation was used, the prevalence of CKD was reduced by 2.4 percent. Furthermore, in individuals with MDRD eGFR of 45-59 mL/min per 1.73 m^2, the CKD-EPI creatinine equation reclassified 34.7% to eGFR of 60-89 mL/min per 1.73 m^2 and 1.2% to eGFR of 30-44 mL/min per 1.73 m^2. Individuals reclassified to a higher eGFR category had 0.80, 0.73, and 0.49 lower adjusted risks for death, cardiovascular disease, mortality, respectively, than those not reclassified. Overall net reclassification favored the CKD-EPI over the MDRD for the three outcomes[86].

Rule *et al*[89] evaluated the association of CKD risk factors (urine albumin, lipid profile, uric acid, hypertension, diabetes and smoking) with eGFR based on Cr and/or CysC and compared them with iothalamate mGFR in 1150 subjects with a mean age 65 year and mean mGFR of 80 mL/min per 1.73 m^2. Authors concluded that the association between most of the risk factors was stronger for CysC than SCr and CysC was a better predictor for risk stratification and management of CKD than SCr eGFR[89].

These data demonstrates that the CKD-EPI equation is superior for GFR estimation leading to fewer false-positive diagnoses of CKD. In addition the CKD-EPI equation translates in a decreased prevalence of CKD and is associated with a more precise risk prediction for outcomes and prognosis. The KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, based on this evidence recommends that CKD be diagnosed, classified, and staged by eGFR and suggests CKD-EPI should be utilized as the preferred equation[1].

Other endogenous biomarkers for kidney function

Blood urea nitrogen: BUN increases as GFR declines however is less valuable than the SCr since the BUN can vary independently of the GFR. The production rate of urea is not stable and increases with rich protein diets or tissue breakdown such as bleeding, muscle trauma or steroid administration. On the other hand a very low protein diet or liver failure can decrease BUN without affecting GFR[32,90].

B2-microglobulin: B2-microglobulin (B2-M) is a small molecule of 11.8 kDa and constitutes a class Ⅰ HLA, is present in all nucleated cells in the body, and has a large quantity of immune cells like lymphocytes and monocytes. It has the characteristic that it is freely filtered in the glomeruli and is reabsorbed and metabolized in the proximal tubule[91]. Levels of B2-M are elevated in kidney disease, in addition to other conditions such as malignancies, autoimmune diseases, infections and aging[92]. There is data to demonstrate that plasma B2-M is a good endogenous marker of GFR and that in the context of GFR decline the increase of serum B2-M occurs prior than SCr. B2-M has been associated with death in a cohort of 1034 elderly subjects and appeared to be superior than CysC, even after adjustment for known risk factors[93,94]. Lack of further studies in the last decade however has limited the utility of this biomarker in clinical practice.

KIDNEY DAMAGE

The kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g). Damage usually precedes alterations in functions. For instance it is known that albuminuria precedes the decrease in

eGFR, hence the importance to count with markers of renal damage in stages that are blind for current markers of renal function decline. In theory this could facilitate early diagnosis, guide interventions and monitor disease progression.

Albuminuria

Albumin excretion rate (AER) can be determined in 24 h urine collections or in spot collections. Increases should be confirmed in at least two of three samples, within a period from 3 to 6 mo[11]. Microalbuminuria, or incipient nephropathy, is defined as an AER of 20-200 μg/min in timed samples, or 30-300 mg/24 h in 24 h samples, however spot collections are accurate enough that they can replace 24 h collections and these are now strongly recommended by the most recent guidelines[1,95].

The corresponding values that define microalbuminuria in a urine sample are AER > 30 mg/24 h or an albumin-creatinine ratio (ACR) of 30-300 mg/g (0.3-3 mg/mmol). Higher values indicate macroalbuminuria, also called clinical nephropathy[1]. Taking these values into account the prevalence of microalbuminuria in 4101 individuals of NHANES (1999-2000) with ACR 30-300 mg/g and ACR > 300 mg/g was 7.3% and 1.7% in men and 10.4% and 0.9% in females, respectively[96].

The threshold of ACR > 30 mg/g to define kidney damage has been validated as a risk factor for adverse events in different populations. In high risk patients for CKD, the ACR > 30 mg/g is has demonstrated to be a risk factor cardiovascular (CV) death and all cause mortality, progression of kidney disease, acute kidney injury (AKI) and kidney failure[97,98]. Likewise, these findings have been reproduced in low risk cohorts. In more than 1 million participants from 21 cohorts, ACR > 30 mg/g and ACR > 300 mg/g were associated with higher risk for death (HR of 1.6 and 2, respectively). Moreover the risk for CV mortality was two-fold higher with ACR > 30 mg/g compared to those with ACR of 5 mg/g and this risk persisted after adjustment for GFR and other known risk factors. This risk also applies to ACR levels < 30 mg/g. In study of Waheed *et al*[99], ACR of 10 mg/g compared to 5 mg/g was associated with all cause mortality. This however may not necessarily reflect kidney damage and may be a marker of endothelial dysfunction.

On the basis of the linear association of albuminuria with progression of CKD, end stage renal disease (ESRD), and all cause of mortality independent of eGRF, albuminuria staging has been added in the 2012 KDIGO guidelines.

Combination of biomarkers

Combining albuminuria with eGFR improves the prediction of CKD progression. This was demonstrated in the Nord-Trondelag Health (HUNT-2) study that included 65589 participants, where albuminuria and eGFR independently predicted kidney disease progression and the combination of both markers was superior to predict those subjects at highest risk for ESRD development[100]. In a large prospective cohort involving more than 26000 subjects, the authors evaluated whether combining eGFR creatinine, CysC, and urine ACR could improve risk prediction when compared with eGFR alone. In this cohort the adjusted mortality risk was six fold higher in patients with CKD identified by all three markers and was also three fold higher in patients with CKD defined by both eGFR Cys-cr, compared to those with CKD defined by eGFR creatinine alone. The risk for CKD progression to kidney failure was higher among patients with CKD defined by all three markers. The authors concluded that adding CysC to SCr and ACR was superior for prediction for kidney disease progression and death[101].

New biomarkers for kidney damage

Although albuminuria is a powerful biomarker, it may occur after the damage has occurred or may not be present in other types of kidney damage such as tubulointerstitial disease and hypertensive kidney disease. This has led to the search for new biomarkers that are also non-invasive and could better correlate with the etiology of the kidney disease. Moreover; early identification of patients with CKD could allow implementing early interventions to reduce CVD or CKD progression. In the next few paragraphs we describe the most promising biomarkers in CKD (Table 2) and its utility (Table 3).

Kidney injury molecule

Kidney injury molecule (KIM-1) is a transmembrane protein is a type 1 transmembrane protein whose expression has been upregulated after kidne injury[102,103]. KIM-1 is an early biomarker for proximal tubular damage since it is expressed in the urine during the first 12 h of the tubular injury[104]. Experimental and clinical studies have demonstrated high KIM-1 expression in areas of fibrosis and inflammation. In murine models with polycystic kidney disease, KIM 1 is highly expressed in renal tubules, it associates with interstitial fibrosis in human allografts and in type 1 diabetes mellitus regression of microalbuminuria has been associated with lower urinary levels of KIM-1[105-108].

Persistent expression of KIM-1 has been associated to inflammation characterized by high monocyte chemoattractant protein-1 (MCP-1) levels[109]. In contrast, in experimental models, mice with mutant KIM-1 are protected from fibrosis and had lower inflammatory markers[110]. In a retrospective analysis of 107 diabetic type 1 with CKD stages 1-3 (AER > 500 mg/24 h) followed for 5-15 years, 63% of those subjects with higher KIM-1 levels (> 97 pg/mL) progressed to ESRD whereas only 20% of patients with lower levels progressed. In addition baseline plasma KIM-1 levels correlated with rate of eGFR decline after adjustment for baseline urinary albumin-to-creatinine ratio, eGFR, and Hb1Ac[111]. KIM-1 may represent a promising marker for the future. Larger

Table 2 Novel biomarkers in chronic kidney disease

Biomarker source	Ref.	Population/type of study	Commentaries
u-LFABP Urinary	Nielsen *et al*[190]	227 newly diagnosed type 1 diabetic patients/longitudinal	Baseline u-LFABP levels predicted development of microalbuminuria (HR = 2.3, 95%CI: 1.1-4.6), and predicted mortality (HR = 3.0, 95%CI: 1.3-7.0)
NAG Urinary	Kern *et al*[191]	87 type 1 diabetics with microalbuminuria and 174 controls/ longitudinal	Baseline NAG independently predicted microalbuminuria (OR = 1.86, $P < 0.001$) and macroalbuminuria (OR = 2.26, $P < 0.001$) but risk was attenuated in multivariate models
CTGF Urinary	Nguyen *et al*[192]	318 type 1 diabetic patients and 29 control subjects/cross sectional	U-CGTF was significantly higher in diabetic nephropathy than micro o normoalbuminuria. U-CGTF correlated with albuminuria and GFR
IL-18 Kidney tissue	Miyauchi *et al*[193]	12 type 2 diabetes with overt nephropathy and 7 patients with MCD/ cross sectional	IL-18 expression in tubular cells was observed highly observed (83%) in patients with diabetes but only observed in 14.3% of MCD
ApoA-Ⅳ Plasma	Boes *et al*[194]	177 non-diabetic patients with mild to modetare renal CKD/longitudinal	Baseline ApoA-Ⅳ was a significant predictor of disease progression (HR = 1.062, 95%CI: 1.018-1.108) and patients with level above the median had significantly faster progression compared with patients with level below median ($P < 0.0001$)
CD14 mononuclear cells Urinary	Zhou *et al*[195]	16 patients with autosomal dominat polycystic kidney disease/longitudinal	Baseline urinary CD14 mononuclear cells correlated with 2 yr change in total kidney volume in males
NGAL	Bolignano *et al*[121]	33 patients with glomerulonephritis and proteinuria > 1 g per day/cross sectional	u-NGAL was higher in glomerulonephritis compared with controls and significantly correlated with serum creatinine and urinary protein excretion
Urinary	Smith *et al*[124]	158 patients with CKD stages 3 and 4/longitudinal	u-NCR was associated with a higher risk of death and initiation of renal replacement therapy
Urinary	Bolignano *et al*[125]	96 white patients with CKD/ longitudinal	Baseline urinary and serum NGAL were predictors of CKD progression
Urinary/serum	Shen *et al*[119]	92 patients with chronic glomerulonephritis CKD stage 2-4, and 20 control subjects/longitudinal	s-NGAL levels were higher compared to controls and negatively correlated with the eGFR Patients with sNGAL level > 246 ng/mL had a poor 2 yr renal survival compared with the control group
Serum	Bhavsar *et al*[123]	286 participants from the ARIC and 143 matched controls/longitudinal	Higher quiartiles of NGAL (but no KIM-1) were associated with incident CKD
KIM-1 Serum	Krolewski *et al*[111]	107 diabetic type 1 with CKD 1-3 (AER > 500 mg/24 h)/longitudinal	Baseline plasma KIM-1 levels correlated with rate of eGFR decline KIM-1 levels (> 97 pg/mL) correlated with progression to ESRD
Urinary	Peters *et al*[109]	65 patients with Proteinuric IgAN and 65 control subjects/longitudinal	In patients with IgAN uKIM-1 excretion was significantly higher than controls uKIM-1 is independently predictor of ESRD
FGF-23	Nakano *et al*[134]	738 Japanese patients with CKD stages 1-5/longitudinal	Levels of FGF-23 associated with kidney function decline or initiation renal replacement therapy
Serum	Fliser *et al*[137]	227 non diabetic patients with CKD stages 1-4/longitudinal	FGF-23 was an independent predictor of CKD progression
	Lee *et al*[138]	380 patients with type 2 diabetes/ longitudinal	Levels of FGF-23 was associated with increased risk of ESRD and was a significant risk factor for all cause mortality

u-LFABP: Liver-type fatty acid-binding protein; NAG: N-Acetyl-b-O-glucosaminidase; CTGF: Connective tissue growth factor; IL-18: Interleukin-18; ApoA-Ⅳ: Apolipoprotein A-Ⅳ; NGAL: Neutrophil gelatinase associated lipocalin; MCD: Minimal change disease; ARIC: Atherosclerosis Risk In communities; IgAN: IgA nephropathy; u-NCR: u-NGAL to creatinine ratio; eGFR: Estimated glomerular filtration rate; FGF-23: Fibroblast growth factor 23; CKD: Chronic kidney disease; KIM-1: Kidney injury molecule; AER: Albumin excretion rate; GFR: Glomerular filtration rate; U-CGTF: Urinary-connective tissue growth factor; u-NGAL: Urinary-NGAL; s-NGAL: Serum-NGAL; ESRD: End stage renal disease.

Table 3 Utility of new biomarkers in chronic kidney disease

Biomarker	Origin	Outcome assessed
Urinary liver-type fatty acid-binding protein	Proximal tubule	Diabetic Nephropathy: Microalbuminuria and mortality
Urinary N-Acetyl-b-O-glucosaminidase	Proximal tubule	Diabetic Nephropathy: Albuminuria
Urinary connective tissue growth factor	Proximal tubule	Diabetic Nephropathy: Glomerular filtration rate decline
Interleukin-18	Tubulointerstitial	Diabetic Nephropathy: Albuminuria
Apolipoprotein A-Ⅳ	Intestinal enterocytes	CKD: CKD Progression
Urinary CD14 mononuclear cells		Polycystic kidney disease: Kidney volume
Neutrophil gelatinase associated lipocalin	Proximal and distal tubule	Glomerulonephritis: GFR and proteinuria CKD: CKD progression, renal replacement therapy and mortality
Kidney injury molecule-1	Proximal tubule	CKD: CKD progression and renal replacement therapy
Fibroblast growth factor-23	Osteocytes and osteoblasts	Diabetic Nephropathy and others CKD: CKD progression and mortality
Urinary retinol binding protein 4	Proximal tubule	Congenital or acquired tubular dysfunction: Proximal tubule dysfunction

CKD: Chronic kidney disease.

studies however are still warranted before KIM-1 could be applied routinely in clinical practice.

Neutrophil gelatinase-associated lipocalin: Neutrophil gelatinase-associated lipocalin (NGAL) is a lipocalin iron-carrying protein of 25 kDa and is part of the well-defined super family of proteins called lipocalins, is expressed by tubular renal epithelial cells following tubulointerstitial injury[112-114]. NGAL has been an established marker for acute kidney injury however its role in CKD is less studied[115-119]. In patients with IgA nephropathy urinary NGAL level was higher compared to controls and was also associated with disease severity[120]. In patients with glomerular proteinuria above 1 g/24 h and in patients with polycystic kidney disease, NGAL levels were higher compared to controls and significantly correlated to SCr[121,122]. NGAL has also been associated to incident CKD progression in adults. In a community based population of 286 subjects, NGAL was evaluated as an independent risk factor for incident CKD. Those in the highest quartile of NGAL had a higher risk for incident CKD, effect that was attenuated after adjustment for creatinuria and albuminuria[123]. In a cohort of 158 adults with stage 3 or 4 CKD, urinary NGAL to creatinine ratio was associated with mortality and renal replacement therapy and this risk was independent of kidney and CV risk factors[124]. Similar results were found in a cohort of 96 CKD patients followed for 18.5 mo where plasma and urinary NGAL predicted CKD progression after adjustment for eGFR[125].

Thus far there is evidence to support that NGAL levels either in plasma or urine can predict kidney disease progression independent of GFR, however the data is limited by the number of participants and larger studies are needed before establishing this biomarker in clinical practice.

Fibroblast growth factor 23: Fibroblast growth factor 23 (FGF-23) is 32-kDa phosphaturic protein secreted by bone osteocytes. Among its functions is to promote phosphate excretion, decrease calcitriol production and suppress parathyroid hormone[126-128]. In CKD the increase of FGF-23 level precedes the decline in vitamin 1,25-$(OH)_2$ vitamin D3 and the increase of PTH level. Although FGF-23 is higher in patients with moderate to severe CKD, there is data to support that the rise of FGF-23 occurs earlier in the disease. In the past decade several studies have found an association between high FGF-23 levels, kidney disease progression and mortality in subjects with CKD[129-132]. In a cohort of 227 non diabetic patients with CKD followed for more than 4 years, FGF-23 was an independent risk factor for kidney disease progression. Likewise Semba *et al*[133] in 701 healthy women (mean eGFR 60 mL/min × 1.73 m^2), and Nakano *et al*[134] in 738 Japanese patients with CKD stages 1-5 (mean eGFR 35 mL/min × 1.73 m^2) reported that increasing levels of FGF-23 associated with decline in kidney function or initiation renal replacement therapy after a follow-up of 2 and 4.4 years, respectively. In addition, in patients undergoing renal replacement therapy, elevated FGF-23 levels have been associated with CV outcomes such as left ventricular hypertrophy and increased risk of mortality[133-138]. It is important to mention that this association has been independent of phosphate levels and CKD stage.

Asymmetric dimethylarginine: Asymmetric dimethylarginine (ADMA) is an aminoacid of 202 Da, it is normally synthesized intracellularly and eliminated through the urine. One of its adverse effects is the inhibition of the nitric oxide synthases and this mechanism has been associated to adverse cardiovascular side effects[139,140]. As kidney function deteriorates ADMA levels increase and this has been associated to kidney parenchymal damage through the decrease in dimethylarginine-dimethylamino-hydrolase[141,142]. ADMA has been associated to CKD progression. In the diabetic and non diabetic population, ADMA levels are higher as GFR declines and are associated with rapid kidney function decline[143,144]. In a recent study of 164 CKD patients followed for one year, elevated ADMA and markers of oxidative stress were strong predictors of progression in patients with CKD stages 3-4[145]. Moreover, ADMA has been associated to death and CV events in the CKD population[146,147]. Some authors had considered ADMA to be the "missing link" between cardiovascular disease and CKD[139]. Whether counteracting the effects of ADMA in CKD should be explored as a strategy to prevent cardiorenal complications would need to be confirmed in larger studies.

MCP-1: MCP-1 belongs to the group of inflammatory chemokines[148,149]. Expression of MCP-1 is up regulated in kidney diseases that have a sustained inflammatory response, such as in diabetic nephropathy and lupus nephritis[150,151]. Studies have demonstrated glomerular and tubular kidney cells release MCP-1 in response to high glucose levels and urine levels of MCP-1 are increased in diabetic nephropathy[152,153]. Likewise MCP-1 levels in urine are over expressed in active lupus nephritis[151-154]. Emerging evidence suggest that MCP-1 has a significant role in the pathogenesis of many kidney diseases and urinary MCP-1 is a promising biomarker with diagnostic and prognostic implications[155-157].

Urine retinol-binding protein 4: Urine retinol-binding protein 4 (uRBP4) is a 21 KDa protein derived of plasma RBP4 (pRBP4), is an integrant of the lipocalin family and is produced mainly in the liver but also in the adipose tissue where it performs as an adipokine that has been linked to insulin resistance and obesity[158,159]. Unlike other biomarkers such as NGAL and KIM-1, uRBP4 is currently the most sensitive functional biomarker of proximal tubule. pRBP4 is filtered at the glomerulus and completely reabsorbed in the proximal tubule. In addition, it is known that variation levels of pRBP4 (secondary to nutrition, vitamin A levels, liver disease and infection) have small effect on uRBP 4 as a

biomarker[160]. Sensitivity for uRBP4 however decreases as kidney function declines due to false positives that occur in the presence of glomerular disease[161]. This marker was been useful in several diseases related with proximal tubule dysfunction, either hereditary, such as Fanconi syndrome, dent type 1 syndrome and lowe syndrome[162], or acquired conditions that directly affect proximal tubule such as drug toxicity in human immunodeficiency virus, cadmium toxicity, plasma cell dyscrasias, AKI diagnosis and other renal tubulointerstitial diseases[163]. Amer *et al*[164] assessed the prognostic value in renal transplantation of a panel of urinary proteins in 221 patients at 1 year post transplant and reported that patients with glomerular lesions had higher albuminuria than patients with normal histology, and in patients with tubulointerstitial disease, uRBP4 has over expressed. In addition, uRBP4 was a risk factor for long term allograft loss and this risk was independent of kidney biopsy histology and albuminuria[164].

Future directions

Advances in technology during the last decade have enlightened our knowledge regarding genetic regulatory pathways. A fast growing arena are the microRNAs (miRNAs), the current number of miRNAs in humans are estimated to be between 700 and 1000, and they have been implicated in several physiological events as well pathologic process, including kidney disease[165]. miRNA have selective expression by different organs, and the kidney expresses mostly miRNA 192, 194, 204, 215 and 216 which have been implicated in proliferation, migration and structure of renal cells[166,167]. Little changes in these molecules have implications in kidney function, for instance it is know that deletion of the miRNA 30 family decreases renal cells, affects blood pressure and develop vascular damage and extensive fibrosis[168]. Other miRNAs are related with diverse pathophysiologic process, miRNA 155 is associated to blood pressure control through down regulation of type 1 angiotensin Ⅱ receptor[169,170], miRNA 192 and 200 families are related to fibrotic damage in diabetic nephropathy manly by regulation of transforming growth factor beta[171], miRNA 15, 17 and 31 are associated with cystogenesis in polycystic kidney disease[172], and finally miRNA 142, 155 and 223 are increased in acute rejection related to activation of epithelial cells and blood mononuclear cells[173], and can discriminate between acute humoral rejection and cellular rejection[174]. MiRNA expression pathways have also been evaluated as diagnostic biomarkers in other pathologies. In a study of lupus nephritis patients miRNA 27 and 192 in urine could identified in renal biopsies of lupus patients with nephritis[175]. The knowledge of miRNA in health and disease remains with several questions concerning its regulation, production and specific target. In addition most studies have measured miRNA in tissue and therefore become cumbersome to measure in clinical practice. Studies evaluating its utility in plasma and urine are urgently needed. Nonetheless this is a rapidly growing field and future research may provide a better understanding of the pathophysiology in kidney disease and may reveal potential diagnosis and therapeutic options.

Not only in the area of proteomics (NGAL, KIM-1, *etc.*) and transcriptomics (miRNAs) have the kidney markers evolved, the latest piece added to the puzzle corresponds to metabolomics, and as it name points out, is the measure of end products of basic metabolic molecules. These end products could improve the utility of other type of biomarkers[176]. Currently, metabolomics in kidney disease have mainly been studied in uremia, renal cell carcinoma, glomerulonephritis, diabetes mellitus, polycystic kidney disease and drug related nephrotoxicity. For instance in patients with drug related nephrotoxicity, end products from amino acids and simple sugars increase in urine before tissular changes become apparent. The latter has been described with antibiotics[177], and immunosupression therapy, for example, the increase of metabolomic end products during the first month after cyclosporine predicts kidney damage[178]. Similarly metabolomics has been associated to several metabolic profiles (mainly amino acids, derivatives of sugar and phospholipids) that could be useful in the diagnosis and prognosis of different types of renal disease as diabetic nephropathy, IgA nephropathy and other glomerulonephritis, in addition to diagnosis, metabolomics offers a promising future in the area of pharmaco-metabolomics, which could lead to personalized therapeutic targets[179]. At this point metabolomics main limitation is related to problems with specificity and technical variability and is not ready to be implemented in clinical practice.

CONCLUSION

During the last century, SCr has been the most used biomarker to screen and diagnose kidney disease. SCr however has several limitations and should be utilized only in estimating equations. The CKD-EPI is more generalizable and performs better than the MDRD estimating equation, especially in the healthy population. More recently the GFR estimating equation that combines SCr and CysC has demonstrated to be superior than equations that use either SCr or CysC alone, and is recommended in specific conditions, such as when confirmation of CKD is required. Albuminuria remains one of the strongest risk factors for outcomes and the combination of SCr, CysC and urinary albumin to creatinine ratio improves risk stratification predicts CKD progression and mortality.

In the last decade several other promising biomarkers have emerged. However, although these biomarkers are highly sensitive and specific and have allowed an earlier diagnosis of kidney disease with promising results; none of them have been validated to make clinical decisions upon their positivity. These biomarkers should have the potential to indicate injury type or the specific site of harm. It is improbable however that one biomarker would be sufficient to guide intervention upon their result. Larger and long term studies are warranted before applying these biomarkers in clinical practice. The CKD

Biomarkers Consortium has 15 ongoing studies with the aim to develop and validate novel biomarkers for CKD. In the meantime current biomarkers in CKD should be cautiously implemented acknowledging its strengths and limitations.

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REVIEW

ACE and ACE2 in kidney disease

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Abstract

Renin angiotensin system (RAS) activation has a significant influence on renal disease progression. The classical angiotensin-converting enzyme (ACE)-angiotensin Ⅱ (Ang Ⅱ)-Ang Ⅱ type 1 (AT1) axis is considered to control the effects of RAS activation on renal disease. However, since its discovery in 2000 ACE2 has also been demonstrated to have a significant impact on the RAS. The synthesis and catabolism of Ang Ⅱ are regulated *via* a complex series of interactions, which involve ACE and ACE2. In the kidneys, ACE2 is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from Ang Ⅰ and the catabolism of Ang Ⅱ to produce Ang 1-7 are the main functions of ACE2. Ang 1-7 reduces vasoconstriction, water retention, salt intake, cell proliferation, and reactive oxygen stress, and also has a renoprotective effect. Thus, in the non-classical RAS the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis. This review examines recent human and animal studies about renal ACE and ACE2.

Key words: Angiotensin-converting enzyme; Angiotensin-converting enzyme 2; Diabetic nephropathy; Kidney disease; Renin angiotensin system

Core tip: In the kidneys, angiotensin-converting enzyme 2 (ACE2) is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from angiotensin Ⅰ (Ang Ⅰ) and the catabolism of Ang Ⅱ to produce Ang 1-7 represent the main functions of ACE2. Ang 1-7 reduces vasoconstriction, water retention, salt intake, cell proliferation, and reactive oxygen stress, and also has a renoprotective effect. Thus, in the non-classical renin angiotensin system the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis. This review examines recent human and animal studies about ACE and ACE2 expression in various renal diseases.

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INTRODUCTION

The renin-angiotensin system (RAS) has a significant influence on renal disease progression. Angiotensinogen

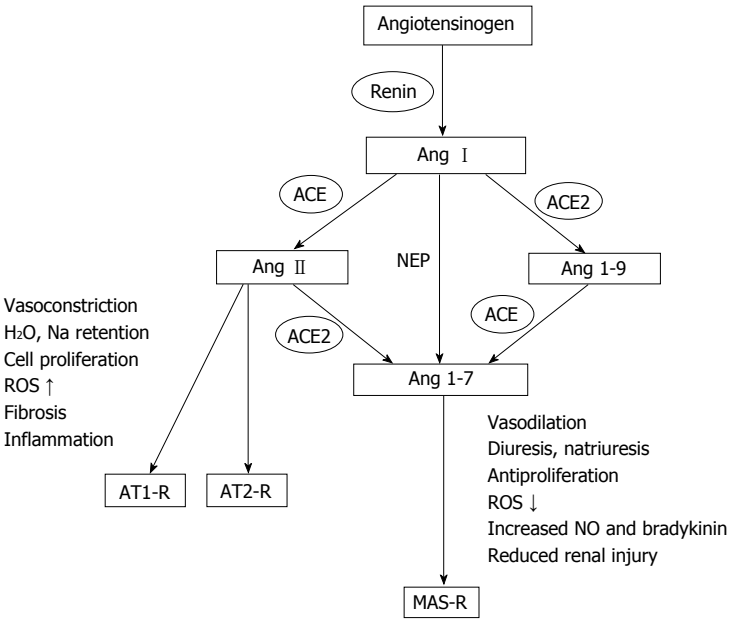

Figure 1 Roles of angiotensin-converting enzyme and angiotensin-converting enzyme 2 in the renin angiotensin system. Angiotensinogen is cleaved by renin to form angiotensin Ⅰ (Ang Ⅰ), which is converted to Ang Ⅱ by ACE. The main function of ACE2 is to synthesize inactive Ang 1-9 from Ang Ⅰ and to produce the vasodilatory and antiproliferative molecule Ang 1-7 from Ang Ⅱ. Ang Ⅰ acts a substrate for neprilysin, which cleaves it to form Ang 1-7. Ang Ⅱ binds to the Ang Ⅱ type 1 receptor (AT1-R) and AT2-R. The Mas receptor (MAS-R) is a specific receptor for Ang 1-7. The ACE2-Ang 1-7-MAS axis counteracts the effects of the ACE-Ang Ⅱ-AT1 axis. ACE: Angiotensin-converting enzyme; NO: Nitric oxide; NEP: Neprilysin; ROS: Reactive oxygen species.

is broken down by renin to give angiotensin Ⅰ (Ang Ⅰ), and angiotensin-converting enzyme (ACE) subsequently converts Ang Ⅰ to angiotensin Ⅱ (Ang Ⅱ). In the kidneys, the ACE-Ang Ⅱ type 1 (AT1) axis (the classical RAS) promotes sodium and water retention, oxidative stress, vasoconstriction, cell proliferation, inflammation, and fibrosis (Figure 1). In 2000, ACE2[1,2] was discovered, which indicated that the RAS is more complex than was previously imagined. The synthesis of inactive Ang 1-9 from Ang I and the catabolism of Ang Ⅱ to form Ang 1-7, which binds to the Mas receptor (its specific membrane receptor) and counteracts the effects of Ang Ⅱ, are the main functions of ACE2[3,4]. Ang Ⅰ is also a substrate for neprilysin, which cleave it to produce Ang 1-7[4]. In the non-classical RAS, the ACE2-Ang 1-7-Mas axis counteracts the effects of the ACE-Ang Ⅱ-AT1 axis. Specifically, it induces natriuresis, reduced oxidative stress, vasodilation, antiproliferative activity, and diuresis by upregulating the concentrations of nitric oxide and prostaglandins. These processes contribute to protecting the kidneys from damage (Figure 1)[4-7]. Ang 1-7 is also metabolized by ACE[4]. Furthermore, accumulating evidence indicates that the ACE/ACE2 ratio regulates the production and accumulation of Ang Ⅱ and that ACE2 deficiency leads to higher Ang Ⅱ concentrations[8-13].

This review examines recent studies about the roles of renal ACE and ACE2 in various conditions (Table 1) and potential treatments for renal disease that target these molecules.

RENAL DISTRIBUTION OF ACE AND ACE2

In mouse kidneys, the apical brush borders of the proximal tubules exhibit colocalization of ACE and ACE2, but this is not the case in the glomeruli[14]. Whilst ACE2 is expressed in podocytes and less strongly glomerular mesangial cells, only endothelial cells have been found to express ACE[14].

In agreement with the findings of animal studies, the apical brush borders of the proximal tubules in human kidneys exhibit marked ACE and ACE2 colocalization, and both ACE and ACE2 were also detected in the glomeruli although at lower concentrations[15,16]. Furthermore, in immunoelectron microscopy studies of human kidneys, it was confirmed that ACE is expressed at the proximal tubule brush borders and in glomerular endothelial cells[17]. ACE has also been detected within renal vascular endothelial cells[18]. On the other hand, no previous studies have used immunoelectron microscopy to examine the distribution of ACE2 in human kidneys.

Glomerular ACE2 expression was found to be markedly weaker than that observed in the proximal tubules in both diabetic and normal control kidneys in all of the species examined in the above-mentioned studies.

RENAL ACE/ACE2 RATIO

ACE2 counteracts the effects of ACE by catabolizing

Table 1 Renal angiotensin-converting enzyme and angiotensin-converting enzyme 2 protein expression in various diseases

Diseases	Species	Glomerular ACE	Tubular ACE	Glomerular ACE2	Tubular ACE2	Ref.	Year
Type 1 DM	Rats	↑	↓	↑	↓	Tikellis *et al*[30]	2003
Type 2 DM	Mice	ND	↓	ND	↑	[1]Ye *et al*[9]	2004
Type 2 DN	Mice	↑	↓	↓	↑	Ye *et al*[14]	2006
Type 1 DM	Rats	↑	↑	↓	↑	Moon *et al* [31]	2008
Type 2 DN	Mice	ND	ND	↓	↑	Chodavarapu *et al*[32]	2013
Type 2 DN	Humans	ND	ND	↑	↑	Lely *et al*[34]	2004
Type 2 DN	Humans	↑	↑	↓	↓	Mizuiri *et al*[15]	2007
Type 2 DN	Humans	↑	↑	↓	↓	Reich *et al*[16]	2007
Primary glomerulopathy	Humans	ND	ND	↑	-	Lely *et al*[34]	2004
IgA nephropathy	Humans	↑	↑	↓	↓	Mizuiri *et al*[36]	2011
Hypertension	Rats	ND	ND	↓[1]		Prieto *et al*[41]	2011
Hypertension	Humans	↑[1]		↓[1]		Koka *et al*[45]	2008
Nephrosclerosis	Humans	-	↓	-	↓	Wang *et al*[22]	2011
Subtotal nephrectomy	Rats	ND		↓[1]		Velkoska *et al*[46]	2009
Subtotal nephrectomy	Rats	↑[1]		↓[1]		Eräranta *et al*[47]	2012

[1]No description about expression pattern of glomerular and tubular ACE and ACE2. DM: Diabetes mellitus; DN: Diabetic nephropathy; ND: Not done; ACE: Angiotensin-converting enzyme; -: No changes.

Ang Ⅱ to produce Ang 1-7. The balance between the effects of these two molecules affects the renal RAS, and hence, the ACE/ACE2 ratio might represent the key parameter that is driving the regulation of the renal RAS[8-13]. In healthy conditions, ACE2 activity rises along with ACE activity, but imbalances can develop under disease conditions[12]. It is suggested that the main priority of the RAS is to achieve an appropriate balance between ACE and ACE2 activity. The ACE-Ang Ⅱ-AT1 axis has been suggested to have detrimental effects on the RAS, whereas the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis and seems to play a renoprotective role[19].

Various studies have used kidney injury models to investigate the ACE/ACE2 ratio. For example, Ye *et al*[9] demonstrated that higher ACE2 concentrations and lower ACE protein concentrations in the renal tubules had renoprotective effects in the early stages of the condition experienced by *db/db* mice (rodent model of type 2 diabetes) without nephropathy. In a recent study, significantly increased concentrations of ACE2 mRNA, ACE mRNA, and Ang Ⅱ were detected in the plasma and renal cortical tissue of streptozotocin (STZ)-treated rats (rodent model of type 1 diabetes) compared with the control rats[20]. In addition, the STZ-treated rats exhibited the greater increase in ACE mRNA as opposed to ACE2 mRNA compared with the normal controls[20]. In rats, the consumption of a high salt diet led to a higher glomerular ACE/ACE2 ratio, resulting in kidney damage and oxidative stress[8]. Hamming *et al*[21] reported low sodium intake or ACE inhibition does not affect renal ACE2 despite large changes in renal ACE in healthy rats and they suggested that renal ACE inhibition and dietary sodium restriction affect renal ACE and ACE2 *via* different mechanism.

In humans, kidney biopsies have indicated that hypertensive patients have higher ACE/ACE2 mRNA ratios[10]. In addition, we detected high ACE/ACE2 ratios in patients with type 2 diabetes and overt nephropathy; thus, such changes might play a role in renal damage[15]. In a study of patients with hypertensive nephrosclerosis, Wang *et al*[22] detected a correlation between the glomerular ACE/ACE2 protein ratio and the extent of glomerulosclerosis and an inverse correlation between the glomerular ACE/ACE2 protein ratio and the estimated glomerular filtration rate (eGFR)[22]. Conversely, no associations were detected between the tubulointerstitial ACE/ACE2 ratio and histological or clinical parameters[22].

Batlle *et al*[12] suggested that as ACE and ACE2 are regulated *via* different mechanisms and the ACE/ACE2 ratio could be misleading[12]. Pohl *et al*[23] demonstrated that whilst the ACE2 is expressed along the entire renal tubular segment ACE is only expressed in the brush-border membrane of the late proximal tubules and they suggested that surface expression of ACE and ACE2 differed as a function of endocytosis[23].

Together, the findings of these studies indicate that increases in the ACE/ACE2 ratio induced *via* the ACE-Ang Ⅱ-AT1 axis have a significant influence on the development of severe kidney damage. On the other hand, renal ACE/ACE2 ratio data should be interpreted carefully, as ACE and ACE2 are regulated *via* independent mechanisms.

CHRONIC KIDNEY DISEASE

In dogs, Mitani *et al*[24] found that chronic kidney disease (CKD) kidneys exhibited weaker ACE immunoreactivity than normal kidneys and detected a negative association between ACE expression and renal tissue damage. However, both upregulated and downregulated ACE2 expression were detected in dogs with CKD, and ACE/ACE2 immunoreactivity did not exhibit a close relationship with renal tissue damage[24]. Burrell *et al*[25] suggested

that renal ACE2 deficiency and a lack of cardiac ACE2 activation might influence the progression of cardiac and renal tissue damage in rats with CKD. Furthermore, the detrimental cardio-renal effects of CKD were only partially abrogated by long-term ACE inhibition[25]. However, the CKD rats used in their study might not have been a suitable CKD model, as they were killed 28 days after subnephrectomy. Dilauro *et al*[26] found that renal ACE2 expression was reduced in a mouse model of early CKD, and this led to increased albuminuria *via* an AT1 receptor-dependent blood pressure-independent mechanism.

In a recent study, Roberts *et al*[27] found that hemodialysis CKD patients exhibited lower plasma ACE2 activity than pre-dialysis CKD patients, and female hemodialysis patients displayed lower plasma ACE2 activity than male hemodialysis patients. The lower plasma ACE2 activity exhibited by dialysis patients might slow the catabolism of Ang Ⅱ, which may be responsible for the high prevalence of cardiovascular disease among these patients[27]. Human plasma is known to contain an endogenous ACE2 inhibitor[28]. However, Wysocki *et al*[29] reported that this cannot explain the lower plasma ACE2 activity seen in dialysis patients as the removal of the inhibitor by dialysis resulted in higher plasma ACE2 concentrations. Recently, it has been indicated that plasma ACE2 has renoprotective effects in CKD, but more studies of the plasma ACE2 concentrations of pre-dialysis and dialysis CKD patients and healthy subjects are necessary to confirm this.

Diabetic nephropathy

Diabetic nephropathy is a representative disease of CKD and is linked to activation of the renal RAS, resulting in Ang Ⅱ-induced tubular and glomerular damage. In a study involving diabetic rats, Tikellis *et al*[30] observed reduced renal expression levels of ACE and ACE2 mRNA, higher glomerular ACE and ACE2 protein expression levels, and lower tubular ACE and ACE2 protein expression levels at 24 wk after the administration of STZ. Furthermore, the rats' ACE2 protein expression levels rose after treatment with an ACE inhibitor[30]. In a study examining *db/db* mice without nephropathy, Ye *et al*[9] detected higher ACE2 protein expression and lower ACE protein expression in the animal's renal tubules, which resulted in renoprotective effects. However, as the study had involved young *db/db* mice with early stage diabetes, they could not rule out the possibility that the ACE2 expression levels of the mice might subsequently fall as nephropathy developed[9]. Moreover, they speculated that reduced ACE2 expression and upregulated ACE expression gradually induce kidney damage in diabetes[9]. Ye *et al*[14] also examined the glomeruli of *db/db* mice with established diabetic nephropathy and observed upregulated ACE protein expression and downregulated ACE2 protein expression. In the glomeruli of rats with STZ-induced diabetes, Moon *et al*[31] noted stronger and weaker ACE and ACE2 staining in the glomeruli, respectively, at 8 wk after the administration of STZ, but observed increase in both ACE and ACE2 staining in the tubules compared with the control rats. In a study of *db/db* mice, Chodavarapu *et al*[32] detected reduced glomerular ACE2, increased tubular ACE2 and ADAM17, and suspected ectodomain shedding of active renal ACE2 in the urine.

In humans, we observed downregulated ACE2 expression and upregulated ACE expression in both the glomeruli and tubulointerstitium of diabetic patients with overt nephropathy, which led to the diabetic patients having significantly higher ACE/ACE2 ratios than the controls ($P < 0.001$) (Figure 2)[15,33]. We also detected a positive correlation between the ACE/ACE2 ratio and the serum creatinine, fasting blood glucose, proteinuria, hemoglobin A1c, and blood pressure values and an inverse correlation between and the eGFR ($P < 0.001$)[15]. In addition, Reichi *et al*[16] observed decreased ACE2 expression and increased ACE expression in the glomeruli and tubules in biopsy samples collected from patients with type 2 diabetes-induced kidney disease. Conversely, Lely *et al*[34] detected upregulated ACE2 expression in the glomerular and peritubular capillary endothelia in all types of primary and secondary renal disease as well as renal transplant patients; however, they only examined 8 diabetic patients and did not concentrate on the variation in ACE2 expression between biopsy samples from diabetic nephropathy patients and normal renal tissue from patients underwent surgery for renal tumors. In a real time PCR study in which 8 diabetic patients with overt proteinuria were compared with 66 non-diabetic patients with renal disease, ACE mRNA expression was significantly increased, but ACE2 mRNA expression was not significantly changed in the diabetic patients[35]. The differences between the results obtained in human studies of type 2 diabetic nephropathy and those obtained using *db/db* models of diabetes without nephropathy[9] might have been due to the different stages of diabetes, as no human studies of early stage diabetes have been conducted.

Taken together, human biopsy studies have suggested that upregulated ACE expression and downregulated ACE2 expression are seen at both the glomerular and tubular levels in established diabetic nephropathy. Whilst most animal studies detected associations between diabetes and increased ACE expression and decreased ACE2 expression in the glomeruli, tubular ACE and ACE2 expression were demonstrated to be significantly decreased and increased, respectively.

Primary glomerular disease

In the study by Lely *et al*[34], increased ACE2 expression was detected in the glomerular and peritubular capillary endothelia in all primary renal diseases (including 8 cases of IgA nephropathy, 5 cases of focal glomerulosclerosis, and 18 cases of membranous glomerulopathy), but no

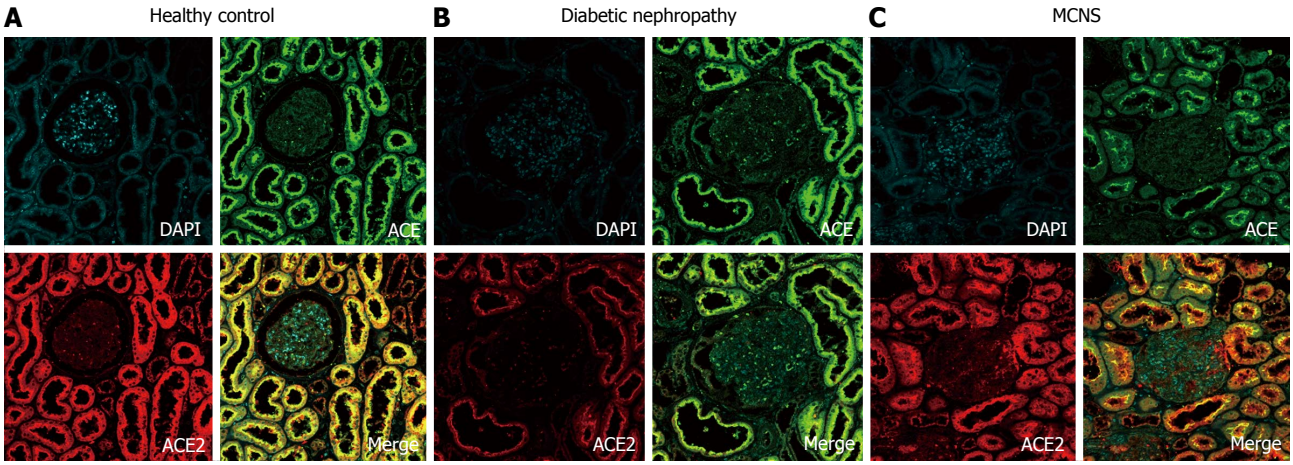

Figure 2 Images obtained with confocal microscopy of triple immunofluorescence staining of angiotensin-converting enzyme (green), angiotensin-converting enzyme 2 (red), and nuclei (DAPI; blue) in kidney specimens. In healthy subjects, marked co-localization of ACE and ACE2 (yellow) was observed in the apical brush borders of the proximal tubules (A). Both ACE and ACE2 were also present in the glomeruli, but the glomeruli exhibited weaker staining than the proximal tubules (A). In diabetic kidneys, stronger ACE expression and weaker ACE2 expression were detected in the proximal tubules, and no marked colocalization of ACE and ACE2 was observed (B); however, similar ACE and ACE2 staining patterns were seen in patients with minimal change nephrotic syndrome (MCNS) and healthy controls (C). Adapted with permission from Mizuiri *et al*[33]. ACE: Angiotensin-converting enzyme.

variations in ACE2 expression were detected between the different renal diseases. In a previous study, we detected lower ACE2 and higher ACE expression in the glomeruli and tubules of 30 patients with IgA nephropathy than in those of 20 healthy controls[36]. In addition, patients with membranous nephropathy, but not those with minimal change nephrotic syndrome, also displayed downregulated tubular ACE2 expression[36]. We excluded patients that were taking ACE inhibitors or AT1-receptor blockers, which might partly explain the differences between our findings and those of Lely *et al*[34]. In a study examining kidney biopsies from patients with focal segmental glomerulosclerosis and patients with chronic allograft nephropathy Reich *et al*[16] demonstrated that the ACE2 and ACE mRNA levels of these samples did not differ from those of the control samples.

We suggest that upregulated ACE expression and downregulated ACE2 expression represent a generalized response to kidney damage[36], but further studies of ACE and ACE2 expression in the kidneys based on human renal biopsy samples are necessary to confirm this.

HYPERTENSION

The renal RAS acts independently of the systemic RAS and is suggested to aid blood pressure control[37]. Imbalances between Ang 1-7/ACE2 and Ang Ⅱ/ACE expression downregulate Ang 1-7 synthesis and promote renal Ang Ⅱ expression and hypertension. Crackower *et al*[38] found that all hypertensive rat strains exhibited significantly downregulated ACE2 mRNA and protein expression. Tikellis *et al*[39] found that the kidneys of spontaneously hypertensive rats demonstrated significantly upregulated ACE2 expression and activity at birth and that at the onset of hypertension tubular ACE2 expression decreased, but glomerular expression paradoxically rose. Furthermore, in ACE2-deficient mice, a link was detected between severe hypertension and the excessive renal accumulation of Ang Ⅱ[40]. In the kidneys of Goldblatt hypertensive rats, upregulated renin expression in the collecting duct was found to be associated with upregulated ANG Ⅱ and ACE expression and downregulated ANG 1-7 and ACE2 expression[41]. Samuel *et al*[37] found that in obese Zucker rats, renal Ang Ⅱ expression was upregulated and ACE2-AT(2)R-MasR axis expression was downregulated by high Na consumption. ACE2 increases the synthesis of Ang 1-7 from Ang Ⅱ, which helps to avoid the excessive accumulation of Ang Ⅱ[42]. Furthermore, strategies such as increasing ACE2 activity and promoting Ang Ⅱ catabolism have been found to be useful for ameliorating hypertension[43]. In addition, recombinant ACE2 has been demonstrated to increase plasma ACE2 after Ang Ⅱ infusion, which results in blood pressure and plasma Ang Ⅱ normalization[44].

In a study of human renal biopsy samples in which total RNA was examined, the ACE to ACE2 ratio was found to be significantly higher in hypertensive subjects than in the controls[10]. In a study involving real-time PCR and immunohistochemistry, Koka *et al*[45] found that hypertensive nephropathy and hypertensive cardiopathy displayed significantly increased ACE expression and decreased ACE2 expression. Furthermore, Wang *et al*[22] reported that hypertensive patients exhibited significantly reduced protein expression levels of ACE and ACE2 in the tubulointerstitium compared with the controls, whereas there was little difference between the glomerular ACE and ACE2 protein expression levels

of the two groups.

The above studies indicate that ACE and ACE2 are important for keeping the RAS in balance, and variations in ACE and ACE2 expression might be associated with hypertension.

SUBTOTAL NEPHRECTOMY

In rats, Velkoska *et al*[46] found that subtotal nephrectomy resulted in the lowering of ACE2 activity; however, these changes were reversed by ACE inhibition. In 5/6 nephrectomized rats, Eräranta *et al*[47] found that dietary phosphate loading led to greater tissue damage and upregulated renal ACE expression. It can be said that renal ACE2 expression is downregulated after subtotal nephrectomy, which might increase the deleterious effects of Ang Ⅱ on the kidneys.

CLINICAL IMPLICATIONS OF CIRCULATING ACE2 AND URINARY ACE2

Only a small number of studies have examined the activity of circulating ACE2 in humans. Soro-Paavonen *et al*[48] demonstrated that type 1 diabetes patients with micro- or macrovascular disease display higher circulating ACE2 activity, suggesting that ACE2 might act to counteract the effects of such conditions. Soler *et al*[49] found that plasma ACE2 activity can be assessed in kidney transplant recipients and is positively correlated with age and the serum levels of urea, γ-glutamyl transferase, glycosylated hemoglobin, creatinine, aspartate transaminase, and alanine transaminase. It is also reported that soluble ACE2 activity correlates with hypertension and soluble ACE concentration decreases, while soluble ACE2 concentration increases in systolic heart failure[50]. The above studies indicate that plasma ACE and ACE2 levels might be useful biomarkers of the RAS in renal and coronary diseases.

In order to identify biomarkers of kidney disease progression, urine is readily collected and available. Soluble ACE2 has been detected in human urine[51], which might have been due to the excretion of the protein from renal cells or from plasma *via* glomerular filtration. Using Western blotting and an enzyme-linked immunosorbent assay, we found that patients with diabetic nephropathy had higher urinary ACE2 protein levels than the healthy controls[52]. In addition, Park *et al*[53] demonstrated that the urinary ACE2 concentration is strongly correlated with type 2 diabetes mellitus and is an independent predictor of microalbuminuria. Renal transplant patients with diabetes have also been found to have increased urinary ACE2 levels[54]. The conversion of membranous ACE2 into its soluble form is partially dependent on tumor necrosis factor-α convertase (ADAM17), which is responsible for increased ectodomain shedding of ACE2 at least *in vitro*[52,55]. Patients with renal disease exhibit increased expression levels of ADAM17[56]. We propose that ACE2 ectodomain shedding is associated with reduced renal ACE2 expression in patients with diabetic nephropathy[52]. A recent study demonstrated that insulin treatment reduced ADAM17 and ACE2 shedding in the kidneys of diabetic Akita mice[57]. The above findings suggest that the urinary ACE2 protein level and ACE2 activity are useful as biomarkers of diabetic nephropathy.

ACE2 AS A THERAPEUTIC TARGET IN KIDNEY DISEASE

ACE1 temporarily downregulates Ang Ⅱ expression, and Ang Ⅱ receptor blockers increase Ang Ⅱ levels. Both types of molecule suppress Ang Ⅱ activity incompletely; therefore, combining RAS inhibitors with ACE2 activators might lead to more complete downregulation of the RAS[12]. Qudit *et al*[58] found that human recombinant ACE2 (hrACE2) slowed the progression of diabetic nephropathy and lowered NADPH oxidase activity and blood pressure. In addition, their *in vitro* study indicated that the protective effect of hrACE2 is derived from upregulated Ang 1-7 expression and downregulated Ang Ⅱ expression[58]. Another study demonstrated that whilst hrACE2 treatment was effective at increasing plasma ACE2, it did not affect renal or cardiac ACE2 activity[44].

Xanthenone (XNT)[59] and diminazene (DIZE)[60] are ACE2 inhibitors. Hernández Prada *et al*[59] found that the acute *in vivo* administration of XNT to spontaneously hypertensive rats led to improvements in their cardiac function and reduced their blood pressure. Jarajapu *et al*[60] proposed that the short-term administration of XNT or DIZE to diabetic patients with complications is not effective at treating diabetic endothelial progenitor cell dysfunction. Moreover, recently Haber *et al*[61] confirmed a lack of enhancement of ACE2 enzymatic activity by XNT and DIZE *in vitro* and *ex vivo* experiments in both mice and rat kidney. Therefore, the suggestion that ACE2 activators have supra therapeutic effects on kidney disease than classical RAS inhibitors is speculative at present, although enhancing ACE2 activity might represent a new therapeutic strategy for Ang Ⅱ overactivity.

CONCLUSION

ACE and ACE2 play significant roles in the RAS, and both enzymes are strongly expressed in the kidneys, where their actions aim to achieve a balance between the ACE-Ang Ⅱ-AT1 axis and ACE2-Ang 1-7-Mas axis. In addition, the renal ACE/ACE2 ratio seems to have a significant impact on a variety of diseases including diabetes, hypertension, IgA nephropathy, and subtotal nephrectomy.

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MINIREVIEWS

Lipid abnormalities in kidney disease and management strategies

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Abstract

Patients with kidney diseases continue to experience significant cardiovascular disease (CVD) morbidity and mortality. Although there are many important risk factors playing a role in the pathogenesis of CVD in chronic kidney disease (CKD) patients, dyslipidemia (elevated triglycerides, elevated oxidized low-density lipoprotein and low/dysfunctional low high-density) represents one of the modifiable risk factors. Renal failure patients have unique lipid abnormalities which not only have complex role in pathogenesis of CVD but also cause relative resistance to usual interventions. Most of the randomized trials have been in hemodialysis population and data from CKD non-dialysis, peritoneal dialysis and renal transplant populations is extremely limited. Compared to general population, evidence of mortality benefit of lipid lowering medications in CKD population is scarce. Future research should be directed towards establishing long term benefits and side effects of lipid lowering medications, through randomized trials, in CKD population.

Key words: Chronic kidney disease; Dyslipidemia; Statins; Cardiovascular disease; Renal transplant recipients; Hemodialysis; Peritoneal dialysis

Core tip: Burden of cardiovascular disease and dyslipidemia continues to be high among patients with kidney diseases. Our review includes unique lipid abnormalities specifically affecting patients with kidney diseases. We have included comprehensive review of the latest evidence of the dyslipidemia treatment for each subgroup [*i.e.*, chronic kidney disease (CKD) not on dialysis, CKD on dialysis and Kidney transplant recipients] and current guidelines from Kidney Diseases: Improving Global Outcomes.

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INTRODUCTION

Chronic kidney disease (CKD) has become a public health problem with a global prevalence of around 8%-16%[1] and with an estimate of more than 10% (*i.e.*, > 20 million) prevalence in the adult United States population[2]. Data from National Health and Nutrition Examination Survey showed that CKD prevalence among ages 60 and above increased from 18.8% in 1988-1994 to 24.5% in 2003-2006[3]. According to United States Renal Data System (USRDS) a total of 112788 patients initiated dialysis in 2011[4]. Cardio vascular diseases (CVD) remain the number one cause of death among patients with kidney diseases[5,6]. USRDS 2013 Annual Data Report indicates that CKD patients not only have higher rates of congestive heart failure, acute myocardial infarction (MI) and cerebral vascular accident compared to non-CKD patients, but they also have lower survival rates compared to non-CKD patients. This survival further decreases with severity of CKD[7]. Similarly, renal transplant recipients (RTR) have elevated CVD mortality with estimated 10-year risk of 21.5%, due to effect of CKD, post-transplant allograft function and effects of various immunosuppressant medications[8,9].

Dyslipidemia is a well-established risk factor for CVD in the general population but this relationship is not straightforward in CKD population. While dyslipidemia is associated with CVD in pre-dialysis CKD[10] and hemodialysis population[11], data regarding its association in peritoneal dialysis patients is lacking[12]. With an ever increasing CKD burden worldwide, providing treatments for modifiable risk factors, like dyslipidemia, becomes an essential component for improving outcomes. In this review, we will examine various lipid abnormalities associated with kidney diseases and current evidence regarding various treatments.

Reverse epidemiology

Relationship between dyslipidemia and survival has not been consistent among patients with kidney disease. "Reverse epidemiology", terminology first coined in 2003, refers to the findings of increase survival among dialysis patients with high body mass index, obesity and hypercholesterolemia[13]. Authors suggested that survival bias, presence of malnutrition and inflammation and time discrepancies of risk factors possibly explain these findings[13]. While some studies have shown that lower cholesterol was associated with an increase in mortality[13,14], other studies have concluded that among dialysis patients without malnutrition/inflammation and among black dialysis patients, hypercholesterolemia is associated with an increase in cardiovascular mortality[15,16]. Chawla *et al*[17] reported data from the cohort of non-diabetic CKD (non-dialysis) patients, cholesterol levels were not associated with CVD mortality.

Lipid profile in kidney diseases

There are both qualitative and quantitative abnormalities seen in the lipid profile of patients with kidney disease[18]. Some of these abnormalities also differ between spectrums of kidney diseases. With impaired renal function and reduced clearance, abnormal removal is major contributor of lipid abnormalities. Common initial abnormalities include hypertriglyceridemia and low high-density (HDL) cholesterol. Elevated triglyceride levels (TG) can be attributed to increased concentration of Apolipoprotein C-Ⅲ[19] and also to the reduced activity of lipoprotein lipase[18].

HDL cholesterol, generally considered as "good" cholesterol, usually plays a role in anti-inflammatory, anti-oxidation and reverse cholesterol transport processes in normal individuals. In CKD patients, these activities are severely affected due to variety of factors[20]. With advanced renal failure, there is decreased production of apolipoprotein A-1 (which leads to decreased HDL levels) and decreased production and activity of lecithin-cholesterol acyltransferase which further decreases HDL levels and maturation of HDL cholesterol[21]. There are functional changes noted in HDL cholesterol in patients with renal failure. Anti-oxidant and anti-inflammatory properties of HDL cholesterol are compromised due to reduced activities of paraoxonase and glutathione peroxidase in renal failure patients[20,21]. Furthermore, oxidative stress can result in dysfunctional HDL which has rather pro-inflammatory effects[22]. Studies in hemodialysis patients have shown that dysfunction of HDL is not only associated with multiple co-morbidities and poor quality of life[23], but also with an increased risk of CVD events and CVD mortality[24]. In summary, patients with renal failure develop certain functional and structural abnormalities in HDL cholesterol which makes them prone to develop atherosclerosis and thus contributing to their CVD burden.

CKD patients also have reduced levels of lipoprotein lipase, hepatic lipase and defective very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) receptors. This leads to accumulation of VLDL, intermediate-density lipoprotein and chylomicron remnants which are susceptible to oxidization. These oxidized products are usually atherogenic and play a role in CVD pathogenesis in this population[20]. CKD patients frequently develop secondary hyperparathyroidism which also has an impact on lipid abnormalities[25]. It has been postulated that this usually occurs due to an increase of intracellular calcium concentration in hepatocytes by elevated parathyroid hormone in CKD patients[26]. Studies have shown a role of parathyroidectomy in reducing triglyceride levels in CKD patients[26,27].

Among renal transplant recipients, it has been seen that lipids and lipoprotein profile ratios were more beneficial when the TG levels were less than 150 mg/dL and apoA1 was greater than 150 mg/dL when compared to the opposite[28]. They are also on immunosuppressive medications, many of which affect the lipid profile adversely. In most renal transplant centers in the United States, kidney transplant patients receive induction

Table 1 Common lipid profile in patients with kidney disease[18,33,34]

	CKD not on dialysis	Hemodialysis	Peritoneal dialysis	Transplant patients
Total cholesterol	Normal or elevated	Normal or low	Elevated	Elevated
Triglycerides	Elevated	Elevated	Elevated	Elevated
LDL cholesterol	Normal or elevated or low	Normal or low	Elevated	Elevated
HDL cholesterol	Low	Low	Low	Normal

CKD: Chronic kidney disease; HDL: Low high-density; LDL: Low-density lipoprotein.

Table 2 Brief summary of randomized clinical trials in patients with kidney diseases[9,35,46,47]

Trial	Study population	Intervention	Follow-up	Major findings
ALERT (2003)	Renal transplant recipients (n = 2102)	Fluvastatin (40 mg/d) *vs* placebo	Mean 5.1 yr	Fluvastatin group had reduced major cardiac events and cardiac death but this was not statistically significant No effect seen on all-cause mortality
4D (2005)	Hemodialysis patients with DM type Ⅱ (n = 1255)	Atorvastatin (20 mg/d)	Median 4 yr	Atorvastatin did not have significant effect on CV death, non-fatal MI, non-fatal stroke and all-cause mortality
AURORA (2009)	Hemodialysis patients aged 50-80 yr (n = 2776)	Rosuvastatin (10 mg/d) *vs* placebo	Median 3.8 yr	Rosuvastatin had no significant effect on CV mortality, non-fatal MI, non-fatal stroke and all-cause mortality
SHARP (2011)	CKD not on dialysis (n = 6247) Hemodialysis (n = 2527) Peritoneal dialysis (n = 496)	Simvastatin 20 mg/d plus ezetimibe 10 mg/d *vs* placebo	Median 4.9 yr	Simvastatin plus ezetimibe significantly decreased major atherosclerotic event but had no major effect on CV mortality or all-cause mortality. Results were available for only entire population (both dialysis and non-dialysis)

ALERT: Assessment of lescol in renal transplantation; AURORA: Assessment of survival and cardiovascular events; SHARP: Study of heart and renal protection; CKD: Chronic kidney disease; CV: Cardiovascular; MI: Myocardial infarction; DM: Diabetes mellitus.

immunosuppression followed by ongoing use of various combination of immunosuppression class of medications including corticosteroids, calcineurin inhibitors (tacrolimus, cyclosporine) and mammalian target of rapamycin (mTOR) antagonists (sirolimus, everolimus)[29]. Steroids commonly cause insulin resistance and hyperinsulinemia which is associated with hypercholesterolemia[30]. Cyclosporine has been noted to decrease hepatic clearance of LDL as well as increase the synthesis of VLDL and decrease the secretion of bile salts causing increase in cholesterol levels. Tacrolimus increases the incidence of new onset diabetes after transplant (NODAT) which in turn is associated with an increased risk of atherosclerotic cardiovascular events[31]. mTOR inhibitors inhibit the activity of lipases, thereby increasing the circulating lipoproteins; they also decrease the fatty acid uptake into the adipose tissue leading to a decrease in plasma lipid clearance adding to the dyslipidemia[32].

Broadly, common lipid abnormalities among the patients with kidney diseases can be summarized in Table 1.

MANAGEMENT STRATEGIES

CKD patients not on dialysis

Statins: In general population, statins are clearly associated with decreasing CVD events and mortality, however results in CKD population have been variable. Most of the data regarding statins in CKD (not on dialysis) comes from subgroup/post hoc analysis and meta-analysis. Only one randomized trial, Study of Heart and Renal Protection (SHARP) trial, evaluated statin therapy with major cardiovascular events[35]. SHARP trial included 6247 patients with CKD not on dialysis, with mean glomerular filtration rate (GFR) of 26.6 mL/min per 1.73 m^2. Patients were randomly assigned to simvastatin 20 mg daily plus ezetimibe 10 mg daily *vs* placebo. The primary outcome was first major atherosclerotic event with median follow up of 4.9 years. Final results were available for the entire study group (both non-dialysis and dialysis), and it showed a significant reduction in the risk of major atherosclerotic event (RR = 0.83, P = 0.0021); non-hemorrhagic stroke (RR = 0.75, P = 0.01) and reduction for the need for revascularization procedure (RR = 0.79, P = 0.0036) in simvastatin/ezetimibe group. There was no significant difference between the two groups for major coronary events and it did not show any significant difference in progression to end-stage renal disease (ESRD) among non-dialysis patients (Table 2).

A 2014 meta-analysis by Palmer *et al*[36], which included 50 studies and 45285 patients, showed that statins consistently reduced CVD events and death rates in CKD patients not on dialysis. It showed that, when compared to placebo, statins reduced overall mortality (RR = 0.79 with 95%CI: 0.69-0.91 in 10 studies and 28276 patients), cardiovascular (CV) mortality (RR = 0.77, 95%CI: 0.69-0.87 in 7 studies and 19059 patients), CV events (RR = 0.72, 95%CI: 0.66-0.79 in 13 studies and 36033 patients), and myocardial infarction (RR = 0.55, 95%CI: 0.42-0.72 in 8 studies and 9018 patients). This meta-analysis did not show any consistent effect of statin on progression of CKD.

Post hoc analyses of three randomized trials

Table 3 Kidney diseases: improving global outcomes recommended doses of commonly used statins, based on doses used in trials, in patients with estimated glomerular filtration rate < 60[9,35,45-47]

	Dose (mg/d)
Fluvastatin	80
Atorvastatin	20
Rosuvastatin	10
Simvastatin/ezetimibe	20/10
Pravastatin	40
Simvastatin	40
Pitavastatin	2

(CARE, LIPID and WOSCOPS) have also shown that pravastatin reduced cardiovascular event rates (HR = 0.77, 95%CI: 0.68-0.86) in patients with moderate CKD; and this was similar to the patients without CKD[37]. Interestingly, subgroup analysis of JUPITER trial showed that rosuvastatin decreased cardiovascular event rates as well as overall mortality in patients with moderate CKD even in the absence of hyperlipidemia (LDL < 130). However, this study originally excluded patients with diabetes and advanced CKD[38]. Other meta-analyses of trials (randomized trials in CKD population plus sub-group analysis of trials of general population) have persistently shown the beneficial effect of statins[39-41].

There has been a suggestion that statins might have been associated with decreased decline in renal function[42]. However, not only majority of data is from secondary analysis; the results have been contradictory as well[43]. As stated above, SHARP trial (only randomized trial in this population) did not show any effect of stain on renal progression. Recent meta-analysis by Nikolic *et al*[44] showed improvement in GFR with statin use with the most benefit observed between year 1 and year 3 of statin therapy.

Recommendations for use: Kidney diseases: improving global outcomes (KDIGO) 2013 guidelines[45] recommend treatment with statins for CKD patients (not on chronic dialysis or had transplantation) $\geqslant$ 50 years of age who have estimated GFR (eGFR) below or above 60 mL/min per 1.73 m^2. For patients between ages of 18-49, KDIGO currently recommends statin therapy if they have known coronary disease, diabetes, prior history of ischemic stroke and if their cumulative 10-year risk of coronary death or non-fatal MI is greater than 10%. Statins are generally well tolerated; main side effects include hepatotoxicity and muscle toxicity including myopathy, myalgia and rhabdomyolysis. The incidence of these side effects has not been higher in CKD population compared to general population. For patients with eGFR $\geqslant$ 60 mL/min per 1.73 m^2, there is no dose adjustments required for CKD patients. KDIGO recommends using doses, used in randomized trials for particular statins, for the patients with eGFR below 60 (Table 3).

Fibrates: Fibrates mainly have effects on reducing triglyceride levels and increasing HDL cholesterol levels. Fibrates can decrease triglyceride levels by 18%-45%[48] and increase HDL cholesterol by 10%[49]. However in patients with CKD, their overall effect on cardiovascular risk has not been proven consistently. K/DOQI guidelines in 2003 recommended use of fibrates for the prevention of pancreatitis in patients with hypertriglyceridemia but in their latest 2013 guidelines, this recommendation has been removed[50].

In post-hoc subgroup analysis of VA-HIT trial, gemfibrozil was evaluated for secondary prevention of cardiovascular events in patients with CKD[51]. Gemfibrozil therapy reduced the composite outcome of coronary death, non-fatal MI and stroke but overall mortality was unchanged. Gemfibrozil group had higher incidence of increase in serum creatinine compared to placebo. Other major trials, evaluating effects of fibrates on cardiovascular risk, either had a very small proportion of patients with kidney disease[52] or CKD patients were entirely excluded[53]. At present KDIGO recommends against use of combination of statin and fibrates in CKD patients due to increased adverse events.

KDIGO recommends therapeutic life style changes in patients with hypertriglyceridemia, although the evidence for this is weak. These include weight reduction, dietary modification, increase physical activity, reduced alcohol intake and treatment of hyperglycemia. KDIGO recommends that fibrates can be considered in patients with triglycerides > 1000 mg/dL[50].

CKD patients on dialysis

Statins: There have been three major randomized clinical trials evaluating effect of statins in dialysis population. First one to be reported was Die Deutsche Diabetes Dialyse study, commonly known as 4D study. In this study, effect of atorvastatin on cardiovascular disease and death was evaluated among 1255 diabetic patients who were receiving maintenance hemodialysis[47]. Groups were assigned to receive atorvastatin 20 mg or matching placebo. At median follow-up of 4 years, despite decrease in LDL cholesterol by 42% within first four weeks, atorvastatin use did not significantly impact the primary endpoints of cardiovascular death, non-fatal MI and non-fatal stroke. Interestingly, atorvastatin group had higher incidence of fatal stroke. Atorvastatin also did not have significant effect on all-cause mortality. Various factors have been attributed to these findings, including the fact that the entire patient population was diabetic, had significant cardiovascular disease burden at baseline, relatively lower dose of atorvastatin, and probable limited role of statin once ESRD occurs. Subsequent post hoc analysis of 4D trial by März *et al*[54] showed that atorvastatin reduced fatal and non-fatal cardiac event and all-cause mortality in the particular group when pre-

Table 4 Kidney disease: Developing global guidelines recommendations for dyslipidemia treatment among chronic kidney disease groups

CKD groups	KDIGO recommendations for dyslipidemia
CKD patients not on dialysis	In adults ≥ 50 yr with eGFR ≥ 60 mL/min per 1.73 m^2, treatment with statins is recommended In adults ≥ 50 yr with eGFR ≤ 60 mL/min per 1.73 m^2, treatment with statins or statins/ezetimibe combination is recommended In adults 18-49 yr, treatment with statins is recommended if they have one or more of the following risk factors: Known coronary disease Diabetes mellitus Prior ischemic stroke Estimated 10-yr incidence of coronary death or non-fatal myocardial infarction > 10%
CKD patients ON dialysis	In adult CKD patients on dialysis, initiation of statin or statin/ezetimibe combination is not recommended In adult dialysis patients who are already on statin or statin/ezetimibe combination at the initiation of dialysis, these agents should be continued
Kidney transplant patients	In adult patients with kidney transplant, treatment with statin is recommended

Adapted from Tonelli *et al*[45]. CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate.

treatment LDL is > 145 mg/dL[54].

A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) trial tried to investigate effects of rosuvastatin in hemodialysis patients[46]. This trial included 2776 hemodialysis patients randomly assigned to rosuvastatin 10 mg daily or matching placebo. Primary end-point was composite of death from cardiovascular cause, non-fatal MI and non-fatal stroke. At median follow up of 3.8 years, rosuvastatin did not have any significant association with primary end-point (HR = 0.96, 95%CI: 0.84-1.11, *P* = 0.59). As seen in the 4D study, rosuvastatin group in this study had a 43% reduction in their LDL levels from baseline at 3 mo. No significant effect on all-cause mortality was seen either. Authors suggested several possibilities contributing to these findings which include exclusion of patients < 50 years of age and those who have been on statin, high percentage of patients leaving the study, and lower than expected yearly cardiovascular event rates. In post hoc analysis of this trial among diabetics (*n* = 731), rosuvastatin did not have significant effect on primary end-point although rosuvastatin group had significant decrease in cardiovascular events (HR = 0.68, 95%CI: 0.51-0.90)[55].

SHARP trial[35] included CKD patients on dialysis as well as not on dialysis. It also included patients on both hemodialysis and peritoneal dialysis. Out of 9270 patients, 3023 were on dialysis and among them 2527 were on hemodialysis and 496 were on peritoneal dialysis. As mentioned earlier, there was a 17% reduction in major atherosclerotic event with the use of simvastatin and ezetimibe but the data on individual components of primary endpoints were only available for whole (dialysis + non-dialysis) population, and not separately. The authors suggested that proportional effect on major atherosclerotic event did not differ between dialysis and non-dialysis patients. The results showed that simvastatin and ezetimibe did not have any significant effect on cardiovascular mortality and all-cause mortality. In sub-group of dialysis patients, there were similar numbers of major atherosclerotic events in simvastatin plus ezetimibe group (15%) as in the placebo group (16.5%) with RR = 0.9 with 95%CI: 0.75-1.08, although this trial did not have sufficient power to see any effect in subgroup analysis. Interestingly, lesser number of dialysis patients used lipid lowering therapy and had lower LDL cholesterol levels at baseline compared to non-dialysis patients (Table 2).

Palmer *et al*[56] presented review of statins in dialysis population in 2013. Their review included 25 studies and 8289 patients. Authors reported that at the dose of simvastatin 20 mg/d or equivalent, statin reduced total cholesterol by 46 mg/dL. But they had no significant effect on major cardiovascular events (RR = 0.95 with CI: 0.88-1.03 in 4 studies with *n* = 7084), cardiovascular mortality (RR = 0.94 with CI: 0.84-1.06 in 13 studies with *n* = 4627), and all-cause mortality (RR = 0.96 with CI: 0.90-1.02 in 13 studies with *n* = 4705). They further noted that the data regarding risk of adverse events were not conclusive and there was not enough information to evaluate difference between hemodialysis and peritoneal dialysis population. It was concluded that statins could not be recommended for the prevention of cardiovascular events among dialysis patients.

Recommendations for use: KDIGO 2013 guidelines[45] recommend not to initiate statin or statin plus ezetimibe in dialysis population based on the results of the above mentioned clinical trials. For the patients who are already on statin or statin plus ezetimibe at the initiation of dialysis, there is no conclusive data available. Nevertheless, at this time, KDIGO recommends to continue these agents and periodically review them (Table 4).

Fibrates: There are no specific randomized trials of the use of fibrates in dialysis population. At present, KDIGO recommendations for hypertriglyceridemia and use of

fibrates remain same for both non-dialysis and dialysis populations.

Kidney transplant patients

Kidney transplant patients are unique in that they are not only on multiple long term immunosuppressive medications but are also prone for infections and malignancy therefore one need to be extremely cautious in adding medications in this unique population. Use of any combination of medication in RTR entails one to be vigilant of the side effect profile as well as the drug interactions. Guidelines are developed based on input from several landmark trials; however paucity of trials in managing lipid abnormalities in RTR does pose a challenge.

Assessment of LEscol in renal transplantation (ALERT) Study was a well conducted multicenter randomized double-blind, placebo controlled trial which included 2102 renal transplant recipient who were treated with fluvastatin or placebo and followed for 5-6 years. At the end of the study Fluvastatin group had a significant lowering of their mean LDL cholesterol, total cholesterol and triglyceride levels compared to placebo, with no significant change in the HDL cholesterol level. Even though the study showed a reduction in the primary endpoint of major adverse cardiac events, it was not statistically significant. However the treatment with fluvastatin led to a reduction in the risk of cardiac death by about 38% and non-fatal Myocardial Infarction by about 32% without any significant difference in the adverse events related to the medication dose. Of note, the power of the trial (to achieve its primary end point) was low; along with that there was increased use of statin in the placebo arm towards the end of the study, both of which could have limited the evidence of the full benefits of the statin drug[9,57-59]. An Extension of the ALERT study reinforced the effective reduction of LDL-Cholesterol as well as the major adverse cardiac events without any safety or tolerability issues even in the cyclosporine treated patients[58].

Another multicenter randomized trial looking into the effect of fluvastatin on acute rejection involved 364 RTR who were given fluvastatin 40 mg and were compared to placebo. Individuals receiving fluvastatin had a reduction in LDL cholesterol level by 18%, total cholesterol (TC) by 10% and an augmented increase in the HDL cholesterol by 6%. There was no increase in adverse events from the use of statin and no reduction in the acute rejection rates in RTR[60]. A recent meta-analysis of statin use in RTR included 22 studies and found the inconclusive effects of statin on all-cause mortality and kidney function. However it did show the significant reduction of TC, LDL cholesterol and concluded the possible benefit of statin in reducing cardiovascular events[61].

As mentioned above, various immunosuppressive combinations are used in RTR and fluvastatin appears to have a less lipid lowering effect in everolimus treated patients which is an mTOR antagonist. Given this information alternative statin therapy or a combination of medication may have to be instituted to better optimize the dyslipidemia. A small study involving 12 RTR on everolimus, when switched from fluvastatin to rosuvastatin showed an additional significant improvement in the lipid panel without affecting the safety and tolerability[62].

Smaller trials have assessed the safety and tolerability of various statins in RTR and some have highlighted the pleiotropic effects of statin on graft survival and improving endothelial dysfunction. Atorvastatin and Simvastatin in spite of their involvement in the cytochrome P450 pathway have been shown to be relatively safe overall in RTR[63].

Based on available data, KDIGO recommends (weak recommendation with moderate quality of evidence) use of statin in renal transplant recipients[45].

CONCLUSION

In summary, patients with kidney diseases have unique lipid abnormalities when compared to general population and they have different clinical implications associated with these abnormalities. Over the time, our understanding has evolved regarding dyslipidemia in CKD patients. Statins remain the first line of treatment for dyslipidemia. Majority of current evidence comes from subgroup/post hoc analysis and meta-analysis, especially in CKD (pre-dialysis), peritoneal dialysis and renal transplant population. Prospective interventional studies are needed in this population to identify subsets of patients who will benefit most and also to assess long term toxicity of statins. KDIGO recommendations provide general principles regarding treatment of dyslipidemia but it should be individualized for each patient.

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MINIREVIEWS

Increasing the use of biocompatible, glucose-free peritoneal dialysis solutions

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Abstract

A major concern inhibiting some clinicians from embracing peritoneal dialysis (PD) as the preferred first modality of dialysis is the effects of PD solutions on the peritoneal membrane. These anatomical and functional changes predispose to complications like peritonitis, encapsulating peritoneal sclerosis and ultrafiltration failure. In recent years, "biocompatible" and glucose-sparing PD regimens have been developed to minimize damage to the peritoneal membrane. Can the use of these more expensive solutions be justified on current evidence? In this review of the literature, we explore how we may individualize the prescription of biocompatible PD fluid.

Key words: Individualized prescription; Biocompatibility; Peritoneal dialysis; Glucose degradation products; Peritonitis; Ultrafiltration failure; Residual renal function

Core tip: There is increasing evidence of benefit for using biocompatible and non-glucose based peritoneal dialysis (PD) fluids. However, cost remains an impediment and perhaps there are selected groups of patients where the cost can be justified. We suggest that biocompatible solutions should be considered for patients with residual renal function and/or expected to remain on PD for a long period. They are particularly helpful for patients with drain-in pains. The targeting of diabetic patients for non-glucose solutions is intriguing given the recent IMPENDIA/EDEN study although vigilance is required to minimize unaware hypoglycemia. It remains to be seen if PD nephrologists are willing to take the same leap of faith that our hemodialysis (HD) colleagues took when they moved from Acetate-based HD solutions to Bicarbonate dialysate. It is possible that economies of scale will reduce the cost of the biocompatible solutions if we use them more frequently.

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INTRODUCTION

Peritoneal dialysis (PD) has been a popular modality of renal replacement therapy since it was introduced in 1978[1]. In comparison to hemodialysis (HD), PD provides a more gradual and continuous method of fluid and solute clearance, with improved preservation of residual renal function and minimal cardiac stress. PD is at least equivalent in terms of survival benefits in the initial phase of dialysis vintage[2]. Furthermore, PD is more cost effective than HD, especially when reduced erythropoietin stimulatory agent requirement and patient transport cost savings are considered[3].

Common complications of PD include peritonitis, technique and ultrafiltration failure. It has been proposed that newer "biocompatible" and "non-glucose" containing PD fluids can reduce these complications[4]. However, these newer PD solutions are more expensive, and the potential cost advantage of PD over HD may be attenuated. We have reviewed the literature to determine if the additional cost of these newer solutions can be offset by reducing complication rates.

It is generally accepted that conventional PD fluids alter the functional and anatomical integrity of the peritoneal membrane over time[5,6]. Glucose degradation products (GDPs), high lactate and low pH levels have been implicated in the pathogenesis of adverse dynamic changes in the peritoneal membrane[7], which then predispose to complications like peritonitis, technique failure, *etc*[8].

Biocompatible PD fluids are produced in multi-compartmented bags that separately store the acidic glucose solution and the bicarbonate buffer solution. This allows the glucose component to be heat sterilized at a low pH thus causing minimal or no caramelization and GDP generation[9]. At the point of use, the acidic glucose compartment is mixed together with the buffer solution to produce a more physiological pH solution, with minimal lactate and GDP concentrations.

ALTERNATIVES TO GLUCOSE AS OSMOTIC AGENTS

Glucose remains a popular osmotic agent in conventional PD solutions due to its low cost, relative safety and effectiveness. Increasing glucose concentration allows for greater ultrafiltration due to the larger osmotic gradient. However, increasing glucose concentrations also means increased glucose absorption, which may result in metabolic abnormalities like hyperglycemia, hyperinsulinemia, obesity and hyperlipidemia[10]. Non-glucose based osmotic agents such as icodextrin (used in Extraneal solution) and amino acids (used in Nutrineal solution) are often used in glucose-sparing regimens to reduce the metabolic impact of glucose absorption. The icodextrin molecule is large sized and does not cross the membrane easily, thus producing a prolonged osmotic gradient and sustained ultrafiltration. The enhanced ultrafiltration achieved with Extraneal results in better fluid balance with improved blood pressure control[11], and a reduction in left ventricular mass[12].

Nutrineal is an amino acid based PD solution which is generally considered equivalent to a 1.5% glucose bag with respect to osmotic power. Although the pH of the solution is 5.5 (low), it contains no glucose and hence is considered biocompatible. No study has shown any mortality benefit with this solution but improvements in nutritional parameters like albumin, transferrin and protein catabolic rate has been observed in some malnourished PD patients[13,14]. Both these non-glucose based PD solutions are licensed to be used once a day.

COST OF BIOCOMPATIBLE PD SOLUTIONS

Table 1 illustrates the cost difference between the various PD solutions. For convenience sake we have included the trade name of the PD fluids most commonly used in the United Kingdom. The catalogue prices of the non-conventional solutions are approximately 50% more expensive than the conventional ones. In the United Kingdom, based on these catalogue prices, continuous ambulatory PD compromising of daily 4 exchanges (CAPD × 4) of Dianeal would cost £5650/year, but × 2 Physioneal, Nutrineal, Extraneal would cost £10860/year. The incremental cost of switching a patient on automated PD from Dianeal to biocompatible glucose sparing regimen is similar. The cost incurred using 4 cycles of Dianeal (1.5%) overnight followed by last fill Dianeal (2.5%) is estimated to be £9420/year. A switch to 3 cycles of Physioneal, 1 cycle of Nutrineal and last fill Extraneal would cost an extra £5000/year (Table 2).

When extrapolating to a PD program of 150 patients the additional cost of prescribing biocompatible, glucose sparing regimen equates to £0.75 M/year. This calculation is somewhat spurious as it is based the on United Kingdom catalog prices which is not the actual price charged to the National Health Service. Nevertheless, as a comparator, the annual salary of a Band 6 nurse in United Kingdom ranges between £25700 to £34500. These figures present a significant dilemma as the same PD program could possibly employ 20 additional fully trained nurses at equivalent cost of changing to glucose sparing biocompatible fluids.

Table 1 Catalog prices of different peritoneal solutions

	United Kingdom (£)		Singapore ($)		Pakistan (Rs)	
CAPD fluid						
Conventional CAPD 2 litre bag						
Dianeal (1.5%)	3.87		10.66		774	
Staysafe (1.5%)	4.24		10.98		812	
Biocompatible CAPD 2 litre bag	£	Increment (%)	Sing $	Increment (%)	Rs	Increment (%)
Physioneal	7.32	89	12.5	17	1464	89
Nutrineal	8.5	120	14	31	1785	131
Extraneal	6.6	70	12.3	16	1200	55
Balance	4.63	9	12.1	10	1020	26
Automated PD Fluid						
Conventional APD 5 litre bag						
Dianeal (1.5%)	8.6		28		1400	
Sleepsafe (1.5%)	7.8		28		1450	
Biocompatible APD 5 litre bag		Increment (%)		Increment (%)		Increment (%)
Physioneal	12.2	42	39	39	2200	57
Sleep balance	12.5	60	40.5	45	2350	62

Source: Fresenius Dialysis Product Catalogue 2013 revised (United Kingdom, Singapore and South Asia); Baxter PD Product List 2014 (United Kingdom, Singapore and Pakistan). CAPD: Continuous ambulatory peritoneal dialysis; APD: Automated peritoneal dialysis; PD: Peritoneal dialysis.

Table 2 Estimated annual cost of peritoneal dialysis fluids based on United Kingdom catalog prices

	United Kingdom (£)	Increment (%)
CAPD		
Dianeal (1.5%) × 4	5650	-
2 × Dianeal, Nutrineal, Extraneal	8340	48
2 × Physioneal, Extraneal, Nutrineal	10860	92
APD		
Dianeal: 1.5% (× 4 cycles) with last fill of 2.5%	9420	-
Dianeal, Nutrineal, Extraneal: (× 3 cycles 1.5%, 1 cycle Nutrineal) with last fill Extraneal	11790	25
Physioneal, Nutrineal, Extraneal(× 3 cycles 1.5%, 1 cycle Nutrineal) with last fill Extraneal	14420	53

CAPD: Continuous ambulatory peritoneal dialysis; APD: Automated peritoneal dialysis.

EVIDENCE OF BENEFIT AND USE OF BIOCOMPATIBLE PD SOLUTIONS

Faced with the reality of current financial constraints can we individualise the use of biocompatible PD fluids?

The balANZ trial[15] was a large well conducted RCT exploring the clinical benefits of biocompatible solutions. Using biocompatible fluids, a significant 33% reduction in peritonitis rates was achieved although other studies have not yielded similar results. We have to consider if employing additional nurses would be more cost effective than biocompatible solutions in reducing peritonitis rates[16].

The balANZ study also suggested that biocompatible solutions may better preserve residual renal function (RRF). Although the primary end point did not reach statistical significance, the rate of decline of RRF was lower in the biocompatible PD fluid arm and time to anuria which was a secondary end-point did reach statistical significance. The importance of delaying onset of anuria should not be underestimated and would support using these more expensive solutions in patients with residual renal function.

One of the strongest drivers for the use of biocompatible solutions is the hope that PD membrane will be preserved, thereby delaying PD technique failure and reducing the development of encapsulating peritoneal sclerosis (EPS). Dialysate concentration of Cancer Antigen 125 (CA-125) is proposed to be an indicator of peritoneal mesothelial cell health[17]. There is evidence to suggest that biocompatible solutions preserve CA-125 levels, implying that they might prevent peritoneal membrane damage induced by the bioincompatible nature of the PD solutions[18,19]. Those most at risk of EPS may benefit from using biocompatible solutions. The incidence of EPS complication increases with time on PD[20]. There is consensus that EPS is very rare in people who were on PD for less than 3-4 years. The Pan-Thames EPS study[21] showed that more than 70% of the patients who developed EPS had a PD vintage of more than 5 years. If one is to use biocompatible solutions to reduce EPS risk, it should be prescribed at outset of PD. One might argue that elderly patients with high co-morbidity and short life-expectancy are unlikely to develop this complication. Perhaps more controversially, young patients with good match prognosis

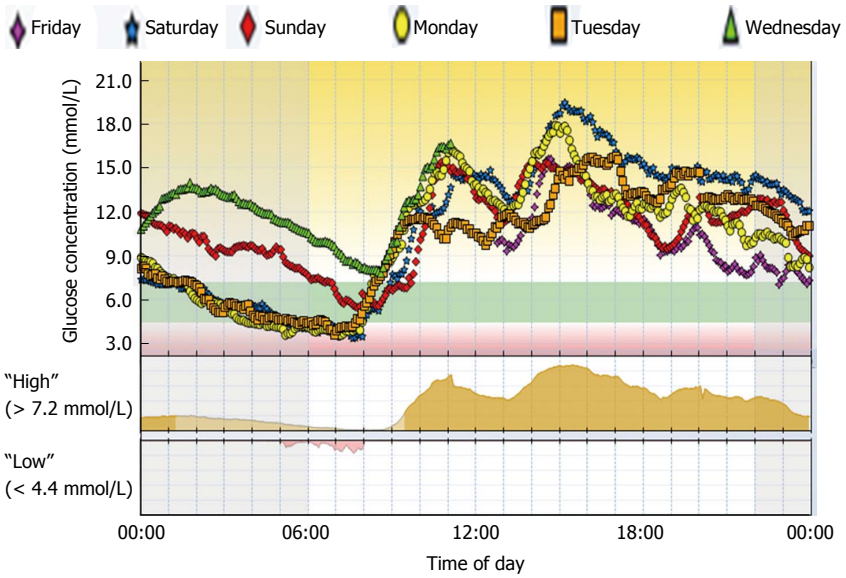

Figure 1 Continuous Glucose monitoring (6 d) of a Diabetic patient on peritoneal dialysis using Extraneal at night (22:00 to 06:00) not only showing hyperglycemia during the day (after 10 am) when glucose peritoneal dialysis solutions used, but also showing significant and regular episodes of hypoglycemia (unaware) suffered by the patient overnight. Continuous Glucose Monitoring demonstrates the merits and risk of using non-glucose based PD solutions (Extraneal). On one hand the overnight Extraneal dwell (from 22:00 to 06:00 h the next day) controlled the blood sugar effectively in comparison to the glucose based PD fluid dwell (from 06:00 till 22:00 h the same day). On the other hand Extraneal is putting the patient at risk of hypoglycemia (between 05:00 and 08:00 h). It is noteworthy that diabetic end stage renal disease patients have an increase incidence of hypoglycemia unawareness. PD: Peritoneal dialysis.

index for transplantation (especially patients with live donors) are also less likely to remain on PD long enough to develop EPS.

Infusion pain with PD fluids is known to affect treatment compliance and quality of life[22]. This pain is ascribed to the low pH of conventional PD solutions and the use of biocompatible PD fluids instead has shown to alleviate this discomfort in a randomized controlled trial[23].

GLUCOSE BASED *VS* NON-GLUCOSE BASED PD FLUIDS

The use of hypertonic 3.86%-glucose bags appears to precede the development of ultrafiltration failure (UF) and impaired osmotic conductance which are important predictors of PD technique failure and EPS[24]. Replacing 3.86% hypertonic solutions with Extraneal would be a reasonable strategy. The role of icodextrin for patients who have high transport characteristics exhibiting UF failure is well established, and recommended in the International Society of Peritoneal Dilaysis guidelines. However, it is not clear if reducing glucose exposure further by substituting Nutrineal for 1.36% glucose solutions will have clinically significant effects on peritoneal membrane preservation. Whilst inadequate solute clearance and ultrafiltration failure are undoubted causes of PD technique failure, patient and carer "burn out" is probably equally important. In this situation, biocompatible solutions will not help but diverting resources to providing more nursing support may be more effective in helping such patients continue on PD.

There are other obvious reasons for minimizing glucose load in the PD solution. Li *et al*[25] (on behalf of the IMPENDIA and EDEN study groups) reported a significant improvement in glycemic and lipid control with the use of glucose sparing PD fluids in the diabetic population. Could better glycaemic control have been achieved through more meticulous diabetic treatment if the additional resources were devoted to providing a comprehensive diabetic service? We suggest an additional caveat: not only should we be concerned about hyperglycaemia but hypoglycemia unawareness might be more dangerous leading to cardiac instability (an association between unaware hypoglycaemia and prolonged electrocardiogram QT-dispersion has been found in non-dialysis patients[26]). Hypoglycaemia unawareness is certainly something that we have found in diabetic patients that undergo routine continuous glucose monitoring. Figure 1 provides an example of diurnal hourly variations in interstitial glucose concentrations in a diabetic patient using nocturnal icodextrin to minimize overnight glucose exposure.

CONCLUSION

It is very ironic to note that HD faced a similar dilemma when a transition from acetate to bicarbonate buffered dialysate was proposed. Prescribing bicarbonate dialysate was equally controversial as it was more expensive and generally all the supportive data came from *in vitro* studies while *in vivo* studies provided very

little support. Nevertheless, a calculated rational leap of faith was taken and over time bicarbonate buffered HD dialysate has become cost-effective. Furthermore, the superiority of bicarbonate over acetate-based buffer was demonstrated during this time. Although we strongly believe in the potential benefits of PD biocompatible fluids, we acknowledge the pragmatic hesitancy of our colleagues due to associated high premium costs. In such a stalemate situation an approach to individualizing the prescription of biocompatible PD solutions is sensible. There is evidence to support its use in selected patients groups such as those with residual renal function with good life expectancy or patients with drain-in pain. The use of non-glucose PD solutions to improve diabetic control is perhaps more controversial but one hopes that cost will fall as uptake of these solutions increase. We are quite hopeful that in the imminent future the story of biocompatible PD fluids will have a similar conclusion to that of the bicarbonate buffered dialysate in HD.

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Role of β2-microglobulin in uremic patients may be greater than originally suspected

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Abstract

The role of beta2-microglobulin (β2M) in dialysis-related amyloidosis as a specific amyloid precursor was defined in the 1980s. Studies in those years were largely related to β2M amyloidosis. In 2005, for what was probably the first time in the available literature, we provided data about the association between β2M and early-onset atherosclerosis in hemodialysis patients without co-morbidities. In recent years, the role of uremic toxins in uremic atherosclerosis and the interest in β2M as a marker of cardiovascular (CV) and/or mortality risk have grown. In the current literature, clinical studies suggest that β2M is an independent, significant predictor of mortality, not only in dialysis patients, but also in predialysis patients and in the high-risk portion of the general population, and it seems to be a factor strongly linked to the presence and severity of CV disease. It is still unknown whether β2M is only a uremic toxin marker or if it also has an active role in vascular damage, but data support that it may reflect an increased burden of systemic atherosclerosis in a setting of underlying chronic kidney disease. Thus, although there have been some inconsistencies among the various analyses relating to β2M, it promises to be a novel risk marker of kidney function in the awareness and detection of high-risk patients. However, more research is required to establish the pathophysiological relationships between retained uremic toxins and further biochemical modifications in the uremic milieu to get answers to the questions of why and how. In this review, the recent literature about the changing role of β2M in uremic patients will be examined.

Key words: Beta2-microglobulin; Carotid atherosclerosis; Cardiovascular disease; Cardiovascular risk; Coronary artery disease; Hemodialysis; Mortality; Uremia; Uremic toxins

Core tip: Previously, the clinical significance of beta2-microglobulin (β2M) in uremic patients was limited to β2M-derived amyloidosis; in recent years, its role and power has changed and expanded. Although there have been some inconsistencies among the various analyses relating to β2M, the data generally support β2M as a promising novel marker of kidney function by predicting cardiovascular (CV) risk, CV events and overall mortality.

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INTRODUCTION

Beta2-microglobulin (β_2M) forms the non-variable light chain of the Class 1 major histocompatibility complex (MHC). It is found on the surfaces of nearly all nucleated cells. It is a non-glycosylated polypeptide with a molecular weight of 11.729Da[1,2]. Due to its small size, β_2M is present in the glomerular filtrate of the normal kidney. It was originally discovered as a component present in the urine of patients with tubular proteinuria. It has been estimated that there are 10^5-10^6 β_2M molecules/cell in human lymphocytes. When MHC is degraded, the MHC-associated β_2M is released into circulation[2-4]. This results in a constant production of free β_2M at a level of 0.13 mg/h × body weight in kilograms under normal conditions[2]. β_2M is found in low concentrations as a conformationally less restricted free monomer in blood and other biofluids, including synovial fluid. The normal physiological function, if any, of the freely circulating β_2M is unknown[2]. The β_2M synthesis rate in healthy individuals ranges from 2 mg/kg per day to 4 mg/kg per day, with a half-life of 2.5 h, and plasma concentrations vary between 1 mg/mL and 3 mg/mL[5].

Elevated β_2M levels are observed in chronic renal failures, lymphoproliferative disorders, inflammations, infections and other conditions as well, with high cell turnover[2]. A relationship has been noted between tumor burden and β_2M[6]. Concerning its use in oncology, β_2M levels correlate with the disease stage and poorer prognosis in patients with multiple myeloma and chronic lymphocytic leukemia[6,7]. It is also the most important predictor of treatment-free survival and overall survival of patients affected by lymphocytic leukemia and in most cases of lymphatic neoplasia. Additionally, serum β_2M levels can help to predict outcome in patients > or = 60 years with untreated acute myeloid leukemia[6,8]. Therefore, it is emphasized that β_2M may be the subject of future target therapy in cancer research[9].

Given that β_2M elimination is achieved *via* glomerular filtration, it is not surprising that plasma levels are inversely related to the glomerular filtration rate. Levels can be elevated as much as 60-fold in anuric patients with end-stage renal disease[5]. β_2M is a well-known, frequently studied representative marker of middle molecule uremic toxins and its role in dialysis-related amyloidosis as a specific amyloid precursor was defined in the 1980s[10]. Previous studies have largely been related to β_2M amyloidosis. However, its relationship with vascular risk was not identified until 2005. That year, to our knowledge for the first time in the available literature, we provided data on this relationship. We showed that, besides well-known cardiovascular (CV) risk factors, β_2M levels were independently related to carotid artery intima media thickness (C-IMT) in non-diabetic hemodialysis (HD) patients who had no clinical evidence of atherosclerosis[11]. Since then, the number of studies concerning this relationship has increased and now β_2M's direct pathophysiological role in vascular disease and its power as a predictor of overall and CV events are more predominant in the literature. In this review, the recent literature about the changing role of β_2M in uremic patients will be examined.

β_2M-DERIVED AMYLOID

In long-term dialysis patients, retention of β_2M produces a disease related to the deposition of β_2M amyloid fibrils around large joints such as shoulders and hips. In 1980, Assenat *et al*[12] were the first to report finding amyloid in the material they had excised from their patients' carpal tunnels and in 1985, Gejyo *et al*[13] established a novel type of amyloidosis in patients undergoing HD. Initially, it was believed to occur only in patients on chronic HD; therefore, it was called "dialysis-associated amyloidosis". However, it soon became clear that amyloidosis could develop in patients on any type of renal replacement therapy and even in uremic and predialysis patients. Therefore, it came to be referred to as β_2M-derived amyloid or in line with general amyloid terminology, as A β_2M-amyloid[5].

Pathogenesis

The exact mechanism of amyloidogenesis in dialysis patients remains unclear; however, elevation of circulating β_2M levels may not be the only cause of β_2M-derived amyloid. Recent studies have emphasized that in addition to substrate retention, biochemical modification of the β_2M molecule (such as oxidative modification) in the uremic milieu may potentiate its pathogenicity[14]. Glycosylated β_2M, a modified microglobulin, has been found in amyloid deposits as advanced glycation end products. This may further enhance the development of the lesions by both stimulating the secretion of cytokines and acting as chemoattractant and an apoptosis-delaying agent for monocytes. However, it remains unknown whether this modification plays an active role or is merely a long-term transformation of long-lived amyloid fibrils[14-17].

One of the other pathogenetic concepts in β_2M-derived amyloidosis is limited proteolysis and partial breakdown of native β_2M[5]. In recent years, a cleavage product form of β_2M that has a deletion of lysine at position 58 on the molecule ΔK58-β_2M and behaves differently from normal β_2M has been demonstrated in the sera of 20%-40% of dialysis patients[18]. Although it is conformationally unstable and amyloidogenic *in vitro*, it was suggested that it could play a role in β_2M amyloid fibrillogenesis[19]. However, it was not detected by 2D electrophoresis in the *ex vivo* amyloid fibrils of

two patients affected by dialysis-related amyloidosis[20]. Based on this result, the authors concluded that the process of amyloid deposition "in a target tissue requires that the fibrillogenic protein attains the amyloidogenic conformation at the right site, at the right time, and at the right concentration". They further speculated that ΔK58-β2M might be more susceptible to degradation than to amyloid deposition. In contrast, a N-terminal truncated species lacking six residues, ΔN6-β2M which was highly amyloidogenic *in vitro* was not detectable in plasma[20]. The list of factors that have been shown to influence the conformation of intact β2M is very long. However, many of the *in vitro* conditions that are highly favorable for amyloid formation from normal β2M are not encountered *in vivo* because of the possibility that several factors may interplay in different ways *in vivo*[5].

Clinical manifestations/diagnosis

This is a systemic type of amyloidosis but clinical manifestations of the disease are largely confined to the musculoskeletal system with carpal tunnel syndrome, spondyloarthropathies, hemarthrosis, joint pain and immobility. Late in the course of the disease, systemic deposition can occur, principally in the gastrointestinal tract and heart. Ninety percent of the patients may have the disease pathologically but not manifest clinical symptoms. Additionally, clinical symptoms are often nonspecific and easily mistaken for other articular disorders. The manifestations of β2M appear gradually over the course of years, between two and ten years after the start of dialysis in the majority of patients[2,5,21].

There are some suggestive findings, but no pathognomonic clinical or radiological findings exist in β2M amyloidosis. The gold standard diagnostic technique to demonstrate positive Congo Red staining and the presence of β2M is biopsy. Diagnostic material usually has to be obtained from synovial membranes or bone lesions[5,21].

Treatment

There have been many studies addressing the effects of different dialysis membranes on serum β2M levels. Generally, it is recommended that non-cuprophane, high-flux dialyers should be used for patients with evidence of or at risk for β2M amyloidosis[21]. High-volume hemodiafiltration and ultrapure dialysate were also reported to be associated with increased β2M removal, lower serum concentrations and reduced inflammation[21-23]. However, dialysis of any kind or with any membrane is incapable of removing sufficient quantities of β2M to completely prevent the deposition of amyloid and, with the exception of kidney transplantation, no currently available therapy can stop the disease progression of β2M amyloidosis or provide symptomatic relief[21].

β2M AND CARDIOVASCULAR RISK

In recent years, progressively more studies have been conducted with the aim of showing the involvement of uremic toxins and endothelial dysfunction in several aspects of uremic atherosclerosis[24]. Related to this, interest has grown in β2M as a marker of kidney function and CV risk.

Pathogenesis

The role of serum β2M in the pathogenesis of CV disease is still not clearly known. However, there have been some suggestions. For example, β2M appears to damage vessels by participating in amyloid formation in the vascular wall[25]. Also, retained uremic solutes, such as β2M advanced glycosylated end products which have been substrates for oxidative injury, seem to further contribute to the proatherogenic milieu of uremia[14]. Additionally, it has been demonstrated that some uremic toxins inhibit endothelial proliferation and wound repair in uremic patients[26]. In the presence of uremic serum, endothelial progenitor cells, which contribute to vessel repair and neovascularization, undergo a decrease in their ability to migrate[27]. The influence of the uremic milieu was confirmed by the observation that high serum levels of β2M and indole-3-acetic acid were associated with low numbers of circulating CD34+CD133+ endothelial progenitor cells[28]. Another study investigated whether β2M was proinflammatory by inducing oxidative burst in leukocytes; β2M was not found to be a factor for induction of leukocyte free radical production[29]. However, the involvement of β2M in the inflammatory process and its association with vascular risk is still an area of interest deserving attention.

Carotid atherosclerosis

In 2005, we investigated the associations of different risk factors with C-IMT, which had been an early marker of atherosclerosis, in "healthy" non-diabetic HD patients who had no clinical evidence of atherosclerosis[11]. In multivariate regression analysis, age, β2M, C-reactive protein and left ventricular hypertrophy were independently related to C-IMT. Elevated levels of β2M were found to be correlated not with the inflammatory markers but with the time patients had been in a uremic state. As we explained, although elevated plasma β2M was a well-known characteristic of chronic renal failure, that correlation may be just an epiphenomenon rather than a causal relationship, or β2M levels may indirectly influence uremia-related CV risk factors, or β2M *per se* may contribute to atherogenesis. As these were probably the first data about the importance of β2M as a CV risk factor in uremic patients, our findings necessitated confirmation in additional, larger scale studies. In 2006, using the same patient group, we assessed the determinants of the progression of C-IMT over the course of one year[30].

As in our former study, β2M was independently related to C-IMT at baseline; however, age and sex were the only independent predictors of the progression in C-IMT from baseline to the 12 mo stage. Subsequent studies in the general population showed that β2M was independently and significantly associated with total mortality and adverse CV outcome in patients with prevalent asymptomatic carotid atherosclerosis.

Peripheral arterial disease

In 2007, Wilson *et al*[25] researched patients in the general population with and without peripheral arterial disease (PAD) and analyzed their plasma. The peak intensity of a 12 kDa protein was higher in patients with PAD. Western blot analyses and immunoaffinity studies confirmed that that protein was β2M and circulating β2M in PAD patients was elevated and correlated with the severity of the disease. Another study found no relationship between β2M and the augmentation index, either in patients with PAD or in healthy subjects. However, it did demonstrate that among patients with PAD, elevated plasma β2M levels were associated with higher aortic stiffness irrespective of CV disease risk factors[31]. Subsequent studies did not support this association between β2M and PAD[32]. Additionally, no changes were found in β2M levels in PAD patients after exercise on a treadmill, thus challenging the initial hypothesis by Wilson *et al*[25] of an increase in β2M levels in patients with PAD due to repeated bouts of ischemia-reperfusion[33]. Although the conflicting results mostly pointed to a non-specific elevation of β2M in patients with a high vascular risk, it was concluded that β2M levels may not indicate the presence of PAD, but may instead reflect an increased burden of systemic atherosclerosis in a setting of underlying chronic kidney disease (CKD)[31,32,34].

Coronary artery disease

In 2007, we evaluated the determinants of coronary artery disease (CAD) other than conventional risk factors in nondiabetic HD patients[35]. Patients with CAD were compared to those without and, although β2M levels were higher in CAD patients (5.4 ± 1.4 mg/d *vs* 4.8 ± 1.5 mg/dL), the difference between the groups was not found to be statistically significant. The association between CV risk markers and arterial calcification in patients with CKD at Stages 3 and 4 had only recently been studied and β2M was found to be associated with coronary artery calcification beyond some other inflammatory biomarkers[36]. In addition, β2M, along with cystatin C and C-reactive protein, were found to predict mortality and improve risk classification and discrimination for a high-risk cohort undergoing coronary angiography[37].

Acute heart failure

In a study evaluating the prognostic role of serum β2M in heart failure, patients with severe renal dysfunction were excluded and a higher baseline serum β2M concentration was found to be the most powerful predictor of cardiac events and cardiac mortality in acute heart failure patients with creatinine ≤ 3.0 mg/dL. Furthermore, the baseline serum β2M concentration had a superior ability to distinguish cardiac event risk in acute heart failure patients compared with creatinine-based renal parameters[38].

Left atrial size

A linear correlation was found between the circulating levels of β2M and cystatin C and left atrial diameters. Additionally, left atrial diameters were negatively related to creatinine clearance in two study groups, one with CAD and the other without[39].

Arterial stiffness

Arterial stiffness occurs due to loss of compliance of the vascular wall. It is a prominent feature of vascular ageing and strongly predicts CV and total mortality. β2M has been shown to be related to arterial stiffness in the general population[40]. In HD patients, β2M levels were found to be positively associated with pulse pressure, which is a result of arterial stiffness. Additionally, β2M levels were positively associated with insulin resistance[41].

β2M AND ALL-CAUSE AND CARDIOVASCULAR MORTALITY

The HEMO study on 1704 HD patients showed that the predialysis serum β2M predicted mortality. After making statistical adjustments for the number of years on dialysis and for residual kidney function, for every 10 mg/L increase in the β2M level, there was a corresponding increase of 11% in mortality. The specific causes of death that account for this increased mortality have not been determined[42]. Another study evaluated the association of β2M levels in 490 HD patients with their clinical outcomes by dividing them into two groups according to their serum β2M levels[43]. Mortality from all causes in the higher β2M group was found to be significantly higher compared to that in the lower β2M group. These results demonstrated that serum β2M was a significant predictor of mortality in HD patients, independent of HD duration, diabetes, malnutrition and chronic inflammation[43].

The impact of β2M was studied in patients with CKD at different stages not yet on dialysis[44]. Baseline β2M levels were associated with vascular calcification but not with arterial stiffness. Higher β2M levels were independently associated with overall and CV mortality, with CV events in the whole cohort, and with CV events in the predialysis cohort. Furthermore, serum β2M was identified as an independent predictor of all-cause mortality in a population-based sample of older adults. Also, β2M was identified as a novel risk marker for adverse CV outcomes in patients with carotid

atherosclerosis[45].

β2M and infectious mortality in hemodialysis patients

The HEMO Study Group examined the association of serum β2M levels and dialyzer β2M kinetics with cause-specific mortality. They focused on cardiac and infectious diseases which were the most common causes of death. There was no statistically significant association in that study between cumulative mean predialysis serum β2M levels and cardiac mortality. However, in the entire cohort, each 10 mg/L increase in serum β2M level was associated with a 21% increase in the rate of infectious mortality[46].

β2M and mortality and graft loss

The association between post-transplant serum β2M and the outcomes following kidney transplantation were investigated. Serum β2M at discharge was a potent predictor of long-term mortality and of graft loss in kidney transplant recipients, providing information on the allograft function beyond that of serum creatinine[47].

ENCAPSULATING PERITONEAL SCLEROSIS

This is a serious complication in peritoneal dialysis patients. β2M was found to be a useful screening test for the onset of encapsulating peritoneal sclerosis and β2M and the accumulation of middle-molecular uremic toxins were thought to be related to the pathophysiology of this disease[48]. Recently, the accumulation of advanced glycation end products and β2M in the fibrotic thickening of the peritoneum in long-term peritoneal dialysis patients was investigated. The proportion of β2M-expressing areas was found to be elevated in long-term peritoneal dialysis patients, which may be a marker of peritoneal injury[49].

β2M AS A NOVEL MARKER OF KIDNEY FUNCTION AND RISK PREDICTION

Recently, there have been studies which evaluated whether novel biomarkers could add any information to improve risk prediction in patients at moderate and high risk. Data have provided that β2M, cystatin C and C-reactive protein predict mortality and improve risk classification and discrimination for a high-risk cohort. β2M and, to a lesser extent, beta trace protein, shared cystatin C's advantage over serum creatinine-based estimated GFR in predicting outcomes, including kidney failure. Thus, β2M shows promise as a novel filtration marker of kidney function for risk prediction of all-cause and CV mortality[50-52].

CONCLUSION

Previously, the clinical significance of β2M in uremic patients was limited to β2M-derived amyloidosis; in recent years, its role and power have changed and expanded. Although there were some inconsistencies among the various analyses relating β2M to clinical outcomes, the data generally support β2M as a promising novel marker of kidney function by predicting CV risk, CV events and overall mortality. The exact role β2M plays in CV events and why it predicts CV and high risk of morbidity and mortality is still unclear. Further studies are needed to clarify the role of β2M in uremic patients.

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Review on renal recovery after anatrophic nephrolithotomy: Are we really healing our patients?

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Abstract

The main goals for urinary stone treatment are to preserve renal function, reduce or avoid complications related to calculi, and to render the patient free of calculi as soon as possible. Anatrophic nephrolithotomy (ANL) is a valid and useful alternative for conventional staghorn calculi excision. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of them. Another, great concern is the possibility of reduction on renal function related to the procedure itself. This may be related to nephron injury during nephrotomy and parenchymal closure or to ischemic injury. In this review we assess functional results after anatrophic nephrolithotomy.

Key words: Anatrophic nephrolithotomy; Kidney lithiasis; Kidney stone disease; Percutaneous nephrolithotripsy; Staghorn calculus

Core tip: Anatrophic nephrolithotomy (ANL) is a valid and useful alternative for conventional staghorn calculi excision. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of them. Another, great concern is the possibility of reduction on renal function related to the procedure itself. In this review we assess functional results after anatrophic nephrolithotomy.

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INTRODUCTION

The main goals for urinary stone treatment are to preserve renal function, reduce or avoid complications related to calculi, and to render the patient free of calculi

as soon as possible. Procedures with low morbidity and rapid recovery are also essential in current practice. Guidelines from American Urological Association and European Urology Association state that conventional excision of staghorn stones must be considered only in exceptional cases and that percutaneous nephrolithtomy (PNL) should be the preferred choice[1,2].

The definition of "staghorn calculus" is related to the calculation that fills at least one caliceal group and, mandatorily, the pelvis. If the calculus fills the renal pelvis but not all the caliceal groups, it is recognized as a "partial staghorn calculus". However, if this kidney stone occupies the renal pelvis and at least three quarters of the pyelocaliceal system, it is labeled as "complete staghorn stone". Computed Tomography based morphometric studies may help classify and predict outcomes for staghorn calculus treatment[3,4], nevertheless, it is implicit that the greater the stone more difficult it is to leave the patient without remaining calculi in the collecting system. Several authors showed the relation between stone size and stone clearance. In a recent study, el-Nahas *et al*[5] showed that the stone-free rate for percutaneous nephrolithotomy as monotherapy was 56% and complete staghorn calculus was an independent risk factor for residual stones[5].

Undoubtedly, the main reason for conventional surgery rates decrease is the improvement of techniques such as extracorporeal shockwave lithotripsy (SWL) and endourological procedures (ureteroscopy and PNL)[6-9]. Yet, even with such technological developments, some special conditions are still best handled with conventional surgery, such as complex collecting system anatomy, extremely large stones, extremely poor function of the affected renal unit, or excessive morbid obesity.

Anatrophic nephrolithotomy (ANL) is one of the most used option for conventional staghorn calculus removal. Smith *et al*[10] described the anatrophic nephrotomy and plastic calyrhaphy a procedure in which stone removal and correction of collecting system anomalies was possible. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of then. Another, great concern is the possibility of reduction on renal function related to the procedure itself. This may be related to nephron injury during nephrotomy and parenchymal closure or to ischemic injury. In this review we assess functional results after anatrophic nephrolithotomy.

ANATROPHIC NEPHROLITHOTOMY PROCEDURE

Smith *et al*[10] identified some factors that may contribute for perpetuating renal inflammatory process after stone surgery: poor drainage, renal parenchymal damage, failure to control infection and inadequate removal of calculi. In order to control those issues and to preserve the maximal number of functional nephrons they described the anatrophic nephrotomy and calyrhaphy. The main steps in this procedure are: control of the main renal artery and obstruction of the posterior segment of renal artery, endovenous infusion of methylene blue to highlight the Brödel's white line, obstruction of the renal artery common trunk and creation of the condition of hypothermic ischemia, nephrotomy along the anterior border of the posterior calyces (approximately 0.5 to 1 cm posterior to Brödel's white line), calculus extraction, reconstruction of the pyelocaliceal system, and closure of the renal capsule[10]. The first 100 consecutive cases using this technique were published by Boyce *et al*[11] and showed 95% stone-free rate. Serum urea nitrogen obtained to assess renal function and serum creatinine has improved or remained stable in all but 2 patients. Other authors also published their results regarding renal function. Thomas *et al*[12] used 131 I hippuran scanning to assess renal function of thirteen patients operated on with classic ANL with a mean follow up of 13.6 mo. Thirteen percent decrease in renal function of the kidneys undergoing ANL surgery was reported. Nonetheless, total renal function assessed by effective renal plasma flow level remained normal in the postoperative stage. Compensatory hypertrophy may explain the unchanged total renal function as a 13% increase in the contralateral kidney was reported.

Studies in patients with solitary kidney may help to understand changes in renal function without the compensatory effect of the contralateral kidney. With a mean follow-up of 6 years, patients with solitary kidneys operated on with classic ANL were evaluated by Stubbs *et al*[13] and associates. No changes in pre- and post-operative serum creatinine was observed. However, creatinine clearance showed a small increase from 52 to 55 mL/min, but it was not statistically significant.

MODIFIED ANATROPHIC NEPHROLITHOTOMY

Several modifications of the classical approach have been described usually without defining the intersegmental plane[14-19]. Kijvkai *et al*[18] compared standard ANL and modified ANL and concluded that the standard procedure preserved more renal function than the modified[18]. Table 1 describes results of modified ANL in regard to renal function assed by scintigraphy.

In 2003, Kaouk *et al*[20] studied laparoscopic ANL for the management of staghorn renal stone in pigs[20]. After injecting polyurethane in the pyelocaliceal system to create a staghorn calculus model the animals were submitted laparoscopic nephrolithotomy. Glomerular filtration rate (GFR) was assessed before and four to five weeks later with diethylene triamine pentaacetic acid (DTPA) renal scans. The mean total GFR rised from 26.4 mL/min to 54.8 mL/min. A case series was first reported by Simforoosh and associates in 2008[21]

Table 1 Renal function after modified anatrophic nephrolithotomy

Ref.	*n*	Parameter	Renal function improvement/stabilization	Renal function decrease	Percent reduction
Belis *et al*[15]	13	131-iodine hippuran	100%	0%	-
Morey *et al*[16]	16	DMSA	18.8%	81.2%	4%
Melissourgos *et al*[17]	24	DMSA	62.5%	37.5%	4%
Kijvikai *et al*[18]	15 (7 St/8 Mod)	DTPA	0%	100%	9% St/27, 2% Mod
Ramakrishnan *et al*[19]	26	DMSA	87%	13%	-

DMSA: Dimercaptosuccinic acid; DTPA: 99mTc-diethylenetriaminepentaacetic acid; St: Standard; Mod: Modified.

with an update in 2013[22]. Stone-free rate was 88%. Mean pre-operative serum creatinine level rised from 1.20 mg/dL to 1.31 mg/dL in the postoperative period, but without statistically significant difference. Researcher described a stone-free rate of 63% in eight patients evaluated. Tree patients were submitted to preoperative 99mTc-DTPA renography to asses renal function 3 mo after surgery. Renal function decreased 4%, 12%, and 4% on the operated kidney of each patient.

Robot-assisted laparoscopic ANL (RANL) has also been described. Ghani *et al*[23] tried to replicate the conventional technique with ice-slush hypothermia. Follow-up at 1 mo demonstrated no change in renal function as estimated by creatinine clearance. King *et al*[24] evaluated seven consecutive patients submitted to RANL. Renal function was estimated by the Modification of Diet in Renal Disease study equation. In five of six patients estimated GFR was unchanged and improved in one patient (19 mL/min per 1.73 m^2 preoperative *vs* 25 mL/min per 1.73 m^2 postoperative).

PERCUTANEOUS NEPHROLITHOTOMY *VS* ANATROPHIC NEPHROLITHOTOMY

Several studies have assessed the impact of PNL on renal function[25-32]. Usually there is an immediate decrease on renal function after surgery with return to baseline on long term. Improvement or stabilization of renal function may occur because of better drainage, infection and inflammation resolution after surgery. On the contrary, renal function may decrease because of several injury mechanisms. Patient comorbidities, direct injury by kidney puncture and tract dilation, ischemia, inflammation and fibrosis are some of the possible mechanisms implicated on renal function deterioration.

Wilson *et al*[33] tried to quantify the level of parenchymal injury after stone treatment in an animal study. Percutaneous nephrolithotomy accounted for the largest amount of microscopic lesions, although, it was less than 2% of total renal volume and did not affected total renal function. Moskovitz *et al*[26] evaluated renal units separately and identified a remarkable reduction in the functional volume of the pole that underwent PNL, nevertheless, regional uptake and total renal function remained unchanged[26].

In cases where the amount of calculi is remarkable multiple access tracts may be required during the PNL procedure. It could be expected that the number of access tracts and ancillary procedures used for complete stone clearance could negatively impact on renal function. In regard to multiple tracts, there are few studies that support this hypothesis. El-Tabey *et al*[34] found that multiple punctures were an independent risk factor for renal function deterioration in a cohort of patients with solitary kidney. Hegarty *et al*[35] and Fayad *et al*[36] also noted that multiple tracts carries a risk of adversely affect renal function. Handa *et al*[37], on the other hand, showed that multiple access tracts does not lead to a more severe reduction in renal function[37].

Ancillary procedures such as extracorporeal shock wave lithotripsy (ESWL) and retrograde intrarenal surgery (RIRS) are frequently required for complete clearance of staghorn stones. The number of ancillary procedures to render the patient stone-free may range from 2.1 in partial to 3.7 in complete staghorn stones[1]. Most of the studies addressing PNL and ESWL do not show decrease in renal function[38-41]. Also, combined PNL and RIRS does not seem to adversely impact renal functio[42,43]. Zeng *et al*[43] reported that only 2.7% of patients had renal function deterioration after combined treatment. Nevertheless, the potential deleterious effect of ESWL on kidney structures is well established[44,45] and the combination of PNL may have a greater impact on renal function. In regard to RIRS parenchymal injury is not so evident, even so, more studies with longer follow-up are needed.

Most of the studies shows that renal function is not greatly compromised after PNL (Table 2). Nonetheless, there are no prospective randomized studies specifically comparing PNL and ANL. A well-designed study comparing PNL and open surgery was published by Al-Kohlany *et al*[46]. Eighty-eight renal units were assed, 43 submitted to PNL and 45 to conventional surgery. Modified ANL, extended pyelolithotomy, and combined pyelolithotomy/nephrolithotomy were included. Renal function was assessed with 99mTc-mercaptoacetyltriglycine (MAG3) scans and no significant decline in the operated renal unit was observed, although, results were not segregated by technique. Shen *et al*[47] also compared PNL and open surgery in a prospective randomized study. Renal function was assessed with serum and urinary b2-microglobulin and they found no difference between groups. As in Al-Kohlany *et al*[46]

Table 2 Renal function after percutaneous nephrolithotomy

Ref.	*n*	Follow up	Parameter	Renal function improvement/stabilization	Renal function decrease
Ekelund *et al*[25]	11	14 d	DTPA	73%	27%
Moskovitz *et al*[26]	88	1.5-24 mo	SPECT/DMSA	Total percent uptake unchanged	Decreased functional volume of the treated region
Tok *et al*[27]	711	12-24 h	eGFR	13% improvement in the geriatric group	2% decreased in the non-geriatric group
Kuzgunbay *et al*[28]	16	51.1 mo	Serum creatinine	75%	25%
El-Nahas *et al*[29]	122	12 mo	Tc99m MAG3	91.5%	8.5%
Nouralizadeh *et al*[30]	94	48 h	eGFR	0%	100%
Akman *et al*[31]	272	37.3 mo	eGFR	79.6%	20.4%
Ozden *et al*[32]	69	45.7 mo	eGFR	85%	15%

DTPA: 99mTc-diethylenetriaminepentaacetic acid; SPECT/DMSA: Single photon emission computed tomography; eGFR: Estimated glomerular filtration rate; Tc99m MAG3: Technichium99 metastable Mercaptoacetyltriglycine.

study, results were not segregated by technique.

DISCUSSION

Renal function improvement may occur after stone treatment. Possible mechanisms related to increase in renal function are the relieve in obstruction, resolution of infection and inflammatory process, and compensatory hypertrophy of the remaining tissue[12]. Nevertheless, the stone-extraction procedure may itself negatively compromise the functional condition of the surgically treated kidney. Decreased renal function after percutaneous nephrolithotomy may occur because of parenchymal damage during needle puncture and tract dilation. Ischemic injury may also arise if there is inadvertent injury to major vessels, although, it is not so common.

In regard to anatrophic nephrolithotomy decrease in renal function may occur because of direct injury to parenchymal tissue, leading to a permanent scar at the site of nephrotomy. Another possible mechanism is the ischemia-reperfusion injury related to occlusion of renal artery and vein. Protection measures as ice-slush hypothermia and mannitol have been used, as well as restriction of ischemia time to no longer than 30 min. However, the impact of those measures on renal function are not fully known.

It seems that the type of methodology used to assess renal damage influences the postoperative results. When functional markers are employed, kidney damage is temporary and usually mild. Examples of functional markers are renal plasma flow, GFR, serum creatinine, and estimated GFR. However when cellular damage and morphological assessment are considered, renal damage becomes more evident. In most surgeries postoperative renal function is preserved and even when renal dysfunction is observed, it is usually negligible. Nevertheless, information about long term follow-up is scarce, as well as the the cumulative impact of multiple procedures.

As previously addressed PNL is the standard treatment for staghorn stones. Nevertheless, there are some limitations with this approach. The Clinical Research Office of the Endourological Society (CROES) PNL Global Study and the British Association of Urological Surgeons Section of Endourology have reported the efficacy of PNL for treatment of patients with staghorn stones[48,49]. The CROES study group analyzed outcomes of 1466 patients with staghorn calculi compared with 3869 patients with nonstaghorn stones undergoing PNL. They found that patients with staghorn stones more frequently underwent multiple punctures (16.9% *vs* 5.0%) and had lower complete stone-free rates (56.9% *vs* 82.5%). The United Kingdom study group reported on 299 patients who underwent PNL for staghorn calculi demonstrating an intraoperative complete stone-free rate of 59% and 47% on formal postoperative imagin[49].

When the number of less invasive procedures exceeds what is considered reasonable, we must consider the conventional surgery[1,2]. With the advances in laparoscopic and robotic assisted methods replication of the open technique is possible with less morbidity. The main drawbacks of open surgery as bleeding, longer recovery and morbidity related to flank incision may be overcome with laparoscopic/robotic approach.

Although a definitive conclusion can not be drawn from the available literature in regard to which one is the best approach to treat complete staghorn stone, percutaneous nephrolithotomy still is the first option. Nevertheless, in carefully selected cases anatrophic nephrolithotomy may achieve optimal outcomes.

CONCLUSION

Although parenchymal damage after anatrophic nephrolithotomy is of concern renal dysfunction is usually clinically insignificant. Comparative studies of the available modalities are scarce as well as long term follow-up and the impact of multiple procedures.

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MINIREVIEWS

Appropriate kidney stone size for ureteroscopic lithotripsy: When to switch to a percutaneous approach

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Abstract

Flexible ureteroscopy (fURS) has become a more effective and safer treatment for whole upper urinary tract stones. Percutaneous nephrolithotomy (PNL) is currently the first-line recommended treatment for large kidney stones ⩾ 20 mm and it has an excellent stone-free rate for large kidney stones. However, its invasiveness is not negligible considering its major complication rates. Staged fURS is a practical treatment for such large kidney stones because fURS has a minimal blood transfusion risk, short hospitalization and few restrictions on daily routines. However, as the stone size becomes larger, the stone-free rate decreases, and the number of operations required increases. Therefore, in our opinion, staged fURS is a practical option for kidney stones 20 to 40 mm. Miniaturized PNL combined with fURS should be considered to be a preferred option for stones larger than 40 mm. Moreover, URS is an effective treatment for multiple upper urinary tract stones. Especially for patients with a stone burden < 20 mm, URS is a favorable option that promises a high stone-free rate after a single session either unilaterally or bilaterally. However, for patients with a stone burden ⩾ 20 mm, a staged operation should be considered to achieve stone-free status.

Key words: Ureteroscopy; Lithotripsy; Laser; Kidney calculi; Nephrostomy; Percutaneous

Core tip: Flexible ureteroscopy (fURS) has become a more effective treatment for large and multiple kidney stones. However, as the stone size becomes larger, the stone-free rate decreases, and the number of operations required increases. We herein review the appropriate kidney stone size for ureteroscopic lithotripsy and for situations when it should be combined with percutaneous surgery. In our opinion, staged fURS is a practical option for stones 20 to 40 mm. Miniaturized percutaneous nephrolithotomy combined with fURS should be considered to be a preferred option for stones larger than 40 mm.

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INTRODUCTION

The technical developments and patient requests for rapid stone removal have led to changes in clinical stone management. In the past 30 years, kidney stone treatment has shifted from open surgery to percutaneous surgery, and this has almost been replaced by shock wave lithotripsy (SWL). However, during the last decade, the limitations of SWL for some situations have become evident, and flexible ureteroscopy (fURS) has become more available. The demand for imperative complete stone removal has led to a shift toward endourology. The fURS and related instruments are still evolving. This evolution has made it possible to treat urinary stones in all locations, while decreasing the morbidity associated with the active intervention. However, as the stone size become larger, the stone-free rate of fURS monotherapy decreases, and the number of operations required increases. A percutaneous approach should be considered preferentially for larger stones. In this review, we discuss the possibilities and limitations of ureteroscopic lithotripsy in terms of the kidney stone size and stone number.

INDICATION FOR ACTIVE TREATMENT OF KIDNEY STONES

In general, there is a consensus that small stones may be treated with conservative management. In contrast to ureteral stone, most kidney stones are asymptomatic. It is questionable for small stones, especially in the lower pole, if treatment is required. The natural history and the risk of progression of such kidney stones have not been well evaluated. However, stone growth, potential obstruction, associated infection and pain are clear indications for the treatment of such kidney stones. Several authors have reported a significant rate of incidents during the follow-up of kidney stones (Table 1). For example, Glowacki *et al*[1] have reported that symptomatic events developed in 31.8% of patients, and spontaneous passage occurred in 15.0%, while surgical interventions were required in 16.8%. Burgher *et al*[2] reported that 77% of asymptomatic kidney stones became larger, and 26% required surgical intervention. Hübner *et al*[3] reported that an infection developed in 68% of asymptomatic kidney stones, and 45% had become larger after 7.4 years of follow-up. They also suggested that 83% of kidney stones require surgical intervention within the first five years after the diagnosis. Inci *et al*[4] observed that 33.3% of lower pole kidney stones had become larger within 52.3 mo of follow-up, while only 11% required surgical intervention. In a prospective randomized controlled trial with a 2.2-year of follow-up, Keeley *et al*[5] reported that there is no significant difference between SWL and observation, when they compared with asymptomatic kidney stones < 15 mm regarding the stone-free rate, symptoms, requirement of intervention, and renal function. Although some authors recommended prophylactic treatment for these asymptomatic kidney stones, conflicting data have been reported about the issue[6]. Other indications for active removal of kidney stones are shown in Table 2.

URETEROSCOPIC LITHOTRIPSY FOR LARGE KIDNEY STONES

Percutaneous nephrolithotomy (PNL) is currently the first-line recommended treatment for large kidney stones ⩾ 20 mm[7,8]. PNL yields an excellent stone-free rate for large kidney stones. However, its invasiveness is not negligible due to its considerable major complication rates. The puncture and dilation of a nephrostomy tract, although it is an essential process in PNL, may induce renal parenchymal damage, blood loss, or visceral injury. A recent global study of PNL reported the major complication rates, which included significant bleeding in 7.8%, renal pelvis perforation in 3.4%, and hydrothorax in 1.8%[9]. Blood transfusions were necessary in 5.7% of the patients. Postoperative high-grade fever occurred in 10.5%. The conventional prone position during the surgery may induce the respiratory problems.

Recently, fURS has become an effective treatment for kidney stones throughout all renal calyces. URS is an endoscopic surgery performed through the natural orifice. Thus, renal parenchymal damage is unlikely after URS[10]. The flexible ureteroscopes and their peripheral equipment have rapidly improved over the past few years. URS with holmium laser lithotripsy yields a same or better outcome than SWL for ureteral stones, as well as small kidney stones[7,8]. Some authors have reported the treatment outcomes of fURS for large kidney stones. For example, Grasso *et al*[11] reported their treatment outcomes of 45 patients with kidney stones larger than 20 mm, with a 76% stone-free rate after the first session of fURS. Second sessions were needed in 15 patients, and the stone-free rate increased to 91% without major complications. This primary remarkable result was supported by additional studies with similar findings[10,12-15]. We summarized the results of the updated studies in Table 3. For larger stones (> 20 mm), fURS monotherapy has achieved an excellent stone-free rate, although its outcome depends on the operator's skills and it may require staged procedures.

We previously reported the treatment outcome of large kidney stones (mean cumulative stone diameter: 31 mm) with an overall 90% stone-free rate after a mean 1.4 session of fURS[10]. In particular, we satisfactorily achieved a 100% stone-free rate in a cohort of

Table 1 Natural history of asymptomatic kidney stones

Ref.	Study type	No. of patients	Follow-up	Disease progression (stone growth)	Symptomatic episode	Need for intervention
Glowacki *et al*[1]	Retrospective	107	31.6 mo	NA	31.8%	16.8%
Hübner *et al*[3]	Retrospective	80	7.4 yr	45%	68%	83%
Keeley *et al*[5]	Randomized prospective	200	2.2 yr	NA	21%	10%
Burgher *et al*[2]	Retrospective	300	3.26 yr	77%	NA	26%
Inci *et al*[4]	Retrospective	24	52.3 mo	33.3%	41.7%	11%

NA: Not available.

Table 2 Indications for active stone removal of kidney stones

Kidney stones
Stone growth
Patients at high risk for stone formation
Obstruction caused by stones
Infection
Symptomatic stones (*e.g.*, pain, macrohematuria)
Stones ⩾ 15 mm
Stones < 15 mm, if observation is not the option of choice
Patient preference
Comorbidity
Social situation of the patient (*e.g.*, profession or travelling)

14 patients with kidney stones 20-40 mm, that included 64% (9/14) of cases with complete stone-free status. Our overall stone-free rate is favorable and equal to that of PNL. In our study, three patients (15%) developed a high-grade fever after the surgery. One patient with a struvite stone developed sepsis after the first session. It is impossible to completely avoid postoperative infections because the bacteria spread into the irrigation fluid during the surgery. Thus, surgeon should make an effort not to push up the intrarenal pressure and to keep the proper drainage flow, as well as the administration of antibiotics[10]. In our opinion, too long operation also apparently increases complication rates. When the operation time goes over 120 min, we usually terminate the surgery and retry the next session. This strategy was supported by a recent report which analyzed large patient cohort from a Japanese nationwide database. The authors suggested that longer operation time (> 90 min) increased the risk of complication[16].

Consequently, staged fURS is a practical option for the treatment of large kidney stones. Staged fURS has little blood transfusion risk, and is associated with a short hospitalization and few restrictions on daily routines. Moreover, the latest digital ureteroscopes, whose image quality is excellent, can promise better treatment outcome[17]. However, as the stone size becomes larger, the stone-free rate of fURS monotherapy decreases, and the number of operations increases. In our study, the stone-free rate for kidney stones > 40 mm dropped down to 67% after a mean 1.8 session, compared with a 100% stone-free rate for stones 20-40 mm after a mean 1.3 session[10]. Therefore, in our opinion, the percutaneous approach should be considered to be a preferred option for stones larger than 40 mm.

MINI-PERCUTANEOUS NEPHROLITHOTOMY COMBINED WITH FLEXIBLE URETEROSCOPY

Kidney stones larger than 40 mm should be treated primarily by PNL. In recent years, the new surgical technique named as "miniperc-PNL (mini-PNL)" or "tubeless PNL," which utilizes a smaller nephrostomy tract (⩽ 18 Fr), was developed. It is expected to prevent the major complications which frequently occurred in conventional standard-PNL (24-30 Fr). Jackman *et al*[18] reported the efficacy of a 13 Fr "miniperc" technique using a ureteroscopy sheath for nine adult patients. They concluded that the "miniperc" can offer advantages associated with hemorrhage, postoperative pain and the hospital stays. This report has been supported by several experts[19-23]. Knoll *et al*[23] evaluated the outcome of standard-(26 Fr) *vs* mini-PNL (18 Fr). They reported a prospective, nonrandomized series of consecutive 50 patients with a solitary kidney stone (lower pole or renal pelvis). After mini-PNL, if uncomplicated, the patients was not left a nephrostomy. Alternatively, a double-J catheter was placed anterogradely and the nephrostomy tract was closed with thrombin-matrix. After standard-PNL, all patients were left 22 Fr nephrostomies. While the stone-free rates were comparable (mini-PNL, 96% *vs* standard-PNL, 92%), mini-PNL showed the advantages of a shorter hospital stay and less postoperative pain. Although the benefits of mini-PNL are still controversial[24], this new less-invasive type of PNL can replace standard PNL for the treatment of large kidney stones, as well as complete staghorn stones. In addition, ultra-mini PNL (11-13 Fr) and micro-PNL (4.85 Fr) were developed and reported their effectiveness of the treatment for 10-20 mm sized kidney stone by some experts[25,26]. These new developed miniaturized PNL are expected to be new standard treatment options.

Furthermore, the simultaneous approach with fURS and PNL in the Galdakao-modified supine Valdivia (GMSV) position has been reported. The double approach (retrograde and antegrade) is expected to be superior to a single antegrade approach with PNL[27,28]. The advantages of the GMSV position enables the good versatility of stone manipulation along the whole upper urinary tract. The GMSV position can make use of combined or subsequent transurethral and percutaneous access to the urinary tract. The GMSV

Table 3 Treatment outcomes of ureteroscopy for large kidney stones

Ref	Study type	No. of patients	Mean stone diameter	Mean number of operation	SFR after the 1st operation	SFR after the 2nd operation
Ricchiuti *et al*[12]	Single center, retrospective	23	3.1 cm	1.43	56.5%	73.9%
Breda *et al*[13]	Single center, retrospective	15	2.2 cm	2.3	60%	86.6%
Riley *et al*[14]	Single center, retrospective	22	3.0 cm	1.82	23%	86.4%
Hyams *et al*[15]	Multi center, retrospective	120	2.4 cm	1.18	83%	97.5%
Takazawa *et al*[10]	Single center, retrospective	20	3.1 cm	1.4	65%	95%

SFR: Stone free rate.

position does not need to change the patient position. Also, it provides better descending drainage, retrieval of the stone fragments from percutaneous tract, and decompression of the intrarenal pressure. Scoffone *et al*[27] reported their experiences with 127 patients who were treated by a simultaneous approach with fURS and standard-PNL (ECIRS: Endoscopic Combined Intra-Renal Surgery) in the GMSV position. The tract was conventionally dilated to 24 Fr or 30 Fr. The mean length of the operation was 70 (range 25-225) min. The stone-free rate was 81.9% after the first session and 87.4% after the second session. Although the overall complication rate was relatively high (38.6%), there was no visceral injury and no anesthetic problems. The anatomical changes related to the supine position do not increase the risk of PNL complications. Although there were some difficulties in the surgeon's manipulations, which are associated with the longer access tract and more limited access field, supine PNL may have some benefits over prone PNL.

A synchronous approach with fURS and mini-PNL (ECIRS) has been suggested to be useful. Hamamoto *et al*[29] reported their treatment outcomes of mini-ECIRS (in the prone split-leg position), mini-PNL (18 Fr tract) and conventional standard-PNL (30 Fr tract). Although their study was nonrandomized and the patient position was prone, the stone-free rate of mini-ECIRS (81.7%) was superior to mini-PNL (38.9%) and standard-PNL (45.1%). Blood loss during the surgery was significantly lower in mini-ECIRS and mini-PNL than standard-PNL. Mini-ECIRS has a good versatility and will be an effective treatment for large kidney stones.

URETEROSCOPIC LITHOTRIPSY FOR MULTIPLE KIDNEY STONES

From some reports describing the outcome of SWL, about 20%-25% patients have multiple stones[30-32]. The stone-free rates after SWL for multiple stones are significantly lower than for a single stone, which dropped down from 70% to only 40%[30]. Many authors reported that the stone number was a significant predictor for the stone-free rates after SWL in their multivariate analyses[8,30-34]. In recent years, URS has been demonstrated its effectiveness and safety for upper urinary tract stones, and the indication has been expanding[35-37]. URS can directly access to the target stones throughout the whole upper urinary tract, regardless of laterality, and actively clear away the stone fragments[38]. This is a great advantage of URS superior to SWL. Therefore, URS may be an ideal treatment for multiple stones that promises a higher stone-free rate than SWL after a single surgery.

As well as fURS, SWL has been considered to be a recommended treatment for small to intermediate kidney stones[8]. The SWL has some advantages: good patient's acceptance, short convalescence, and little need of anesthesia during the treatment. However, the outcome of SWL is susceptible to many factors: stone size, stone position, stone composition, and the distance from skin to stone[30-34]. Particularly, the "multiple stones" is a strong unfavorable factor that impacts on the stone-free rates as well as recurrence-free rates after the treatment. Abe *et al*[30] described in their large cohort study that the stone-free rates after SWL for multiple stones dropped down to 41% compared with 71% for solitary stone. The "multiple stones" was the strongest adverse factor for stone recurrence in their analyses.

PNL is another treatment option for multiple kidney stones. Multiple kidney stones sometimes grow larger in different calices. In such cases, multiple percutaneous tracts are needed for access to the target stones. However, multiple percutaneous tracts may induce blood transfusion risk and the patient's discomfort[39,40].

Flexible ureteroscopes and their peripheral equipments have rapidly improved over the past few years. Nowadays, fURS yields a same or better outcome than SWL for kidney stones[8,36]. In comparison with SWL or PNL, fURS has some advantages for the treatment of multiple kidney stones. The various shaped nitinol baskets enable the removal of stone fragments safely. After the removal of one stone, we can continue the fragmentation of the next stones. Moreover, the latest flexible ureteroscopes and the smallest laser fiber can access to the lower calyx, where the spontaneous passage of residual fragments are hardly expected after SWL. Also, fURS can approach to bilateral upper urinary tract in a single operation[41,42].

There have been some studies of the management of multiple kidney stones by ureteroscopy[43-45]. We summarized the outcomes of the previous reports in Table 4. Breda *et al*[43] studied the results of 51 patients who had multiple unilateral kidney stones. The mean stone number was 3.1 and the mean stone length was

Table 4 Treatment outcomes of ureteroscopy for multiple kidney stones

Ref	Study type	No. of patients	Mean number of stones	Mean number of operation	SFR after the 1st operation	SFR after the 2nd operation
Breda *et al*[43]	Single center, retrospective, unilateral kidney	51	3.1	1.4	64.7%	92.2%
Herrera-Gonzalez *et al*[44]	Single center, retrospective, unilateral kidney	125	3.59	1	74.4%	NA
Huang *et al*[45]	Single center, retrospective, bilateral kidney	25	5.1	1.5	50%	92%

SFR: Stone free rate.

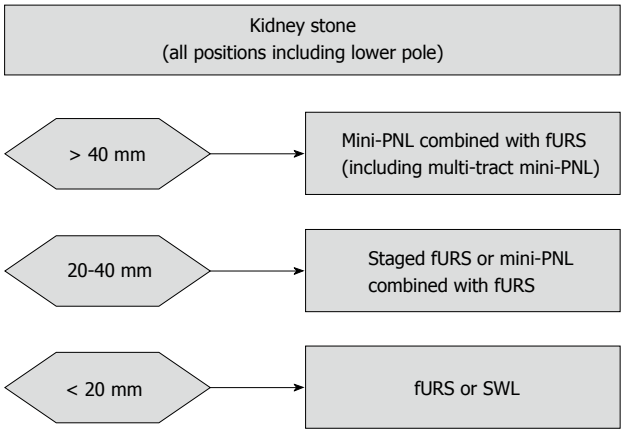

Figure 1 Our proposed treatment algorithm for kidney stones. fURS: Flexible ureteroscopy; PNL: Percutaneous nephrolithotomy; SWL: Shock wave lithotripsy.

6.6 mm. The mean stone burden (cumulative stone length) was 21 mm. The overall stone-free rate was 92.2%, with a mean number of sessions of 1.4. The stone-free rate after one and two sessions in patients with a stone burden ⩽ 20 mm was 79% and 100%, respectively, compared with 52% and 85% in patients with a stone burden > 20 mm. Complications occurred in seven patients (13.6%) including intraoperative bleeding in one, postoperative pyelonephritis in one and a urinary tract infection in three patients.

Herrera-Gonzalez *et al*[44] studied the results of 125 patients with multiple unilateral kidney stones. The mean stone number was 3.59. The mean cumulative stone length was 11.93 mm, and the mean cumulative stone surface was 83.7 mm^2. The overall stone-free rates after a single session was 74.4% The stone-free rates in patients with a cumulative stone surface ⩾ 100 mm^2 was 65.4%, compared with 79.5% in patients < 100 mm^2. Complications occurred in seven patients (5.6%), including urinary tract infections in four, hematuria in two patients, and ureteral perforation in one. The authors concluded that ureteroscopic lithotripsy for multiple kidney stones was an effective treatment.

We also reported the results of 51 patients with multiple stones, although we included both kidney and ureteral stones, either unilaterally or bilaterally, into the study cohort[38]. Our results were equivalent to those in Breda's reports[43]. In our study, the "stone burden" and the presence of "impacted stones" can significantly predict the stone-free rate after the first session of URS, whereas the "stone location" did not significantly influence the outcome. Due to the "impacted stones", if the ureteral mucosa was severely damaged during the procedure, we terminate the surgery in order to prevent a postoperative ureteral stricture. We always place a double-J stent to arrange for the next operation. At the next operation, the access to the residual stones usually becomes easier due to the spontaneous dilation of the ureter by stenting. In our study, we performed same session bilateral URS. We achieved 86% stone-free status after same session bilateral URS with no complication. Some experts has reported the effectiveness of same session bilateral URS[41,42]. Our results supported the adequacy of a same session bilateral URS as a considerable option for bilateral stones when it is performed at the experienced institutions.

We also analyzed our surgical data regarding stone burden[38]. Overall, the mean number of sessions was 1.3, the mean total operative time was 112 min, and stone-free rate after one and two sessions was 80.4% and 92.2%, respectively. The 25 patients with a stone burden < 20 mm had smaller number of sessions, shorter operative time, and higher stone-free rate after the first session than the 26 patients with a stone burden ⩾ 20 mm.

Consequently, fURS is an effective option for multiple stones. Especially for patients with a stone burden < 20 mm, fURS is a favorable option that promises a high stone-free rate after a single session, either unilaterally or bilaterally. However, for patients with a stone burden ⩾ 20 mm, a staged operation should be considered to achieve stone-free status.

CURRENT PROPOSAL FOR ACTIVE REMOVAL OF KIDNEY STONES

Figure 1 shows our proposed treatment algorithm for kidney stones. We select the treatment option with no distinction regarding the stone position (upper/middle pole or lower pole), because the current fURS instruments can easily reach to the all calyces, including the lower calyx, and can clear away the stone fragments by using a basket. Basically, we recommend endoscopic treatment for kidney stones, because residual fragments after SWL frequently do not pass spontaneously and often lead the stone recurrence. Besides, stones composed of calcium oxalate monohydrate, brushite, or cystine are usually resistant to SWL[8]. Depending on the operator's skills

and the stone shape/position/component, stones up to 40 mm can be treated sufficiently by fURS monotherapy, although staged operations may be required. We also recommend using a combination of PNL and fURS for larger stones, especially for staghorn stones, because the fURS can access each calyx, where the percutaneous antegrade approach is difficult. This is associated with a major advantage in terms of clearing the stone burden. Multi-tract PNL has also been evaluated by experts, who reported successful outcomes. However, multi-tract procedures may cause more complications, but if necessary, should be considered for appropriate cases[39,40]. Most upper urinary tract stones should be treated primarily by PNL, URS, SWL or a combination of these techniques. Thus, open or laparoscopic surgery may be a valid primary option in selected cases (*e.g.*, complex stone burden, treatment failed case, anatomical abnormal case.). Recently, the effectiveness of laparoscopic pyelolithotomy for large renal pelvic stone was reported, although further evaluation should be needed[46,47].

CONCLUSION

For large kidney stones, staged fURS is a practical treatment. Staged fURS has little blood transfusion risk, and is associated with a minimal risk of needing a blood transfusion, a short hospitalization and few restrictions on daily routines. However, as the stone size becomes larger, the stone-free rate of fURS monotherapy decreases, and the number of operations increases. Therefore, in our opinion, PNL should be considered to be a preferred option for stones larger than 40 mm. In addition, URS is an effective option for multiple stones. Especially for patients with a stone burden < 20 mm, URS is a favorable option that promises a high stone-free rate after a single session, either unilaterally or bilaterally.

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Time to re-evaluate effects of renin-angiotensin system inhibitors on renal and cardiovascular outcomes in diabetic nephropathy

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Abstract

The use of renin-angiotensin system (RAS) inhibitors, such angiotensin converting enzyme inhibitors/angiotensin-Ⅱ receptor blockers, to slow progression of chronic kidney disease (CKD) in a large group dominated by elderly people in the real world is not supported by available evidence. Large-scale clinical trials had many faults, among them a lack of focus on the elderly. However, it would be difficult to conduct clinical trials of a similar scale in elderly CKD patients. Besides, progression of kidney disease is often slow in elderly persons, and the vast majority of older adults with CKD will die before reaching end stage renal disease. Moreover, since it is not clear that progression of kidney disease, and even of proteinuric diabetic nephropathy, is not inhibited through the use of RAS inhibitors, the most patient-centric goal of therapy for many elderly individuals should be individualized.

Key words: Angiotensin converting enzyme inhibitors; Angiotensin receptor blockers; Dialysis; Chronic kidney disease

Core tip: The use of renin-angiotensin system (RAS) inhibitors, such angiotensin converting enzyme inhibitors/angiotensin-Ⅱ receptor blockers, to slow progression of chronic kidney disease in a large group dominated by elderly people in the real world is not supported by available evidence. Since it is not clear that progression of kidney disease, and even of proteinuric diabetic nephropathy, is not inhibited through the use of RAS inhibitors, the most patient-centric goal of therapy for many elderly individuals should be individualized.

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INTRODUCTION

Renin-angiotensin system (RAS) inhibitors [angiotensin

converting enzyme inhibitors (ACEi) and angiotensin-Ⅱ receptor blockers (ARBs)] have been recommended for reduction of proteinuria and prevention of the progresses of diabetic nephropathy (DN) by national and international guidelines[1-6]. Especially, two landmark trials, the Reduction of Endpoints in non-insulin dependent diabetes mellitus with the Angiotensin Ⅱ Antagonist Losartan (RENAAL)[7] and the Irbesartan Diabetic Nephropathy Trial (IDNT)[8], established the use of ARBs as first-line drugs for hypertensive patients with DN. In line with evidence presented in these trials, ARBs are now widely used for any stage of DN. However, our developed society, examples of which are European countries, the United States, and Japan, is facing a growing elderly population. The evidence referred to in the guidelines was produced more than 10 to 20 years ago when the diabetic population was mainly 50 to 60 years of age. At present, patients with DN are older than previously, suggesting that evidence accumulated earlier does not always hold true. As a general concept, renal function in elderly people is at risk of abrupt and complete inhibition of RAS. Previously, our group proposed that in patients with advanced stage chronic kidney disease (CKD), dose reduction of ACEi was required, especially in elderly patients[9]. In comparison with ACEi, all ARBs are mainly excreted from the bile instead of the kidney[10], supporting the concept that no dose modification is needed for ARBs in advanced stage CKD patients, such as stages 4 and 5 CKD. However, recently, a small dose of ARBs was recommended for patients with advanced stages of CKD[6]. With these issues in mind, this is the best time to reconsider the role of ARBs in the choice of treatment for hypertensive patients with DN. In this mini review, the authors re-examined previous reports that discussed the effects of ARBs on renal and cardiovascular outcomes.

ARE THERE REALLY EFFECTS OF ARBS BEYOND BLOOD PRESSURE LOWERING?

During the past 10 years, in addition to their use for blood pressure reduction, ARBs and ACEi have been administered to reduce proteinuria and to inhibit the progression of renal disease. In both the RENAAL[7] and IDNT[8], unexpectedly, there were very small reductions in blood pressure in patients receiving ARBs compared with patients receiving a placebo. However, in spite of this small reduction in blood pressure, conclusions were drawn regarding factors beyond the blood pressure lowering effects of ARBs on the assumption of a similar blood pressure reduction in both placebo and ARB groups. The possibility cannot be denied that a difference in blood pressure reduction, no matter how small, between groups is important in a large-scale clinical trial[11,12]. Considering these factors, small but not significant average blood pressure changes in a large number of patients cannot be neglected. Between the levels of blood pressure and the frequencies of cardiovascular events[13] there was the log-linear association, indicating that reduction in a systolic blood pressure of 5 mmHg is producing the stroke events by 40% and myocardial infarction by 20% reduction respectively[14]. The cardiovascular endpoints reduction seen in placebo-controlled trials of ACEi or ARBs use is expected from their blood pressure lowering effects, opposing pleiotropic effects of RAS inhibitors on cardiovascular disease (CVD) events. Therefore, it is unlikely that RAS inhibition produces effects beyond lowering blood pressure.

IS THERE A CLOSE RELATION BETWEEN THE LEVELS OF PROTEINURIA OR ALBUMINURIA AND PROGRESSION OF DN?

In the RENAAL, there were a linear relationship between baseline proteinuria and the risk of the primary outcome. Furthermore, every 50% reduction in albuminuria in the first 6 mo produced a reduction of 36% in the primary endpoint and a reduction of 45% in end stage renal disease (ESRD) at the end of study. The authors proposed the renoprotection as reducing proteinuria of losartan but not their lowering blood pressure[15]. Similarly, in the IDNT, every 2-fold increase from the baseline urinary excretion of protein doubled the risk of the primary endpoint. In either treatment groups, this risk was not achieved in half with every 50% reduction in proteinuria at 1 year. These results indicated the amount of proteinuria represented as an intermediate outcome in hypertensive patients with DN[16].

In line with this evidence, an old fashioned dogmatic hypothesis assuming a course of progression of DN stated that, first, microalbuminuria appears as DN and then the estimated glomerular filtration rate (eGFR) starts to decrease[17]. This central dogmatic hypothesis was adopted by the first edition of the CKD guideline of the Kidney Disease Outcomes Quality Initiative[17] and prevailed throughout the nephrology and diabetology world. However, in spite of this guideline, in the real world, general practitioners have been suspicious of this schema. Indeed, Tsalamandris *et al*[18] in 1994 demonstrated that in 40 hypertensive patients with DN followed for more than 7 years, they found 3 different courses of progression of DN over the long term. The first was that in spite of no decrease in the GFR, albuminuria increased; the second was that decreases in the GFR and increases in albuminuria progressed in parallel; and the third was that without any increases in albuminuria the GFR decreased progressively. Similar findings showed that DN is easily able to progress without albuminuria[19]. These data clearly suggest that destruction of other tissue pathways might produce the

decline in renal function. Ten years after the first edition of the CKD guideline, the second version[20] revised and accepted the concept that the levels of albuminuria and progression of DN are not always in parallel and sometimes independently change. This notion should be more greatly emphasized for general practitioners because a larger population of CKD patients with diabetes in the real world is treated by general practitioners than by specialists.

FLAWS IN LARGE-SCALE CLINICAL TRIALS

Onuigbo[21] proposed several serious concerns about randomized controlled trials. First, the discontinuation rates of the trial drugs have been remarkably high. In the RENAAL trial[7], the discontinuation rate of both losartan and placebo was unacceptably high. More than 45% of patients on losartan and more than 50% on placebo discontinued their drug, indicating that the outcome of the trial was not reliable. In contrast, in the ALLHAT trial, only 3.5% of enrolled subjects dropped out throughout the study. In addition to these flaws, both the RENAAL and IDNT trials failed to demonstrate statistically significant reductions in all-cause mortality by ARBs as well as the rate of introduction of dialysis therapy. Secondly, since in the RENAAL there were statistically inconsistence and apparently failed in substantial risk reductions of the doubling of serum creatinine and ESRD and a relatively higher rate of death in the losartan group compared with the placebo group were observed. Thirdly, there has been selection bias for participated patients with preserved renal function at the start of study. Finally, adverse effects, especially potential nephrotoxicity of the trial drug, was not correctly reported.

INCONSISTENCIES IN META-ANALYSES OF RAS INHIBITION IN CKD PATIENTS

Many meta-analyses and review articles have been published with regard to RAS inhibition in CKD patients. Strippoli *et al*[22] evaluated the effects of ACEi/ARBs on renal outcome and all-cause mortality in hypertensive patients with DN. In their analysis, ACEi significantly reduced all-cause mortality (RR = 0.79, 95%CI: 0.67-0.99, P = 0.04) compared with placebo but ARBs did not, although there was strong supportive evidence that ARBs were beneficial, showing a 22% reduction in risk of ESRD and a 42% increase of regression from microalbuminuria to normoalbuminuria. Besides, the effect of all renal outcomes was estimated for favor of ACEi compared with ARBs. Similar findings were reported for CVD outcomes in comparison between ACEi and ARBs. The benefit of ACEi but not of ARBs on all-cause mortality could probably be due to the experimental evidences that bradykinin antagonism of ACEi but not of ARBs, and the selectivity of ARBs could not have an advantage. Despite these findings in 2004, ARBs have been widely used in clinical practice for treatment of patients with DN.

One year after publication of Strippoli *et al*[22], in 2005 Cases *et al*[23] reported a systematic review and meta-analysis of the effect of RAS inhibitors and other antihypertensive drugs on renal outcomes. In their report, comparisons of ACEi or ARBs with other antihypertensive drugs showed a doubling of creatinine (RR = 0.71, 95%CI: 0.49-1.04) and a small benefit on ESRD (RR = 0.89, 95%CI: 0.75-0.99). In hypertensive patients with DN, there was no benefit found in comparative trials of either ACEi or ARBs on the doubling of serum creatinine (RR = 1.09, 95%CI: 0.55-2.15), ESRD (RR = 0.89, 95%CI: 0.74-1.07), GFR, or creatinine values. They proposed that blood pressure lowering effect was a major actions of ACEi/ARBs on renal outcomes conducted as placebo-controlled trials. Therefore, in patients with DN, beyond blood pressure lowering effects still remain unclear. However, considering their data, including data from patients with diabetes in ALLHAT[24], which was not originally designed to investigate the effects of antihypertensive agents for treatment of kidney diseases, it is likely that the mixture of diabetic nephropathy and hypertensive nephrosclerosis could account for the unfavorable effects shown for ACEi. Thus, the importance of the ALLHAT may cancel any effect shown in patients with true DN; therefore, the validity should be cautiously interpreted.

Balamuthusamy *et al*[25] reported a meta-analysis of studies using RAS inhibitors and CVD outcomes in hypertensive CKD patients with proteinuria, which included data from ACEi and ARBs. In that meta-analysis, RAS inhibitors decreased the risk for heart failure (RR = 0.63, 95%CI: 0.47-0.86, P = 0.003) in patients with DN in comparison with the control group. Although there was a decreased risk for myocardial infarction (RR = 0.89, 95%CI: 0.79-1.01, P = 0.06) and an increased risk of stroke (RR = 1.75, 95%CI: 0.96-3.17, P = 0.07) with inhibitors of RAS, the findings were not statistically significant. Based on their analysis, the authors concluded beneficial usage with RAS inhibitors for reduction of the risk of CV outcomes and heart failure in hypertensive patients with DN in comparison with placebo. Moreover, the authors recommended that the RAS inhibitors should be used as the first line antihypertensive drugs for hypertensive patients with diabetes mellitus and proteinuria. However, these results could be cautiously interpreted because a bias with larger numbers affected the findings.

Sarafidis *et al*[26] demonstrated in their meta-analysis that RAS inhibition with ACEi/ARBs in hypertensive patients with DN was related with reductions in the risk for ESRD and the doubling of serum creatinine in comparison with regimens that do not include RAS inhibitors. In addition, these agents did not produce

a reduction of the risk of all-causes mortality was not brought by these agents. In their study, ARBs were reported to reduce the risk of ESRD and the doubling of serum creatinine by 22% and 21% with significance, respectively. In contrast, ACEi were not significantly associated with reduction of 30% for the risk of ESRD but was significantly done with reduction of 29% for the risk of the doubling of serum creatinine. These findings favoring ARBs over ACEi should be interpreted with caution, because the effect on both ESRD and the doubling of serum creatinine were lower in ACEi in comparison with ARBs. These discrepancies might be caused by the two pairs of studies occupying the reported effects of ACEi (Micro-HOPE[27] and DIABHYCAR[28]) and ARBs (RENAAL[7] and IDNT[8]), which are completely different in primary outcomes, participated populations and its study design.

Recently, Sarafidis *et al*[29] summarized that in patients with DN, data from observational analyses and surrogate outcomes (and excluding the data from nondiabetic CKD patients) suggested a blood pressure of < 130/80 mmHg with protein excretion > 0.3 g/d. In non-proteinuric patients with diabetes, the main determinant of blood pressure goals leads to cardioprotection. Diastolic blood pressure < 80 mmHg is warranted, whereas the optimal systolic blood pressure target lies between 130 and 140 mmHg and should be decided on an individual basis, balancing the benefits of stroke reduction and unfavorable risks of hypotension and acute renal failure[30]. However, they proposed that there is no decisive evidence for combined therapy using RAS inhibitors for any type of CKD. Furthermore, sub-analyses from cardiovascular trials suggested no clear-cut benefit of RAS inhibition in hypertensive patients with normo-albuminuria and preservation of eGFR and sometimes produced harm in susceptible individuals.

More recently, Roscioni *et al*[31] postulated that the value of the RAS in the progression of DN has promoted the marketing of a therapeutic strategy to aim every step in the RAS cascade. Blockade of angiotensin Ⅱ by means of ACEi or ARBs is currently considered as the best option to treat DN because the renoprotective capabilities of these agents were well-established.

Among a large number of review articles, the well-designed larger studies dominated the results, whereas small studies had total weights accounting for a small percent of the total results. Thus, even if conclusions of several small studies differed from those of large-scale studies, the results of the large-scale clinical studies would prevail because of the large number of participants.

WHY ARE ARBS NOT RENOPROTECTIVE?

In several reports, Onuigbo of the Mayo Clinic noted that the administration of RAS inhibitors to patients with CKD sometimes produced acute kidney injury (AKI)[32-35]. He could not point out any clear-cut identifiable factors for this phenomenon, although he mentioned that many factors, such as heart failure, hypertension, infections, dehydration, etc. were found to be associated with worsening of renal failure in patients with CKD.

Suissa *et al*[36] assessed the long-term effect of ACEi on the risk of ESRD. They analyzed the data from a population-based cohort of all diabetic patients treated with antihypertensive drugs in the Province of Saskatchewan, Canada, between 1982 and 1986. The patients were followed up to the end of 1997 and identified as cases of end-stage renal failure. Using a nested case-control with the controls matched to each case for age, diabetes type, and duration of follow-up were analyzed. Of 6102 subjects, the 102 cases that developed ESRD were matched to 4129 controls. The adjusted RR of ESRD in relation to thiazide diuretic use, 2.5 (95%CI: 1.3-4.7) for ACEi, 0.8 (95%CI: 0.5-1.4) for blockers and 0.7 (95%CI: 0.4-1.3) for calcium channel blockers were reported. During the first 3 years after the start of follow-up, the RR of ESRD with ACEi use was 0.8 (95%CI: 0.3-2.5), but increased to 4.2 (95%CI: 2.0-9.0) after 3 years. From these data, it is clear that use of ACE-inhibitor use does not reduce the long-term risk of ESRD in diabetes. Their data also suggested that ACEi might actually produce this risk, which contribute to the continuing increases in incidence of ESRD owing to diabetes. These data coming from the real world do not validate the usefulness of ACEi in prevention of progression of DN. In the real world, a recent growth of the proportion of the elderly population is becoming worldwide. Moreover, higher number of elderly patients is brought by the increasing longevity of humans and it is producing subjects with multiple chronic diseases such as hypertension, diabetes, and CKD. These problems increase in morbidity and mortality in the elderly. More than one third of adults in the general population are 70 years over and half of them have CKD[37,38]. Whether evidence supporting current guidelines for the use ACEi/ARBs in patients with CKD can be extrapolated to this large group is unknown. O'Hare *et al*[39] tried to address this question and found that current guidelines addressing ACEi/ARBs use in patients with CKD are funded on evidence with limited relevance to most persons older than 70 years suffered from with CKD. Use of these agents to slow progression in this large group is not supported by available evidence. It is also not clear that slowing the progression of kidney disease represents the most patient-centric goal of therapy for many of these individuals. In elderly persons, renal function is slowly deteriorated and the vast majority of older adults with CKD will die before reaching ESRD[40,41] In a subgroup analysis among patients who were 65 years over and enrolled in the RENAAL trial, losartan was propagated to show renoprotective effect on these older participants. This suggested that this agent has equal efficacy for elderly albuminuric patients. However, the patients in this study was less than 74 years old, indicating that it cannot be applicable for those findings

to patients who are 75 years over[42]. Patients with a mean age of > 65 years were participated in the Telmisartan Randomized AssessmeNt Study in ACE iNtolerant Subjects with Cardiovascular Disease[43]. In this study, the investigators analyzed patients with organ damage but without macroalbuminuria or heart failure who could not tolerate ACEis. Either an ARB (telmisartan) or a placebo were administered to patients in addition to standard treatment and composite renal outcomes (ESRD, doubling of serum creatinine changes in eGFR, or the levels of albuminuria) were examined. Increases in albuminuria were less in patients treated with telmisartan than with placebo (32% *vs* 63%; $P < 0.001$). Furthermore, there was no significant difference in the composite renal outcome between telmisartan and placebo (1.96% *vs* 1.55%). Therefore, it is unlikely that RAS inhibition is effective for patients with DN.

CONCURRENT THERAPY WITH ARBS CAUSES UNRECOGNIZED WORSENING OF RENAL FAILURE IN ADVANCED STAGE OF CKD

Onuigbo *et al*[33] reported that the discontinuation of ACEi and/or ARBs produced reversible AKI in 100 CKD patients and that 75% of these patients were 65 years over, and 23% of these were 80 years over. Also, they examined prospectively the syndrome of worsening renal failure in CKD patients hemodynamically. In 19 of 20 patients the eGFR was increased from 27.8 ± 9.5 to 39.7 ± 14.9 mL/min per 1.73 m^2 after stopping RAS inhibitors. Further, they found that ESRD in the older CKD patients (average age 75.3 years) was frequently coming from patients suffered from unilateral renal artery stenotic lesions with dual kidneys. Similar findings were reported by Ahmed *et al*[44] in 52 patients with advanced stage CKD. Their mean age was 73.3 ± 1.8 years, their average eGFR 16.38 ± 1 mL/min per 1.73 m^2 and urinary excretion of protein 77 ± 20 mg/gCr. Besides, 40 percent had diabetes mellitus. Twelve months after cessation of RAS inhibitors the eGFR increased significantly to 26.6 ± 2.2 mL/min per 1.73 m^2. Of these patients, 61.5% had a more than 25% increase and 36.5% had an increase exceeding 50% in eGFR, although a significant decline in the eGFR slope (-0.39 ± 0.07) in the 12 mo before cessation of RAS inhibitors were found. From these findings in combination, cessation of either ACEi or ARBs could delay the progression of ESRD in the majority of those patients. It is therefore likely that ACEi/ARBs should be used in elderly hypertensive patients with CKD with great caution.

ARE ACEI/ARBS STILL EFFECTIVE IN PREDIALYSIS PATIENTS?

Hsu *et al*[45] examined safety and the adverse effects of ASCi/ARB use for hypertensive patients with advanced CKD and anemia by a population-based longitudinal cohort study. They selected subjects who had a primary diagnosis of CKD and received an erythropoietin stimulating agent. Inclusion criteria was their baseline values for serum creatinine > 6 mg/dL and hematocrit < 28%. From January 2000 through June 30, 2009, 28497 patients were selected. Results showed that use of ACEi/ARB inhibitors significantly reduced the risk of long-term dialysis and the composite outcome, with a hazard ratio (HR) of 0.94 (95%CI: 0.92-0.97) after adjustment for various confounders. In this study, even in patients with DN, ACEi/ARB use reduced the HR of ESRD and the composite outcome of ESRD or death. However, a higher rate of hyperkalemia-associated hospitalization was found among patients treated with ACEi/ARB inhibitors than among nonusers (9.2% *vs* 6.7%), indicating that the use of RAS inhibitors for elderly patients with DN might be dangerous.

ARE ARBS EFFECTIVE IN PATIENTS RECEIVING DIALYSIS?

Heerspink *et al*[46] reported a systematic review and meta-analysis of assessment of blood pressure lowering effects in dialyzed patients. In their analysis, treatment with antihypertensive agents was more closely related with lower risks of CVD events (RR = 0.71, 95%CI: 0.55-0.92, $P = 0.009$), all-cause mortality (RR = 0.80, 95%CI: 0.66-0.96, $P = 0.014$), and CVD mortality (RR = 0.71, 95%CI: 0.50-0.99, $P = 0.044$) than control regimens. Also, their data indicated that there were no differences in blood pressure lowering effects among RAS inhibitors, β blockers, and calcium channel blockers in patients on dialysis. They concluded that the choice of antihypertensive agents might be chosen on the grounds of their tolerability, their side-effect, and other related variables. No specific drugs were recommended. Recently, Iseki *et al*[47] reported that olmesartan, an ARB, did not lower the risks of major CV events or death among patients with hypertension on chronic dialysis. Combining these data, it is suggested that ARBs are not the only antihypertensive drug suitable for patients receiving dialysis. Left ventricular hypertrophy (LVH) is a well-established marker for future occurrence of CVD and an independent predictor of CV events[48-51]. There is some evidence indicating that ARBs could reverse LVH and might confer cardiovascular event risks beyond lowering blood pressure[52-54]. Yang *et al*[55] undertook a meta-analysis to assess the effect of ARBs *vs* placebo or other treatments, as well as ARBs and ACEi in combination, on LVH in patients receiving dialysis. Their study demonstrated that among dialysis patients the ARBs presented a greater regression in the LVM index when compared with the non-ARB users while there was no significant difference in the left ventricular ejection fraction (LVEF) between the two groups. The ARB group had a greater therapeutic value for the left ventricular mass (LVM) index or LVEF without achieving

significance when compared with the ACEi group. No significant alterations were found in the LVM index and LVEF between the ARB and ACEi in combination and the ARB. The authors concluded that ARBs produced a greater reduction in LVH in patients on dialysis. The ARB therapy tended to have favorable effectiveness similar to ACEi; however, the treatment with ARBs and ACEi in combination did not produce additional benefit for LVH in patients on HD. Tai *et al*[56] reported a meta-analysis to examine whether ACEi/ARBs reduced fatal and non-fatal CV events and the LVM in patients receiving HD. In their analysis, in comparison with the control groups, use of ACEi/ARBs did not produce any significant reduction of CV events. ACEi/ARB use resulted in a statistically significant reduction in the LVM (RR = 15.4, 95%CI: 7.4-23.5; $P < 0.001$). From these data, it could be suggested that ACEi/ARBs were effective in reducing the LVM index in patients with CKD accompanied by CVD. These data indicated that ACEi/ARBs are effective to reduce the LVM index in patients receiving HD. While ACEi/ARB use is advocated in peritoneal dialysis (PD) patients, (http://www.kidney.org/PROFESSIONALS/kdoqi/guideline_upHD_PD_VA/index.htm)[57,58] supporting evidence is unclear. Akbari in attempting to answer questions about the efficacy of ACEi/ARBs in patients on PD carried out a systematic review with analysis of randomized controlled trials, in which treatment with ACEi/ARB inhibitors was compared with that with other antihypertensive agents. Their review revealed that there remains no clear cut evidence for the use of ACEi/ARBs for the reduction of mortality and CV events in PD patients; limited data suggested that these agents induce a slow decrease in residual renal function loss. With these facts in mind, ACEi/ARBs can be carefully used in patients on PD.

FUTURE DIRECTIONS

Blood pressure measurements

That measurements of blood pressure in these clinical trials were performed in outpatient clinic might produce erroneous results. Recently, it was shown that blood pressure measurements in medical offices can be considered to be unreliable[59-61] because the mixture of white coat phenomenon and/or masked hypertension cannot be avoided. The recently issued NICE guidelines[62] recommended ambulatory blood pressure monitoring instead of measurement of blood pressure in medical offices[63,64]. Therefore, blood pressure in elderly CKD patients should be measured using home blood pressure[65-68].

Assessment of progression of renal disease

To date, most studies looking at outcomes related to renal disease have not used the renal trajectory as an endpoint. Most previous studies have been employing either a doubling of serum creatinine or time to start of renal replacement therapy. The latter is at most subjective assessment of trajectory[69]. Rosansky[70] proposed the following: change in a patient's eGFR over time (renal function trajectory) is potentially more important when deciding initiation of RRT. In the elderly CKD 4 population with several comorbidities and slow decrease in renal function, the likelihood of death or cardiovascular events prior to the need for RRT should be expected before making arteriovenous access for dialysis.

Newly developed direct renin inhibitor aliskiren

Recently Morishita and Kusano assessed the efficacy of aliskiren on blood pressure control and renoprotection in CKD patients whose proteinuria was not reduced less than 1.0 g daily in spite of administration of ARBs[71]. It is therefore possible that aliskiren produces different action compared with ARBs in hypertensive patients with DN.

CONCLUSION

I would like to propose that it is time for re-evaluation of the use of ACEi/ARBs for patients with DN and that new individualized therapies for elderly people in the real world should be developed.

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ORIGINAL ARTICLE

Case Control Study

Relationship of *MTHFR* gene polymorphisms with renal and cardiac disease

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Abstract

AIM: To investigate the effects of different methylenetetrahydrofolate reductase (*MTHFR*) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial.

METHODS: We challenged the relationship, if any, of MTHFR 677C>T and MTHFR 1298A>C polymorphisms with renal and heart function. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure requiring hemodialysis. MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years, addressing to the increased hazard of hemodialysis, if any, according to the studied MTHFR genetic polymorphisms.

RESULTS: A favorable association with normal renal function of MTHFR polymorphisms, and notably of MTHFR C677T is present independently of the negative effects of left ventricular hypertrophy, increased Intra-Renal arterial Resistance and hyperparathyroidism.

CONCLUSION: *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

Key words: Homocysteine; Glomerular filtration rate; Renal function; Mediterranean diet; Genetic; Methylenetetrahydrofolate reductase polymorphism; Insulin

resistance; Obesity; Left ventricular hypertrophy; Echocardiography

Core tip: We investigated the effects of different methylenetetrahydrofolate reductase (MTHFR) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial, and challenged the relationship, if any, of MTHFR 677C>T and MTHFR 1298A>C polymorphisms with renal and heart function. *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

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INTRODUCTION

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease[1]. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that Folic acid based regimens are not recommended as a generalized approach in the prevention of cardiovascular events in chronic kidney disease[2]. Some polymorphism of the human methylenetetrahydrofolate reductase (*MTHFR*) gene have been associated with increased homocysteine levels: this was suspected to increase risks of cardio-vascular disease[3] (CVD) especially in the natural story of chronic kidney disease[4]. The more common MTHFR polymorphism (nucleotide 677 C>T) results in a thermolabile enzyme, lower folate levels and an inefficient homocysteine metabolism[5]. In recent years evidence has accumulated that the total homocysteine plasma level of patients under different forms of renal replacement therapy is influenced by a common polymorphism at nucleotide position 677 of the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR 677C-->T). Furthermore, compound heterozygosity for the 677T allele and a novel A-->C polymorphism at nucleotide position 1298 of MTHFR was suggested to correlate with a decrease of folate plasma concentrations[6]. Hyperhomocysteinemia appears independent from other risk factors and subsequent reports increased concerns around the related common genetic polymorphism[7] despite earlier studies already challenged this concept[8] since this polymorphism prevalence in the elderly is not lower than in the young[9]. A very relevant question for the putative detrimental role of the allele 677T of the *MTHFR* gene is related to the evidence that this polymorphism is the best explaining protective factor against cervical carcinogenesis[10], and for colonic cancer[11,12], seemingly associated with longer and healthier survival[13]. Nonetheless, according to other studies, MTHFR 677TT homozygous and systolic blood pressure independently influence intima-media thickness[14] as other non-genetic markers[15] and nutritional conditions do[16]. Also mild-moderate renal impairment is associated with mortality, increased left ventricular (LV) myocardial mass[17], lower Ejection Fraction and increased E/A ratio at echocardiography[18]. Insulin resistance accounts significantly for LV mass increase in normotensive individuals[19]. A linear relationship between LV myocardial mass/m^2 (LVMMi) *vs* cardiovascular events, a J-shape relationship between LVMMi *vs* all-cause death[20] and NT-proBNP increase in patients with LV hypertrophy (LVH) suggest a common pathway, through the increase of measured myocardial mass, toward cardiac insufficiency[21]. Relevance of hyper-homocysteinemia stems from many considerations. Among them, in general population with no history of cardiovascular disease, concentrations of homocysteine alone could accurately identify those at high risk of cardiovascular mortality, whereas classic risk factors included in the Framingham risk score do not[22], suggesting the need of intervention[23]. MTHFR polymorphisms[24,25] seemingly intervene, not only inducing hyperhomocysteinemia, within a cluster of different and even interrelated conditions, diseases and indexes. Dietary profiles are the background of any adequate nutrients intake and particularly of a normal B vitamin intake and availability: they can be modified by conditions impairing renal function[26]. *MTHFR* gene-mediterranean diet interaction on homocysteine metabolism was reported: this dietary profile may reduce homocysteine concentrations and consequently influence coronary risk in genetically high-risk individuals by quality and proportion of nutrients[27]. The accompanying body size increase is not invariably detrimental since, actually, patients with established chronic disease benefit of large body size[28]. This finding, defined the obesity paradox, is shared over a variety of cardiovascular, pulmonary, and renal diseases: it challenges the concept about differences for optimal body size in health and disease[29]. The cornerstone is how several metabolic factors affect renal circulation and, as a consequence, renal function. The increase of intra-renal artery resistance, measured by renal artery resistive index (RRI), affects the natural history of atherosclerosis and arterial hypertension, which was found to correlate with LVH and carotid intimal thickening[29], with cardiovascular

risk score and impaired renal outcome and death[30]. Also endocrine factors are very relevant: among them, Parathyroid Hormone intervenes in several mechanisms of disease progression, including LVH[31], impairment of renal function[32] and increase of intrarenal arterial resistance[33,34]. We reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with LVH, high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and RRI. Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate (GFR) and greater hsCRP, iPTH, RRI, and LVH[35]. Even with the limitations of an observational study, the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism[36]. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure. Patients considered were on hemodialysis or on maintenance therapy, and GFR, RRI and LVMM and systolic/diastolic function, dietary profile, hsCRP, iPTH, insulin resistance were assessed.

MATERIALS AND METHODS

We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years. Body mass index (BMI) 27.70 ± 5.76 kg/m^2 consecutively admitted according to the request of their primary care doctors for nutritional assessment and work-up. One hundred and sixty of all subjects were with advanced renal insufficiency, treated by hemodialysis (HD); the other 470 were patients without or with slight-moderate renal impairment, quantified by serum creatinine. These patients were briefly defined No-HD. All patients were managed within a protocol which included medical history, physical examination, nutritional and physical activity assessment, electrocardiogram, Chest X-ray, echocardiography and clinical abdomen and thyroid ultrasound. According to MTHFR genotype, 94 of them were MTHFR C677CC (Wild genotype), 118 heterozygous MTHFR C677CT and 104 homozygous MTHFR C677TT (thermolabile polymorphism) subjects. Of the A1298C subjects 80 were homozygous A1298CC, 76 were heterozygous A1298AC; 158 subjects were with a compound MTHFR heterozygous polymorphism, *i.e.*, both MTHFR A1298AC/C677CT. These data are summarized in Table 1. Routine laboratory tests included virus hepatitis [hepatitis A virus (HAV), HBV, and HCV] and cancer biomarkers (AFP, CEA, Ca125, Ca15-3), thyroid hormones, thyroid stimulating hormone, aspartate Aminotransferase, Alanine Aminotransferase, γ-Glutamyl transpeptidase, ferritin, total protein, and albumin. Human insulin and Folic acid were assayed using immulite 2000 Analyzer, by a solid-phase 2-site chemiluminescent immunometric assay. hsCRP concentrations were assayed by a standard detection limit of 0.175 mg/L (CardioPhase high-sensitivity hsCRP method-Siemens Medical System, Milan, Italy). Homocysteine and B12 Vitamin assay in the blood were performed by ADVIA Centaur® XP Immunoassay (Siemens Medical System, Milan, Italy)[37]. iPTH and NT-proBNP (IMMULITE® 2000 Siemens Medical System, Milan, Italy) were assessed by a solid phase two-site chemiluminescent immunometric assay. PTH values considered normal were < 70 pg/mL for subjects without severe renal insufficiency[38]. Body weight (BW) was measured in light clothing, without shoes, in kilograms, and height (H) was measured in meters, using a scale-integrated stadiometer. BMI was calculated as BW/H2 and patients were categorized as normal weight (< 25.0 kg/m^2), overweight (≥ 25.0 and ≤ 29.9 kg/m^2), and obese (≥ 30.0 kg/m^2). Insulin resistance was assessed by the homoeostasis model-insulin resistance index (HOMA), according to the following formulas: fasting insulin value x fasting blood sugar level/405. The HOMA threshold for insulin resistance is conventionally considered as > 1.7, according to the likelihood ratios for 11-year cardiovascular disease prediction[39]. The Waist-to-Hip (W/H) ratio was assessed in all patients. Ultrasound (US) examinations were performed by echographists unaware of laboratory details at the time of the procedure. An echo-color-doppler machine (Siemens Acuson S2000™, Siemens AG, Muenchen Germany), high resolution, with real-time sectional scan transducers was used. Renal color Doppler echography is performed assessing intra-parenchymal *renal arterial* resistive index, RRI (peak systolic velocity-end diastolic velocity/peak systolic velocity)[40]. First measurement is the size of the left and right kidney. For orientation purposes, perfusion in the whole of the left and right kidneys is then checked using color Doppler ultrasonography and the main trunk of the renal artery is displayed. Three measurements for each kidney are taken by pulsed Doppler within 5 min in the vicinity of the interlobar artery. RRI is calculated as the average value of all measurements taken. RRI threshold to define higher RRI measurements is defined by the 75th percentile derived by measurements of all eligible patients[40]. Echocardiographic studies were performed with two-dimensional guided M-mode echocardiography according to methods established by the American Society of Echocardiography (ASE)[41-44] with transducer frequencies appropriate for body size. Siemens Acuson S2000™, Siemens AG, Muenchen Germany or a GE echo-color-doppler device [GE Logiq7 Expert US, manufactured by GE Medical Systems-Milwaukee-Wisconsin (United States)], high resolution,

Table 1 Differences between methylenetetrahydrofolate reductase groups in all patients

	Wild genotype (*n* = 94)	Heterozygous MTHFR C677T (*n* = 118)	Heterozygous MTHFR 1298 AC (*n* = 76)	Compound heterozygous C677T and A1298C (*n* = 154)	Homozygous MTHFR 1298 CC (*n* = 80)	Homozygous MTHFR 677TT (*n* = 104)	*P*
Age, yr	53.30 ± 11.89	51.59 ± 17.39	56.74 ± 16.55	57.91 ± 17.04	57.85 ± 14.70	50.12 ± 17.09	< 0.0001
BMI, kg/m^2	27.26 ± 5.04	27.28 ± 5.95	28.04 ± 5.84	27.80 ± 6.13	27.01 ± 3.92	28.72 ± 6.61	0.316
GFR	48.84 ± 32.90	66.05 ± 36.70	64.16 ± 36.32	63.31 ± 38.35	61.57 ± 34.91	68.85 ± 27.33	0.002
Triglycerides, mg/dL	109.96 ± 75.73	113.21 ± 57.91	107.63 ± 42.67	128.72 ± 85.29	95.46 ± 37.07	103.94 ± 48.93	0.003
Total cholesterol, mg/dL	191.67 ± 41.71	206.29 ± 52.53	203.84 ± 38.73	198.28 ± 41.37	196.06 ± 54.99	201.54 ± 34.49	0.206
HDL cholesterol, mg/dL	58.11 ± 20.91	55.17 ± 15.75	55.28 ± 18.46	52.52 ± 18.49	52.51 ± 18.50	56.27 ± 16.35	0.175
LDL cholesterol, mg/dL	111.57 ± 34.89	128.47 ± 48.01	127.04 ± 31.67	120.81 ± 35.73	124.46 ± 48.10	124.89 ± 35.30	0.039
AST, U/L	19.50 ± 6.30	23.32 ± 14.23	27.93 ± 17.69	20.49 ± 6.91	21.76 ± 12.26	19.72 ± 5.99	< 0.0001
ALT, U/L	15.82 ± 4.59	16.46 ± 5.38	18.51 ± 5.75	16.46 ± 5.93	16.59 ± 5.74	15.92 ± 5.90	0.031
γGT, U/L	24.63 ± 12.20	33.82 ± 25.96	37.45 ± 38.03	42.71 ± 48.64	28.94 ± 15.17	25.37 ± 16.29	< 0.0001
HOMA	2.00 ± 1.13	3.18 ± 3.49	3.04 ± 2.27	4.04 ± 4.87	2.28 ± 1.07	2.76 ± 2.77	< 0.0001
PTH, pg/mL	84.94 ± 100.37	84.49 ± 170.97	78.37 ± 65.95	86.30 ± 76.75	86.53 ± 95.97	84.83 ± 81.83	0.997
hsCRP, mg/dL	2.58 ± 4.41	2.15 ± 2.79	6.30 ± 13.55	4.30 ± 8.66	3.99 ± 6.42	3.51 ± 4.95	< 0.001
RRI	0.60 ± 0.05	0.59 ± 0.05	0.59 ± 0.04	0.58 ± 0.05	0.59 ± 0.07	0.59 ± 0.06	0.392
EF, %	67.05 ± 8.18	66.94 ± 9.19	65.99 ± 9.15	63.52 ± 12.04	67.15 ± 11.63	66.51 ± 7.26	0.035
E/A	1.15 ± 0.36	1.20 ± 0.26	1.23 ± 0.34	1.09 ± 0.40	1.01 ± 0.26	1.18 ± 0.31	< 0.0001
LVMM/m^2	100.48 ± 54.70	105.44 ± 33.79	107.69 ± 48.47	109.21 ± 41.02	110.38 ± 46.63	97.11 ± 28.69	0.179
AMDS	34.94 ± 2.52	34.97 ± 3.03	33.42 ± 3.88	34.23 ± 3.02	34.93 ± 2.68	34.46 ± 3.18	0.005
Homocysteine μmol/L	17.41 ± 3.00	25.53 ± 8.12	28.58 ± 9.23	18.68 ± 9.01	21.26 ± 9.17	18.83 ± 6.25	< 0.0001

BMI: Body mass index; GFR: Glomerular filtration rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl Transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylenetetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index.

with real-time sectional scan transducers were used. An average of two echocardiographic measurements was taken and the cardiologist reading them was blinded to the clinical information of the patient. Measurements were obtained for LV end-diastolic and end-systolic dimension, septal wall thickness and posterior wall thickness in diastole. LVM was calculated with the method of Devereux *et al*[44] and indexed by dividing by body surface area/m^2. All the exams were stored on digital media for subsequent analysis. LV diameters and wall thickness were measured according the ASE guidelines and LV ejection fraction (LVEF) accordingly[41]. LVEF was considered abnormal if < 50%. GFR is assessed as estimated GFR by the modification of diet in renal disease formula in mL/min per 1.73 m^2, according to the Clinical Practice Guidelines for Chronic Kidney Disease KDOQI[38]. Genotypes of the MTHFR C677T and A1298C polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). DNA was extracted from peripheral blood by a commercially available DNA isolation method (QIAamp DNA Blood Mini Kit QIAGEN, Milan, Italy). Restriction enzyme analysis of amplified product (PCR-RFLP) analysis were carried out for direct genotypes detection of single nucleotide polymorphisms, C667T (rs1801133) and A1298C (rs1801131). PCR products were obtained using specific primers (NCBI Reference Sequence: NG_013351.1): C667T (F5′-GTCCCTGTGGTCTCTTCATCC-3′/R5′-GGTGGCCAAGCAACGCTGTG-3′); A1298C (F5′-CTTCTACCTGAAGAGCAAGTC-3′/R5′-CACATGTCCACAGCATGGAC-3′). Both amplicons were successively digested by HinfI and MboII restriction enzymes for C667T and A1298C respectively, and DNA fragment visualized in a 4% agarose gel stained with Synergy Brand Inc safe (Life Technologies Italia, Monza, Italy); electrophoresis pattern was used to determined MTHFR genotypes[45]. Informed consent was obtained from each patient, relatively also to the use of genetic information, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Statistical analysis

The fit to the Hardy-Weinberg equilibrium was analyzed. Student's *t* test was used to assess the difference between subject with advanced renal insufficiency, treated by

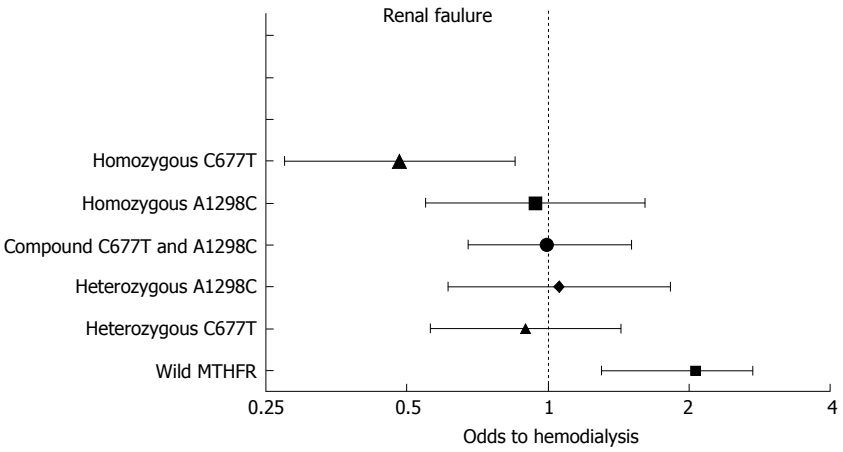

Figure 1 Odds to renal failure-hemodialysis. Comparison of the wild MTHFR genotype in dialysis patients (36/160; 22.5%) *vs* the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with all MTHFR polymorphisms and a four-fold risk *vs* the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status are as follows (hemodialysis patients 160 *vs* No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior. Heterozygous C677T (28/160 *vs* 90/470); *P* = NS. OR = 0.896 (95%CI: 0.561-1.43); Heterozygous A1298C (20/160 *vs* 56/470); *P* = NS. OR = 1.056 (95%CI: 0.612-1.822); Compound Heterozygous C677T and A1298C (40/160 *vs* 118/470); *P* = NS. OR = 0.994 (95%CI: 0.657-1.505); Homozygous A1298C (20/160 *vs* 62/470); *P* = NS. OR = 0.94 (95%CI: 0.548-1.612); Homozygous C677T (16/160 *vs* 88/470); *P* = 0.015. OR = 0.482 (95%CI: 0.274-0.85). MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio; NS: Not significant.

hemodialysis and No-HD group. ANOVA was used to assess the difference in averages between subjects with MTHFR heterozygous, compound and homozygous polymorphism. Descriptive results of continuous variables are expressed as averages (± SD). Two-sided $P < 0.05$ was considered statistically significant. The distributions of MTHFR alleles and genotypes in studied group, *i.e.*, normal-impaired renal function *vs* hemodialysis patients were checked by χ^2 test or Fisher's exact test. Higher quartiles of age, homocysteine, iPTH, RRI, hsCRP and of other continue measures were defined; thereafter, the associations of older age, higher hsCRP, iPTH, RRI, Left Ventricular Hypertrophy (LVMMi $\geqslant$ 135 g/m^2 in men, $\geqslant$ 110 g/m^2 in women[46]) and MTHFR polymorphisms were assessed as odds ratios (ORs) to severe chronic renal failure in hemodialysis with 95%CIs. Statistical analyses were performed using SPSS 18.0 for Windows (SPSS, Chicago, IL), Likelihood Ratio was assessed and sensitivity, specificity and predictivity were calculated by the CEBM Statistics Calculator, by Courtesy of CEBM, and graphs by Prism-Graphpad. Venn Diagram Plotter was used by courtesy of Pacific Northwest National Laboratory.

RESULTS

The differences of averages of measures between patients with MTHFR 677C>T heterozygous and homozygous polymorphism, of heterozygous and homozygous MTHFR 1298A>C polymorphism and of compound heterozygous MTHFR 677C>T/MTHFR 1298A>C polymorphism *vs* wild genotype subjects are shown in Table 1. Glomerular filtration rate is significantly higher in all the polymorphism groups *vs* wild genotype subjects, with figures greater of about 30%-35% more. Difference of age, even significant, are actually minor and, in any case, subjects with polymorphisms are older; homocysteine and low-density lipoprotein cholesterol are slightly higher in the MTHFR 677C>T polymorphism group *vs* wild genotype subjects. There are internal relationships between most measures: a significant linear correlation of GFR *vs* LVMMi (r = -0.37; $P < 0.0001$) is observed. Significant inverse correlation of age *vs* GFR (r = -0.56; $P < 0.0001$) and direct correlations of age *vs* RRI (r = 0.41; $P < 0.0001$), and *vs* LVMMi (r = 0.29; $P < 0.001$) are observed. iPTH shows significant inverse correlation *vs* GFR (r = -0.34; $P < 0.0001$). whereas a direct trend of iPTH is observed *vs* RRI (r = 0.32; $P < 0.001$) and *vs* LVMMi (r = 0.14; $P < 0.05$). No significant correlation is observed both for hsCRP and insulin resistance (HOMA) *vs* GFR, LVMMi and RRI.

Characteristic of study population and differences between HD patients (HD) and No-HD are reported in Table 2.

A significant difference is observed, overall, for the prevalence of wild MTHFR genotype in dialysis patients (36/160; 22.5%) *vs* the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with MTHFR polymorphisms and a four-fold risk *vs* the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status (hemodialysis patients 160 *vs* No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior (Figure 1).

Likelihood ratio was assessed and sensitivity, specificity and predictivity, which were all very weak and substantially non-contributory: Homozygous C677T MTHFR polymorphism displays a Sensitivity of 0.154

Table 2 Characteristic of study population and differences between hemodialysis patients and no-hemodialysis *n* (%)

	Total (*n* = 630)	Dialysis patients (*n* = 160)	Patients with maintained Renal function (*n* = 470)	*P*
Women	336 (53.3)	72	264	0.014[1]
Obese patients	196 (31.1)	24	172	< 0.000[1]
Patients with GFR < 90	514 (81.6)	160	354	< 0.000[1]
NAFLD patients	256 (40.6)	28	228	< 0.000[1]
MTHFR group				
Wild genotype	94 (14.9)	36	58	0.016[1]
MTHFR C677T	118 (18.7)	28	90	
MTHFR 1298 AC	76 (12.1)	20	56	
Compound heterozygous C677T and A1298C	158 (25.1)	40	118	
MTHFR 1298 CC	80 (12.7)	20	60	
MTHFR 677TT	104 (16.5)	16	88	
Age, yr	54.60 ± 16.35	67.48 ± 14.57	50.22 ± 14.51	< 0.0001
BMI, kg/m^2	27.70 ± 5.76	25.29 ± 3.97	28.52 ± 6.04	< 0.0001
Blood glucose, mg/dL	96.42 ± 26.42	95.33 ± 34.80	96.79 ± 22.91	0.545
Blood urea, mg/dL	52.47 ± 35.74	100.45 ± 41.07	36.13 ± 9.40	< 0.0001
Creatinin, mg/dL	2.36 ± 2.98	6.75 ± 2.99	0.86 ± 0.21	< 0.0001
GFR	62.46 ± 35.32	9.28 ± 3.60	80.56 ± 19.38	< 0.0001
Triglycerides, mg/dL	112.16 ± 64.71	131.90 ± 87.21	105.44 ± 53.48	< 0.0001
Total cholesterol, mg/dL	199.72 ± 44.43	175.80 ± 42.67	207.86 ± 42.05	< 0.0001
HDL cholesterol, mg/dL	54.81 ± 18.10	48.20 ± 15.63	57.07 ± 18.34	< 0.0001
LDL cholesterol, mg/dL	122.75 ± 39.63	101.22 ± 33.04	130.09 ± 39.05	< 0.0001
AST, U/L	21.81 ± 11.16	14.38 ± 4.07	24.34 ± 11.66	< 0.0001
ALT, U/L	16.54 ± 5.63	12.75 ± 4.15	17.83 ± 5.49	< 0.0001
γGT, U/L	33.10 ± 32.11	31.78 ± 19.18	33.55 ± 35.46	0.546
Insulin	11.84 ± 9.73	11.44 ± 10.77	11.98 ± 9.36	0.547
HOMA	3.02 ± 3.30	3.08 ± 3.94	3.00 ± 3.05	0.797
PTH, pg/mL	84.58 ± 105.79	162.38 ± 178.81	57.99 ± 36.85	< 0.0001
hsCRP, mg/dL	3.52 ± 7.01	2.62 ± 2.45	3.82 ± 7.98	0.107
Albumin, g/dL	4.60 ± 0.37	4.64 ± 0.35	4.58 ± 0.37	0.119
Albumin, %	62.39 ± 3.60	62.60 ± 3.03	62.31 ± 3.77	0.388
RRI	0.62 ± 0.06	0.68 ± 0.03	0.60 ± 0.06	< 0.0001
EF,%	65.93 ± 9.99	61.03 ± 12.62	67.87 ± 7.95	< 0.0001
E/A	1.14 ± 0.33	1.03 ± 0.39	1.18 ± 0.30	< 0.0001
LVMM/m^2	104.95 ± 42.10	135.37 ± 55.56	93.84 ± 28.91	< 0.0001
AMDS	34.51 ± 3.09	35.93 ± 1.69	34.02 ± 3.31	< 0.0001
Homocysteine, μmol/L	2.1 ± 5.4	36.8 ± 8.5	21.2 ± 7.7	< 0.0001

[1]Pearson χ^2. GFR: Glomerular filtration rate; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylenetetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index; NAFLD: Non-alcoholic fatty liver disease.

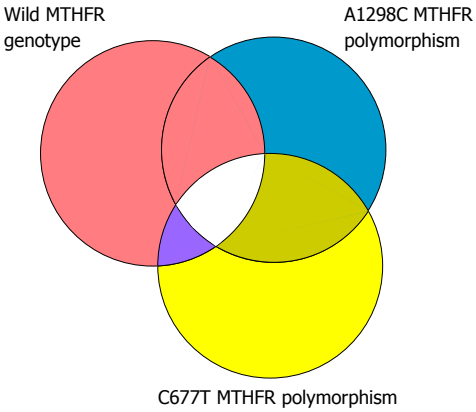

Figure 2 Venn diagram showing proportionally the overlap of methylenetetrahydrofolate reductase genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population. MTHFR: Methylenetetrahydrofolate reductase.

(0.097-0.235), with a Specificity of 0.726 (0.687-0.763); the positive predictive value is 0.1 (0.062-0.156) and the negative predictive value is 0.813 (0.775-0.845); the positive likelihood ratio is LR+ 0.562 (0.351-0.901), the negative likelihood ratio is LR- 1.165 (1.057-1.284). Similarly, for the wild MTHFR genotype, Sensitivity is 0.383 (0.291-0.484); Specificity is 0.769 (0.731-0.802); PPV is 0.225 (0.167-0.296); NPV is 0.877 (0.844-0.903); LR+ is 1.655 (1.227-2.233) and LR- is 0.803 (0.68-0.948).

In Figure 2 the polymorphism overlap is displayed by Venn diagram showing proportionally the overlap of MTHFR genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population.

Odds to LVH (assessed as increased Left Ventricular Myocardial Mass by Echocardiography), by the comparison of the prevalence of LVH within the wild

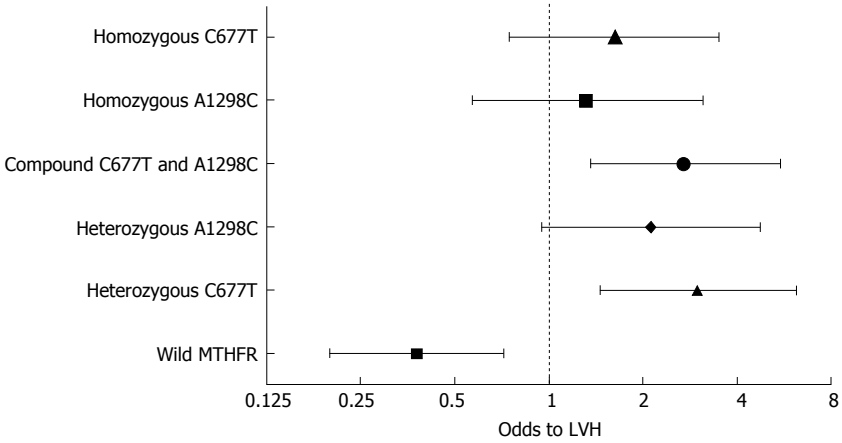

Figure 3 Odds to left ventricular hypertrophy (increased left ventricular myocardial mass assessed by echocardiography). Comparison of prevalence of LVH within the wild MTHFR genotype (12/94; 12.7%) *vs* the polymorphism MTHFR group (131/470; 27.9%): OR = 0.3787; 95%CI: 0.2000 to 0.7171; Z statistic 2.981; *P* = 0.0029, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms. The individual odds of LVH, according to the specific MTHFR polymorphism status are as follows: Heterozygous C677T (12/94; 12.7% *vs* 36/118); OR = 3.0000, 95%CI: 1.4581 to 6.1725, Z statistic 2.985, *P* = 0.0028; Heterozygous A1298C (12/94; 12.7% *vs* 18/76); OR = 2.1207, 95%CI: 0.9490 to 4.7393, Z statistic 1.832, *P* = 0.0669; Compound Heterozygous C677T and A1298C (12/94; 12.7% *vs* 44/154); OR = 2.7333, 95%CI: 1.3581 to 5.5012, Z statistic 2.818, *P* = 0.0048; Homozygous A1298C (12/94; 12.7% *vs* 13/80); OR = 1.3259, 95%CI: 0.5676 to 3.0972, Z statistic 0.652, *P* = 0.5146; Homozygous C677T (12/94; 12.7% *vs* 20/104); OR = 1.6270, 95%CI: 0.7475 to 3.5410, Z statistic 1.227, *P* = 0.2199. LVH: Left ventricular hypertrophy; MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio.

Table 3 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (all patients)

	Wild MTHFR (*n* = 94)	Heterozygous C677T (*n* = 118)	Heterozygous A1298C (*n* = 76)	Compound heterozygous C677T and A1298C (*n* = 154)	Homozygous A1298C (*n* = 80)	Homozygous C677T (*n* = 104)	χ^2	*P*
high RRI	24	30	16	38	28	22	5.746	0.332
EF < 50%	4	4	4	14	4	0	11.188	0.048
E/A > 1	68	100	66	74	42	70	53.497	< 0.0001
LVH (high LVMM)	12	36	18	44	13	20	14.923	0.011

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; LVH: Left ventricular hypertrophy.

MTHFR genotype (12/94; 12.7%) *vs* the polymorphism MTHFR group (131/470; 27.9 %) displays an OR = 0.3787; 95%CI: 0.2000-0.7171; Z statistic 2.981; *P* = 0.0029, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms (Figure 3). The individual odds of LVH, according to the specific MTHFR polymorphism status, confirm substantially this result, *i.e.*, that MTHFR polymorphisms are associated with LVH. Differences are not significant assessing Ejection fraction and Renal Resistive index. The E/A ratio, *i.e.*, the measurement of left ventricular transmitral filling, and index of overall left ventricular distensibility, is higher, *i.e.*, better, is subjects with the wild MTHFR genotype (Tables 3-5).

DISCUSSION

According to our study, the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show an association with chronic renal failure patients requiring hemodialysis which suggests some protective role in comparison with the wild MTHFR genotype. Despite the apparent disagreement with the available studied with renal disease patients, this result is less surprising of what can appear at the first glance. Even with the limitations of an observational study, based on the reappraisal of the information available in our data base investigating within a greater population that includes a subgroup of dialysis patients, we find that the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism[35].

Homocysteine is settled as a putative risk factor for cardiovascular disease[47] and mechanisms for glomerular injury and progression of renal insufficiency are envisaged[48]. Although high-dose folic acid would slow the progression of atherosclerosis and reduce

Table 4 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (chronic renal failure patients-hemodialysis)

	Wild MTHFR (*n* = 36)	Heterozygous C677T (*n* = 28)	Heterozygous A1298C (*n* = 20)	Compound heterozygous C677T and A1298C (*n* = 40)	Homozygous A1298C (*n* = 20)	Homozygous C677T (*n* = 16)	χ^2	*P*
High RRI	20	20	8	28	16	12	10.535	0.061
EF < 50%	4	4	4	12	4	0	9.114	0.105
E/A > 1	24	24	16	8	0	0	72.305	< 0.0001
High LVMM	8	16	4	32	5	4	38.428	< 0.0001

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

Table 5 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (normal renal function patients)

	Wild MTHFR (*n* = 58)	Heterozygous C677T (*n* = 90)	Heterozygous A1298C (*n* = 56)	Compound heterozygous C677T and A1298C (*n* = 118)	Homozygous A1298C (*n* = 62)	Homozygous C677T (*n* = 88)	χ^2	*P*
high RRI	4	10	8	10	12	10	6.833	0.233
EF < 50%	0	0	0	2	0	0	5.798	0.326
E/A > 1	44	76	50	66	42	70	19.848	0.001
High LVMM	4	20	14	12	8	16	13.355	0.02

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

cardiovascular events in patients with chronic renal failure, counteracting effects of hyperhomocysteinemia, is still debated and not demonstrated[49]. Differently, there is a good consistency of data that establishes renal involvement and LV hypertrophy as novel risk factors for morbidity and mortality in diabetes mellitus[50]. Cardiac remodeling, also with increase of LVMM, is a premise toward the development of heart insufficiency[51], which could be redefined also encompassing serological biomarkers[52]. The favorable relevance of adherence to healthier nutritional profile and lifestyle changes is well established and warranted in cardiac disease[53,54] and also, by more recent contributions, in renal disease[55]. In earlier studies[56,57] relationship of MTHFR C677T mutation with renal and cardiac involvement was associated with precocious target organ damage. Actually, in younger subjects[58] and in other reports[59] homozygosity for the C677T mutation is not unequivocally associated with increased risk for cardiovascular disease, irrespective of folate intake. This is confirmed by a recent extensive epidemiological study, in which despite lower serum folate and higher homocysteine, MTHFR 677TT genotype, used as a proxy for lifelong high blood homocysteine concentrations, is associated with a significantly lower risk of CVD mortality[60]. Hyperhomocysteinemia is common in patients with severe heart failure, and plasma homocysteine levels are uniformly elevated regardless of the etiology of heart failure. Elevated plasma homocysteine levels are likely a consequence of heart failure-related renal insufficiency[61]. Moreover, high homocysteine levels in patients with end-stage renal disease were not associated with incidence of vascular access thrombosis[62]. In our study, MTHFR C677T mutation occurs in a population which has still a relatively low prevalence of cardiovascular[5] and renal disease[55]. It is possible that this polymorphism, even associated with greater LVMMi, could have maintained its persistence in human populations by an heterozygosis-mutant advantage mechanism exerted over more critical conditions, including the occurrence of renal insufficiency. All-cause and coronary heart disease death rates are low in cohorts with greater adherence to Mediterranean Diet.

In conclusion, *MTHFR* 677C>T and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms among our dialysis patients in end-stage renal failure; differently, the association with LV hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism related to MTHFR polymorphisms.

ACKNOWLEDGMENTS

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COMMENTS

Background

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that Folic acid based regimens are not recommended as a generalized approach in the prevention of

cardiovascular events in chronic kidney disease.

Research frontiers

A similar finding was reported in fatty liver disease in which it is suggested that methylenetetrahydrofolate reductase (MTHFR) polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure.

Innovations and breakthroughs

The authors reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with left ventricular (LV) hypertrophy (LVH), high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and renal artery resistive index (RRI). Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate and greater hsCRP, iPTH, RRI, and LVH.

Applications

MTHFR gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among the authors dialysis patients in end-stage renal failure; differently, the association with LVH and reduced LV relaxation suggest some type of indirect, or concurrent mechanism.

Peer-review

This is a well written manuscript analysing the effect of *MTHFR* gene polymorphisms on renal and cardiac function.

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SYSTEMATIC REVIEWS

Are phosphodiesterase type 5 inhibitors effective for the management of lower urinary symptoms suggestive of benign prostatic hyperplasia?

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Abstract

AIM: To review the efficacy of phosphodiesterase type 5 inhibitors (PDE5-Is) in lower urinary tract symptoms (LUTS) suggestive of benign prostate hyperplasia (LUTS/BPH).

METHODS: A comprehensive research was conducted to identify all publications relating to benign prostate hyperplasia and treatment with sildenafil, vardenafil and tadalafil. To assess the efficacy, the changes in total international prostate symptom score (IPSS), IPSS subscore including voiding, storage and quality of life (QoL), Benign prostatic hyperplasia Impact Index (BII), maximum urinary flow rate (Qmax) and the International Index of Erectile Function (IIEF) were extracted. A meta-analytical technique was used for the analysis of integrated data from the included studies to evaluate the mean difference in the results.

RESULTS: Total IPSS score, IIEF and BII showed a significant improvement in trials in which LUTS/BPH with or without erectile dysfunction (ED) were compared with the placebo. For LUTS/BPH, the mean differences of total IPSS score, IIEF and BII are -2.17, 4.88 and -0.43, $P < 0.00001$, respectively. For LUTS/BPH with comorbid ED, the mean difference are -1.97, 4.54 and -0.52, $P < 0.00001$, respectively. PDE5-Is appear to improve IPSS storage, voiding and QoL subscore (mean difference = -0.71, -1.23 and -0.33, $P < 0.00001$, respectively). Although four doses of tadalafil (2.5, 5, 10 and 20 mg) failed to reach significance in Qmax (mean difference = 0.22, $P = 0.10$), the 5 mg dose of tadalafil significantly improved the Qmax (mean difference = 0.33, $P = 0.03$).

CONCLUSION: PED5-Is demonstrated efficacy for improving LUTS in BPH patients with or without ED and could be considered to be the first line treatment for LUTS/BPH.

Key words: Phosphodiesterase type 5; Inhibitor; Lower urinary tract symptoms; Benign prostate hyperplasia; Tadalafil

Core tip: The efficacy of phosphodiesterase type 5 inhibitor (PDE5-I) in patients with lower urinary tract symptoms (LUTS) and benign prostate hyperplasia (BPH) has been evaluated and prescribed. Regardless of the significant improvement of total International Prostate Symptom Score and storage subscore, there are controversies about the urine flow rate. Also, we do not know the exact mechanism of how it works in the lower urinary tract. From the meta-analytical data, PDE5-I could be an alternative therapy for LUTS/BPH patients whether or not they have erectile dysfunction. Therefore, well designed large scale clinical trials are required to clarify the efficacy and action mechanisms of PDE5-Is in the management of LUTS/BPH.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a histopathological diagnosis characterized by epithelial cell and smooth muscle proliferation in the transition zone of the prostate leading to nonmalignant enlargement of the prostate, which may result in lower urinary tract symptoms (LUTS), including storage and voiding symptoms[1-3]. BPH is a common disease of aging men. Moderate to severe LUTS secondary to BPH (LUTS/BPH) is predicted to involve 10% to 25% of the contemporary male population (approximately 900 million men) throughout the world[1-3] and it is considered that presumably 1.1 billion males will suffer from LUTS/BPH by the year 2018[4].

It is widely acceptable that BPH is not the exclusive source of LUTS[1-4]. Over the decades, LUTS/BPH treatment paradigms have shifted from surgical interventions to first-line pharmacotherapy for symptom reduction and improvement in quality of life. However, clinical trials of drugs often enroll men based partially on a clinical diagnosis of non-neurogenic LUTS/BPH.

Pharmacotherapy for LUTS/BPH currently consists of alpha-blockers, 5 alpha-reductase inhibitors or combined therapy[1-4]. Although they are proved to be efficacious, these therapies have potential side effects linked to sexual dysfunction, such as reduced libido and ejaculatory disorders, dizziness and hypotension[5]. These side effects may be exacerbated by combination therapy. Phosphodiesterase type 5 inhibitors (PDE5-Is), consisting mainly of sildenafil, vardenafil and tadalafil, are extensively approved for curing erectile dysfunction (ED)[6,7]. Recently, significant improvement in LUTS/BPH has been reported by a large body of clinical studies on PDE5-Is[8-25]. Although improvement of the PDE5-Is mechanisms in LUTS/BPH have yet not been clearly clarified, proposed contributors include inhibition of PDE5 iso-enzymes, present in the bladder, prostate, urethra and supporting vasculature, and consequently elevation in intracellular nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) concentration which functions to inhibit RhoA/Rho kinase signaling pathways, mediates relaxation of the smooth muscle cells in these structures, improves blood perfusion and reduces afferent signaling in the urogenital tract[26-29]. Understanding these complicated mechanisms shows how PDE5-Is play a role in the treatment of LUTS/BPH and is indispensable for health care professionals to optimize both patient screening and treatment. Nevertheless, recent research has shown that PDE5-Is, either as monotherapy or combined with alpha blockers, also enhance LUTS/BPH, presumably *via* relaxation of smooth muscle in the bladder neck, urethra and prostate induced by the NO/cGMP signal pathway.

With the increasing interests in this efficacy, therefore, we systematically reviewed the literature to explore up-to-date evidence on the efficacy of PDE5-Is in LUTS/BPH.

Epidemiological survey: two common conditions in LUTS/BPH and ED?

Two conditions of LUTS associated with BPH and ED that occur with relatively high frequency in aging have triggered a great deal of concern over the last few decades. As the incidence of histopathological stromal-glandular hyperplasia rises, so does the prevalence of moderate to severe LUTS[30]. Correspondingly, the rate of ED also rises with aging. As such, it is not a surprise that many patients with LUTS will also suffer from ED and vice versa. The link between LUTS/BPH and ED has recently been the subject of significant studies[1,31]. Numerous publications have demonstrated a link between ED and LUTS, the epidemiology of which was summarized in a review[32]. It points out that the majority of well-designed longitudinal studies have been proposed to interpret the relationship between ED and LUTS, including varying NO levels, activated RhoA/Rho kinase and atherosclerosis in the pelvis.

A recent abstract from a larger cross-sectional and multinational assessment of LUTS and sexual function was conducted[33]. Logistic regression analysis showed that patients with severe LUTS were estimated to be twice as likely to suffer from erectile dysfunction (OR = 2.0, 95%CI: 1.4, 2.8) and decreased ejaculate (OR = 1.8, 95%CI: 1, 2.5). Furthermore, patients with severe LUTS were 6 fold as likely to complain of discomfort or pain on ejaculation. Another cross-sectional data analysis is from the multinational survey of the aging male (MSAM-7) in which patients aging fifty to eighty years demonstrated high rates of LUTS/BPH in the

United States and Europe (United Kingdom, France, Germany, Netherlands, Italy and Spain)[34]. In this survey, more than 50% of patients were bothered by ejaculatory dysfunction and it also showed that the link between LUTS and ejaculatory dysfunction still existed after controlling for age and other comorbidities.

Clinical studies of PDE5-Is: Are LUTS/BPH and ED independent?

It was speculated that enhancement in LUTS/BPH could be a result of ED improvement because PDE5-Is significantly mitigated the symptoms of LUTS/BPH and ED. As such, a couple of clinical studies have addressed whether the improvement of BPH symptoms is linked to improved ED symptoms[9,34]. In one study of dose-ranging tadalafil with 716 ED patients and 340 non-ED patients, alterations in LUTS/BPH after 3 mo of medication with distinct doses of tadalafil once daily and placebo was analogous in patients with or without comorbidity of ED, demonstrating that the enhancement in LUTS/BPH did not rely on ED alterations[35]. Another tadalafil study confirmed these finding[36]. As a consequence, they are independent of each other even although the mechanism by which PDE5-Is enhance LUTS/BPH could participate in analogous ways with PDE5-Is enhancing ED.

PDE5-I localization in the prostate

Much evidence from experimental research confirmed that the cGMP-degrading PDE5 as well as NO/cGMP signaling pathway are responsible for the regulation of the normal functions of the prostate, regulating proliferation of glandular epithelial cells and smooth muscle as well as stromal connective tissue[29,37]. As early as 1970, the activity of PDE5-Is isolated from human prostate tissue was confirmed by Kuciel and Ostrowski. However, this method could not tender sufficient data on the PDE5 localization in the prostate.

The golden criteria to detect PDE5 distribution in distinct histopathological portions of the prostate was disclosed by immunohistochemistry (IHC). It was demonstrated that cGMP PDE iso-enzyme localized in the glandular zone, the smooth musculature of stroma and blood vessels by utilization of antibodies[35]. It was also shown that PDE5 is detected in tight conjunction with other critical regulators of NO/cGMP pathway. The concentration of tadalafil in the prostate and plasma was 385.7 ± 83.8 and 305.8 ± 41.1 ng/mL, respectively. In addition, the ratio between tissue and plasma was 1.3[38]. Tadalafil and udenafil significantly enhanced the cGMP and cAMP levels in plasma and prostate tissue[38].

PDE5-Is mechanism of action

Briefly, the current postulated action mechanism in improvement of LUTS/BPH includes: (1) ascending NO synthase/NO activity in the prostate; (2) cGMP mediated protein kinase/endothelin inactivation; (3) decreased autonomic hyperactivity of the afferent nerve in the bladder and prostate; and (4) reduction of pelvic ischemia caused by atherosclerosis of pelvic vessels.

MATERIALS AND METHODS

Identification of studies and study design

We searched the following sources from inception to the specified date: (1) the Cochrane Library; (2) MEDLINE; and (3) EMBASE.

The studies in the present review met the following standards: (1) double blinded, clinical controlled trials; (2) LUTS/BPH was involved; and (3) control groups were given a placebo drug. Studies with PED5-Is monotherapy versus an alpha blocker or combination of both were excluded.

To assess the efficacy of PED5-Is, the outcomes of measurement contain at least one of: (1) International prostate symptom score (IPSS); (2) International index of erectile dysfunction (IIEF) score; (3) maximal urinary flow rate (Qmax); (4) IPSS quality of life index (IPSS-QoL); and (5) IPSS irritative (storage) subscore, IPSS obstructive (voiding) subscore and BPH impact index (BII).

Statistical analysis

The meta-analysis used the review manager (Version 5.3, the Cochrane Collaboration, Oxford, United Kingdom). The heterogeneity test was by χ^2 and I^2 ($I^2 \leqslant$ 50%, low heterogeneity; 50% < $I^2 \leqslant$ 75%, moderate heterogeneity; and I^2 > 75%, high heterogeneity). If the heterogeneity was less than 50%, the fixed-effects model was considered to estimate the integrated effect of the outcomes. For moderate or high heterogeneity, a random-effect was used. The continuous value was used as the mean difference with 95%CI.

RESULTS

Clinical trials with PED5-Is for LUTS/BPH

A total of 16 randomized, double blind and placebo-controlled trials investigated the efficacy and safety of tadalafil (n = 14), sildenafil (n = 1) and vardenafil (n = 1) for LUTS/BPH therapy and comorbidities of LUTS/BPH and ED (5 trials: Brock *et al*[39], 2013, Donatucci *et al*[14], 2011, Egerdie *et al*[15], 2012, McVary *et al*[18], 2007 and Porst *et al*[21], 2009, respectively). The characteristics of the studies are summarized in Table 1. The study designs were analogous, followed by up to 4 wk of washout periods in order to eliminate the medications prior to trials.

Efficacy of PDE5-Is of sildenafil, tadalafil and vardenafil

Sildenafil: In 2007, McVary *et al*[10] first reported that 189 patients given sildenafil had improved significantly in total IPSS score (sildenafil *vs* placebo: -6.3 *vs* -1.93, P < 0.0001), IPSS QoL subscore (sildenafil *vs* placebo: -0.97 *vs* -0.29, P < 0.0001), BII (sildenafil *vs* placebo: -2.0 *vs* -0.9, P < 0.001) and IIEF-EF domain

Table 1 Characteristics and qualities of the studies included in the analysis of tadalafil, sildenafil and vardenafil

Ref.	Sample size	Drug (mg)		Duration (wk)	Run-in period (wk)	Inclusion criteria	Publications
		Trial	Control				
Tadalafil							
Brock *et al*[39]	1089	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*BJU Int*
Dmochowski *et al*[13]	200	20	Placebo	12	4	Mean age ≥ 40, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*J Urol*
Donatucci *et al*[14]	427	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*BJU Int*
Egerdie *et al*[15]	606	2.5, 5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Sex Med*
Kim *et al*[16]	102	5	Placebo	12	6	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*LUTS*
McVary *et al*[18]	281	5 + 20	Placebo	6 + 6	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Urol*
Oelke *et al*[19]	343	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[21]	581	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[36]	325	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[20]	1500	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Urology*
Roehrborn *et al*[22]	1058	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45-60, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s, PVR 150-550 mL	*J Urol*
Roehrborn *et al*[12]	1500	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Urol*
Takeda *et al*[24]	610	5	Placebo	12	4	Mean age ≥ 40, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*J Urol*
Yokoyama *et al*[25]	460	2.5, 5	Placebo	12	2	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s, prostate volume ≥ 20 mL	*Int J Urol*
Sildenafil							
McVary *et al*[10]	369	50, 100	Placebo	12	4	Mean age ≥ 45, IIEF ≤ 25, IPSS ≥ 12	*J Urol*
Vardenafil							
Stief *et al*[40]	222	10	Placebo	8	4	Mean age ≥ 45-64, LUTS/BPH ≥ 6 mo, IPSS ≥ 12	*Eur Urol*

IIEF: International index of erectile function; IPSS: International prostate symptom; LUTS/BPH: Lower urinary tract symptoms/benign prostatic hyperplasia; Qmax: Maximum urinary flow rate; PVR: Post-void residual volume.

score (sildenafil *vs* placebo: 9.17 *vs* 1.86, $P < 0.0001$) compared to the placebo group after 12 wk of daily treatment (50 mg for 2 wk, then increased to 100 mg). No significant difference of Qmax was observed between two groups ($P = 0.08$); it is possible that relaxation of the urethra and prostate musculature would tend to enhance urinary flow, but relaxation of the bladder could more or less counteract these effects after administration of PDE5-Is (Table 2).

Vardenafil: In one randomized, double blind, placebo-controlled study, Stief *et al*[40] investigated the efficacy of 10 mg vardenafil in LUTS/BPH patients with or without concomitant ED. After 8 wk of therapy, significant improvement in total IPSS score (vardenafil *vs* placebo: -5.8 *vs* -3.1, $P < 0.05$), IPSS voiding subscore, IPSS storage subscore and IPSS QoL score were observed in the vardenafil group compared to the placebo group ($P < 0.0001$, respectively) (Table 2). Although Qmax was enhanced in vardenafil group, there was no significant difference (vardenafil *vs* placebo: 1.6 mL/s *vs* 1 mL/s) (Table 2). Overall, the most frequent adverse events (AEs) consisted of headaches, flushing and dyspepsia, reported in 32 (29.6%) patients in the vardenafil group and 18 (15.9%) in the placebo group. None of the serious AEs was linked to the vardenafil medication. Nevertheless, it is too soon to consider the underlying role for vardenafil in LUTS/BPH therapy because further data clearly needed to ascertain the benefit-risk details relative to the existing treatment options were not provided.

Tadalafil: A total of 14 randomized, double-blind, placebo-controlled studies have showed the efficacy and safety of once daily tadalafil medication in the management of LUTS/BPH. A one year open label trial demonstrated the sustainability of efficacy and safety of once daily tadalafil long term[14]. The efficacy outcomes are summarized in Table 2.

Brock *et al*[39] (2013), investigating the efficacy of once daily tadalafil in the treatment of LUTS/BPH patients with or without ED, first noted that the effects of therapy in men without ED were analogous to that with ED in LUTS/BPH. In patients without ED, the LUTS/BPH total IPSS score (tadalafil *vs* placebo: -5.4 *vs* -3.3, $P < 0.01$), IPSS voiding subscore (tadalafil *vs* placebo: -3.5 *vs* -2.0, $P < 0.01$) and IPSS storage subscore (tadalafil *vs* placebo: -1.9 *vs* -1.3, $P < 0.05$) from baseline to end points was reduced significantly and IPSS QoL (tadalafil *vs* placebo: -1.0 *vs* -0.7, $P < 0.05$) and BII (tadalafil *vs* placebo: -1.4 *vs* -1.0, $P < 0.05$) were significantly improved. However, a small

Table 2 Least squares mean changes from baseline to end-point in lower urinary tract symptoms/benign prostatic hyperplasia in clinical studies for treatment in subjects with erectile dysfunction and without erectile dysfunction

Ref.	Drug	Remarks	Total IPSS		IPSS voiding subscore		IPSS storage subscore		IPSS QoL subscore		BII		Qmax		IIEF	
	mg		T	P	T	P	T	P	T	P	T	P	T	P	T	P
	Tadalafil															
Brock *et al*[39]	5	ED	-5.7	-3.3	-3.5	-1.9	-2.2	-1.3	-1.1	-0.7	-1.6	-0.9				
	5	No ED	-5.4	-3.3	-3.5	-2	-1.9	-1.3	-1		-1.4	-1				
Dmochowski *et al*[13]	20		-9.2	-5.1	-5.6	-2.8	-3.6	-2.3					0.4	0.5		
Donatucci *et al*[14]	2.5		-5.7	-4.1	-3.8	-2.5	-1.9	-1.6	-1.2	-0.9	-1.3	-1.2			5.5	7.2
	5		-5.0		-2.8		-2.1		-1.1		-1.4				5.3	
	10		-5.7		-3.6		-1.8		-1.3		-1.4				3.7	
	20		-4.6		-3		-2.1		-1		1.2				7.6	
Egerdie *et al*[15]	2.5		-6.1	-3.8	-2.7	-2.2	-1.9	-1.6	-0.9	-0.8	-2.1	-1.2			6.5	1.8
	5		-4.6		-3.6		-2.5		-1		-1.6				5.2	
Kim *et al*[16]	5		-5.6	-3.6									2.5	2.3		
McVary *et al*[18]	5		-6.2	-3.9	-4	-2.5	-2.2	-1.4	-0.7	-0.3	-0.7	-0.4	0.5	0.9	6.7	0.7
	5/20		-7.1	-4.5	-4.4	-2.8	-2.7	-1.8	-0.5	-0.2	-1.3	-0.6	0.5		8.4	1.6
Oelke *et al*[19]	5		-6.3	-4.2	-4.1	-2.6	-2.2	-1.6	-1.3	-1			2.4	1.2		
Porst *et al*[21]	2.5		-4.2	-2.1											8.2	2
	5		-4.7												7.9	
	10		-4.7												6.8	
	20		-3.6												5.4	
Porst *et al*[36]	5		-5.6	-3.6	-3.3	-2.3	-2.3	-1.3			-1.8	-1.3			6.7	2
Porst *et al*[20]	5		-7.9	-5.1												
Roehrborn *et al*[22]	2.5		-5.17	-2.27	-2.94	-1.26	-1.96	-0.99	-0.92	-0.49	-1.38	-0.83	1.96	1.24	8.34	2.2
	5		-3.88		-2.23		-2.07		-0.88		-1.4		1.41		7.98	
	10		-5.21		-3.12		-1.58		-0.74		-0.96		1.64		6.97	
	20		-4.87		-3.13		-1.89		-0.86		-1.45		1.58		5.59	
Roehrborn *et al*[12]	5	Qmax < 10	-5.2	-3.6	-3.2	-2.1							2.8	2.4		
	5	Qmax of 10-15	-6.3	-3.8	-3.9	-2.5							1.4	0.9		
	5	Qmax > 15	-6.8	-2.7	-3.9	-1.2							-1.1	-2.7		
Takeda *et al*[24]	5		-6	-4.5	-2	-1.4	-2	-1.4	-2	-1.4						
Yokoyama *et al*[25]	2.5		-5	-3	-3.3	-1.9	-1.7	-1.1	-0.8	-0.5	-1	-0.8	1.3	2.1		
	5		-5.1		-3.72		-1.5		-0.8		-1.1		1.6			
	Sildenafil															
McVary *et al*[10]	50		-6.3	-1.9					-0.9	-0.3	-2	-0.9	0.31	0.16	9.17	1.86
	100															
	Vardenafil															
Stief *et al*[40]	10		-5.8	-3.6									1.6	1		

IIEF: International index of erectile function; IPSS: International prostate symptom; Qmax: Maximum urinary flow rate; QoL: Quality of life; BII: Benign prostatic hyperplasia impact index; ED: Erectile dysfunction; T: Treatment; P: Placebo.

Qmax improvement was still consistent with the poor link between Qmax and LUTS/BPH in the updated BPH guidelines[41]. The limitation of methodology in choosing an ED or non-ED population is when sexually active patients with LUTS/BPH but no ED history were managed in blind, placebo-controlled trials. Therefore, clinical ED determination alone could not fully exclude ED in this reference groups enrolled for LUTS/BPH.

In another multicenter, randomized, double-blind, placebo-controlled clinical trial with LUTS/BPH patients treated once daily with 20 mg tadalafil for 12 wk, Dmochowski *et al*[13] (2010) pointed out that tadalafil significantly improved total IPSS score (tadalafil *vs* placebo: -9.2 *vs* -5.1, $P < 0.001$), voiding subscore (tadalafil *vs* placebo: -5.6 *vs* -2.8, $P < 0.001$) and storage subscore (tadalafil *vs* placebo: -3.6 *vs* -2.3, $P = 0.006$) compared to the placebo group. Qmax from baseline to endpoints showed a small alteration with no significant difference (tadalafil *vs* placebo: -2.1 *vs* 0.1, $P = 0.33$). In addition, several points should be noted when considering these trials. A relatively high tadalafil dose was used without assessing rigorous intent to treat patients. Thus, the magnitude of improvement investigated in these trials in future clinical utilization should be treated with caution.

Donatucci *et al*[14] completed a double blind, placebo controlled, open-label 12 wk trial of tadalafil (2.5 mg, 5 mg, 10 mg or 20 mg once daily) extended to 1 year. The changes from baseline to endpoint in the total IPSS, IPSS voiding subscore, IPSS storage subscore, IPSS health-related QoL and BII were sustained after one year. Besides, the IIEF-EF was also maintained after 1 year. Higher treatment-induced emergent AEs (57.6% of patients) were observed in the higher dose group but 5 mg tadalafil was well tolerated. Although the efficacy of improvement from baseline or 12 wk to endpoint was noted, the changes from baseline to 12 wk were not reported. Qmax was not evaluated in this trial.

Egerdie *et al*[15] conducted a multinational phase 3 (12 wk) randomized, double blind and control-placebo trial to assess the efficacy of tadalafil 2.5 or 5 mg in the management of LUTS/BPH with ED patients. In this study, both doses of tadalafil significantly improved the IIEF-EF (tadalafil *vs* placebo: 6.5, 5.2 *vs* 1.8, both $P < 0.001$). Improvement with 5 mg but not 2.5 mg in IPSS voiding subscore (tadalafil *vs* placebo: -3.6 *vs* -2.2, $P < 0.001$), storage subscore (tadalafil *vs* placebo: -2.5 *vs* -1.6, $P < 0.001$) and BII (tadalafil *vs* placebo: -1.6 *vs* -1.2, $P < 0.001$) was observed but QoL subscore (tadalafil *vs* placebo: -1 *vs* -0.8, $P = 0.082$) failed to reach a significant difference (Table 2).

Kim *et al*[16] reported a 12 wk randomized, double-blind, controlled-placebo trial of LUTS/BPH in Korean men for once daily tadalafil 5 mg. From baseline to endpoint, the total IPSS and Qmax mean changes were numerically but not significantly improved compared with placebo (tadalafil *vs* placebo: IPSS, -5.6 *vs* -3.6, $P > 0.05$ and Qmax, 2.5 *vs* 2.3, $P > 0.05$).

In 2007, McVary *et al*[18] conducted a trial of 281 men allocated randomly to 5 mg tadalafil once daily for 6 wk with a dose escalation to 20 mg for another 6 wk. There was a significant difference in IIEF-EF (tadalafil *vs* placebo: 8.4 *vs* 1.6, $P < 0.001$), total IPSS score (tadalafil *vs* placebo: -7.1 *vs* -4.5, $P < 0.001$), voiding subscore (tadalafil *vs* placebo: -4.4 *vs* -2.8, $P < 0.0001$), storage subscore (tadalafil *vs* placebo: -2.7 *vs* -1.8, $P < 0.001$) and QoL (tadalafil *vs* placebo: -0.5 *vs* -0.2, $P < 0.001$). However, the difference of Qmax was not significant when comparing tadalafil to placebo (tadalafil *vs* placebo: 0.5 *vs* 0.9, $P > 0.05$).

Oelke *et al*[19] investigated the efficacy of 5 mg tadalafil once daily monotherapy through 12 wk of therapy of LUTS/BPH in a randomized, double- blind, international controlled-placebo study. Total IPSS score significantly improved with tadalafil (tadalafil *vs* placebo: -6.3 *vs* -4.2, $P = 0.001$). Significant improvement in voiding subscore (tadalafil *vs* placebo: -4.1 *vs* -2.6, $P < 0.001$) but not storage subscore (tadalafil *vs* placebo: -2.2 *vs* -1.6, $P = 0.055$) and QoL subscore (tadalafil *vs* placebo: -1.3 *vs* -1.0, $P = 0.022$) was observed from baseline to endpoint in this trial. Qmax increased significantly (tadalafil *vs* placebo: 2.4 *vs* 1.2, $P = 0.009$). Nevertheless, this trial was of 12 wk duration to evaluate the efficacy of LUTS/BPH and did not address longer term efficacy of tadalafil on disease progression. Maybe this kind of trial would trigger great interest in the future.

In a phase 2 to 3, multinational, randomized, double-blind, controlled-placebo study, Porst *et al*[21] (2009) randomly assigned patients to tadalafil 2.5 mg, 5 mg, 10 mg and 20 mg once daily for 12 wk. The least square mean difference of IIEF-EF compared to placebo (the value: 2) was significant for all four doses of tadalafil (2.5 mg dose, 8.2; 5 mg dose, 7.9; 10 mg dose, 6.8, and 20 mg dose, 5.4) (all $P < 0.001$). The mean changes of total IPSS score from baseline to endpoint reached a significant difference (tadalafil *vs* placebo: 2.5 mg, -4.2 *vs* -2.1, $P = 0.015$; 5 mg, -4.7 *vs* -2.1, $P < 0.001$; 10 mg, -4.7 *vs* -2.1, $P < 0.001$, and 20 mg, -3.6 *vs* -2.1, $P < 0.001$). However, Qmax failed to reach significance for treatment groups. The limitation could be the absence of a parallel group without LUTS/BPH as a control reference and it could not summarize the minimum times of sexual intercourse monthly before allocation and the trial duration, which could measure the risk-benefit of once daily tadalafil for IIEF-EF improvement.

In a second randomized, double-blind, placebo-controlled 12 wk study, Porst *et al*[36] pointed out that 5 mg tadalafil significantly improved total IPSS score (tadalafil *vs* placebo: -5.6 *vs* -3.6, $P = 0.004$), voiding subscore (tadalafil *vs* placebo: -3.3 *vs* -2.3, $P = 0.020$), storage subscore (tadalafil *vs* placebo: -2.3 *vs* -1.3, $P < 0.002$), QoL index (tadalafil *vs* placebo: -1.0 *vs* -0.7, $P = 0.013$) and BII (tadalafil *vs* placebo: -1.8 *vs* -1.2, $P = 0.029$) from baseline to endpoint. However, uroflowmetry parameters did not show a significant difference at the endpoint. The IIEF-EF in ED men was significantly improved at 12 wk (tadalafil *vs* placebo: 6.7 *vs* 2.0, $P < 0.001$).

In 2013, Porst *et al*[20] pooled data from 4 multinational, randomized, placebo-controlled clinical trials to investigate 5 mg tadalafil once daily for LUTS/BPH for 12 wk. The pooled data confirmed that tadalafil resulted

Table 3 Outcomes of the meta-analysis of total international prostate symptom score, international prostate symptom score storage subscore, international prostate symptom score voiding subscore, international prostate symptom score quality of life subscore, benign prostatic hyperplasia impact index, maximum urinary flow rate, and international index of erectile function score in lower urinary tract symptoms/benign prostatic hyperplasia or lower urinary tract symptoms/benign prostatic hyperplasia and erectile dysfunction patients

Outcome or subgroup	Studies	Participants	Weight	Statistical method	Effect Estimate (Mean difference, 95%CI)	Heterogeneity χ^2	Heterogeneity I^2 (%)	Overall *Z* value	*P* value
Total IPSS in LUTS/BPH	13	9131	100%	Fixed	-2.17 (-2.42, -1.91)	16.44	0	16.75	< 0.00001
Tadalafil	11	8576	95.5%	Fixed	-2.14 (-2.40, -1.88)	13.27	0	16.18	< 0.00001
Sildenafil	1	341	1.0%	Fixed	-4.40 (-6.87, -1.93)			3.48	0.001
Vardenafil	1	214	3.4%	Fixed	-2.20 (-3.57, -0.83)			3.14	0.002
Total IPSS in LUTS/BPH and ED	6	3626	100%	Fixed	-1.97 (-2.43, -1.51)	12.33	3	8.41	< 0.00001
Tadalafil	5	3285	96.6%	Fixed	-1.88 (-2.35, -1.41)	8.49	0	7.90	< 0.00001
Sildenafil	1	341	3.4%	Fixed	-4.40 (-6.87, -1.93)			3.48	0.001
IPSS storage subscore in LUTS/BPH									
Tadalafil	10	6848	100%	Fixed	-0.71 (-0.85, -0.57)	12.64	0	9.96	< 0.00001
IPSS voiding subscore in LUTS/BPH									
Tadalafil	11	7916	100%	Fixed	-1.23 (-1.41, -1.04)	24.7	15	13.28	< 0.00001
IPSS QoL subscore in LUTS/BPH	8	5999	100%	Fixed	-0.33 (-0.40, -0.26)	8.26	0	8.70	< 0.00001
Tadalafil	7	5648	97.7%	Fixed	-0.32 (-0.40, -0.25)	6.26	0	8.38	< 0.00001
Sildenafil	1	351	2.3%	Fixed	-0.68 (-1.17, -0.19)			2.71	0.007
BII in LUTS/BPH									
Tadalafil	5	3504	100%	Fixed	-0.43 (-0.61, -0.25)	3.89	0	4.64	< 0.00001
BII in LUTS/BPH and ED	4	2561	100%	Fixed	-0.52 (-0.74, -0.29)	8.02	13	4.51	< 0.00001
Tadalafil	3	2210	94.8%	Fixed	-0.48 (-0.71, -0.25)	6.59	9	4.11	< 0.0001
Sildenafil	1	351	5.2%	Fixed	-1.10 (-2.08, -0.12)			2.19	0.03
Qmax in LUTS/BPH									
Tadalafil (2.5, 5, 10 and 20 mg)	9	5034	64.9%	Fixed	0.22 (-0.04, 0.49)	13.43	3	1.65	0.10
Tadalafil (only 5 mg)	7	2876	35.1%	Fixed	0.33 (-0.13, 0.80)	8.24	24	2.14	0.03
IIEF in LUTS/BPH									
Tadalafil	2	2009	100%	Fixed	4.88 (3.31, 8.97)	2.28	0	8.96	< 0.00001
IIEF in LUTS/BPH and ED									
Tadalafil	3	1746	100%	Fixed	4.54 (3.75, 5.33)	7.33	18	11.27	< 0.00001

IIEF: International index of erectile function; IPSS: International prostate symptom score; LUTS/BPH: Lower urinary tract symptoms/benign prostatic hyperplasia; Qmax: Maximum urinary flow rate; QoL: Quality of life; BII: Benign prostatic hyperplasia impact index; ED: Erectile dysfunction.

in improvement in total IPSS score from baseline to endpoint (tadalafil *vs* placebo: -7.9 *vs* -5.1, $P < 0.001$), as well as IPSS QoL index and BII (both $P < 0.01$).

Roehrborn *et al*[22] conducted a 12 wk randomized, double-blind, placebo-controlled, dose-finding study in 10 countries. They randomly assigned the patient to tadalafil 2.5 mg, 5 mg, 10 mg and 20 mg once daily for 12 wk. The least square mean difference of IIEF-EF compared to placebo (the value: 2.2) was significant for all four doses of tadalafil (2.5 mg dose, 5.59; 5 mg dose, 6.97; 10 mg dose, 7.98; and 20 mg dose, 8.34) (all $P < 0.001$). The mean changes of total IPSS score from baseline to endpoint reached a significant difference (tadalafil *vs* placebo: 2.5 mg, -3.9 *vs* -2.3, $P = 0.015$; 5 mg, -4.9 *vs* -2.3, $P < 0.001$; 10 mg, -5.2 *vs* -2.3, $P < 0.001$; and 20 mg, -5.2 *vs* -2.3, $P < 0.001$) and the voiding subscore, storage subscore, QoL index and BII all reached a significant difference ($P < 0.01$, 0.001, 0.05 and 0.05, respectively). However, Qmax failed to reach significance for the medication groups.

In the second study by Roehrborn *et al*[12] (2013), with 5 mg tadalafil for the LUTS/BPH for 12 wk, the effects on the Qmax with LUTS/BPH were investigated. Qmax changes were assessed compared to baseline Qmax. For baseline Qmax < 10 mL/s, increases were higher in tadalafil compared with the placebo group (tadalafil *vs* placebo: 2.8 *vs* 2.4, $P = 0.189$); for Qmax of 10 to 15 mL/s, (tadalafil *vs* placebo: 1.4 *vs* 0.9, $P = 0.044$); and for Qmax > 15 ml/s, (tadalafil *vs* placebo: -1.1 *vs* -2.7, $P = 0.246$).

Takeda *et al*[24] (2014) pooled data of randomized, double-blind, placebo-controlled studies of tadalafil 5 mg from 39 sites in Japan and Korea. Total IPSS score significantly improved with tadalafil (-6 *vs* -4.5, $P = 0.001$). Significant improvement in IPSS voiding subscore (tadalafil *vs* placebo: -4 *vs* -3.1, $P = 0.002$), IPSS storage subscore (tadalafil *vs* placebo: -2 *vs* -1.4, $P = 0.002$) and IPSS QoL subscore (tadalafil *vs* placebo: -1.1 *vs* -0.9, $P = 0.038$) was observed from baseline to endpoint in this trial.

Yokoyama *et al*[25] investigated the effects of tadalafil 2.5 mg and 5 mg in a multicenter, randomized, double-blind, placebo-controlled study from 34 sites in Japan, South Korea and Taiwan. Except for Qmax and BII index, the total IPSS score, voiding subscore, storage subscore and QoL subscore reached a significant difference.

The outcomes of meta-analysis of PDE5-Is on LUTS/BPH from integrated studies

The data were pooled for calculations and computed for integrated analysis. Heterogeneity was not observed (I^2 < 30%) and the fixed effect model was used.

For participants with comorbid LUTS/BPH and ED, the total IPSS, BII and IIEF-EF were divided into two subgroups: subgroup with LUTS/BPH and subgroup with LUTS/BPH and ED. Irrespective of overall group or subgroup analysis, PDE5-Is, especially tadalafil, showed an improvement of total IPSS, BII and IIEF domain (P < 0.0001 or P < 0.00001, Table 3). Changes in the storage, voiding and QoL were also reported (P < 0.00001, Table 3). Changes of Qmax for tadalafil at a dose of 5 mg was calculated in LUTS/BPH patients and showed a significant improvement [0.33 (-0.13, 0.80), P < 0.03, Table 3].

COMMENTS

Background

Lower urinary tract symptoms suggestive of benign prostate hyperplasia (LUTS/BPH) are increasingly frequent in aging men. The majority coexist with erectile dysfunction (ED). Irrespective of coexisting ED, LUTS/BPH patients frequently suffer from a poorer quality of life (QoL).

Research frontiers

Until recently, surgical therapy was the cornerstone of management for male LUTS. As early as 1990s, medical therapy became a possible treatment option for voiding problems. Since then, the surgical option has dropped gradually and currently the first option for treatment of male LUTS is medical therapy. 5-alpha reductase inhibitors and α-blockers have dominated the management of LUTS for many years. Nowadays, new drugs have cast a light on the treatment of LUTS, including PDE5-Is and anticholinergics. In the traditional sense, LUTS occurring with aging has frequently been associated with outlet obstruction in the bladder resulting from BPH, whereas the complaint may be explained by the detrusor overactivity. More recently, increasing evidence has shown that phosphodiesterase type 5 inhibitor (PDE5-Is) could exert improvement in LUTS in aging men who frequently suffer from BPH.

Innovations and breakthroughs

PDE5-Is, including mainly tadalafil, sildenafil and vardenafil, were the first line medication to treat ED patients. More and more randomized controlled trials (RCT) have been done to examine the efficacy of PDE5-Is for treatment of LUTS/BPH. As reported, PDE5-Is might have influenced the terminal decision because of distinct pharmacological profiles and side effects and the enthusiasm for PDE5-Is has decreased due to the lack of objective improvement. Furthermore, urodynamic parameters did not change. More important, coherently explaining the disconnection between objective and subjective changes is still pending. Therefore, it is necessary to determine whether PDE5-Is are effective in the treatment of LUTS/BPH on the basis of a systematic review and meta-analysis of published evidence. Meta-analysis has been increasingly utilized since it was introduced to assess clinical data in the urological community by Peter Boyle. In particular, it could give rise to invaluable insights for benefits. To a large extent, even although a large database was available, some predictive characteristics for responders and non-responders could still not be identified. However, all the convincing studies showed that LUTS was significantly alleviated by the regular use of PDE5-Is. In other words, the available studies on the use of PDE5-Is for the treatment of LUTS are promising. Especially in aging males, there is an increased prevalence of LUTS/BPH. Daily PDE5-Is might be a useful treatment for this condition as such a pharmacological strategy has the potential to become the treatment to manage the aging process of the male urogenital tract. Although the present manuscript underscores that PDE5-Is are a promising therapy for LUTS/BPH from other researchers, a couple of questions are still worthy of considering, including patient selection, durability and health economics, in the case of PDE5-Is for treatment of LUTS. In an ideal world, some situations could inevitably be avoided between doctors and patients while using PED5-Is for patients with any given condition. Firstly, the best candidates should be screened with male LUTS patients alone receiving any given treatment. Secondly, patients should be informed about the potential limitations of PDE5-Is during the treatment of their complaints. Thirdly, who is going to have what kind of treatment and when? In addition, the best practice includes the doctor's choice as well as the patient's.

Applications

PDE5-Is significantly improved total international prostate symptom score (IPSS) score, IPSS voiding score, IPSS storage score, IPSS QoL score and international index of erectile dysfunction score (IIEF)-EF score. Significant improvement of total IPSS score and IIEF-EF score was observed in patients with comorbid ED and BPH. As such, PDE5-Is as the first line for management of ED was also demonstrated to be effective for LUTS/BPH. Therefore, well designed clinical studies of large scales are required to ascertain the efficacy and specific mechanisms of action of PDE5-Is for the management of LUTS/BPH.

Abbreviations

PDE5-I: Phosphodiesterase type 5 inhibitor; LUTS/BPH: Lower urinary tract symptoms suggestive of benign prostate hyperplasia; ED: Erectile dysfunction; IPSS: International prostate symptom score; IIEF: International index of erectile dysfunction score; Qmax: Maximal urinary flow rate; IPSS-QoL: IPSS Quality of life Index; IPSS irritative (storage) subscore; IPSS obstructive (voiding) subscore; BII: BPH impact index.

Peer-review

This is an interesting review regarding the efficacy of PDE5-Is in lower urinary tract symptoms and benign prostate hyperplasia.

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