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Review

Therapeutic strategy towards renal restoration in chronic kidney disease

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Background: The population with end-stage renal disease is increasing. This continued growth is related to: i) diagnostic failure in screening for early chronic kidney disease (CKD) associated with tubulointerstitial fibrosis (TIF), ii) failure in preventing renal disease progression due to lack of understanding of the precise determinants that induce TIF, and iii) delayed treatment which simply slows renal disease progression, but is unable to restore renal function.
***Objective*:** To review therapeutic strategy to restore renal function in CKD stressing fractional excretion of magnesium (FE Mg) as a sensitive biomarker for screening early CKD associated with TIF.
Results: There is much evidence to support the crucial role of renal microvascular disease as the determinant of TIF and disease progression. A unique pattern of hemodynamic maladjustment is characterized by a preferential constriction of the efferent arteriole that induces peritubular capillary flow reduction in CKDs.
Conclusion: The present information leads to a therapeutic strategy to restore renal function in early CKD patients.

Keywords: Chronic kidney disease, fractional excretion of magnesium (FE Mg), hemodynamic maladjustment, renal microvascular disease, tubulointerstitial fibrosis (TIF), vasodilators.

Chronic kidney disease (CKD) is a worldwide public health problem though it is under-recognized in Asia. In the United States, it is the ninth leading cause of death. The number of persons suffering from end-stage renal disease (ESRD) doubled between 1988 and 1997. Medicare-funded programs have increased from approximately 10,000 beneficiaries in 1973 to 340,000 at the end of 1999 [1, 2]. Patients with ESRD consume a disproportionate share of health care resources. The total cost of the ESRD program in the United States was $17.9 billion in 1999, up from $16.7 billion in 1998 - a 7.2% increase. The projected number of ESRD patients by the year 2010 is estimated to be more than 660,000 and the total Medicare ESRD program cost has been estimated to be in excess of $28 billion (US) [3]. In Thailand, according to Chittinandana [4], the prevalence of CKD in the Thai adult populations is estimated to be 9.1 % and 4.6 % by modification of diet in renal disease. In the year 2000, Thailand Renal Replacement Therapy (TRT) registry showed that the prevalence of ESRD patients in Thailand was 1,995 cases [5]. Recent data (unpublished) from TRT registry shows that the prevalence of ESRD is 13,597 cases at the end of 2004. The continued growth of the ESRD program around the world has raised questions as to the source of the patients and diseases driving the growth. This continued growth can be explained partially by recognizing and studying earlier stages of CKD and of risk factors for CKD [6].

In this article, at first, we examine various biomarkers for screening early CKD. Then, we investigate renal microvascular disease and glomerular endothelial dysfunction, asssociated with tubulointerstitial fibrosis (TIF). Finally, we discuss novel strategies to restore renal function in CKD. For abbreviations, see the last section in the text.

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Biomarkers for screening early CKD

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative has recently released *Clinical Practice Guidelines for CKD: Evaluation,*

Classification and Stratification (NFK-K/ DOQI Guidelines for CKD) [6]. Recent studies have estimated the prevalence of CKD in the general population using biomarkers of kidney damage, such as elevated serum creatinine levels, decreased glomerular filtration rate (GFR) and presence of microalbuminuria.

Elevated serum creatinine levels

The prevalence of the serum creatinine levels at (or above) 1.5, 1.7, and 2.0 mg/dL in a sample of 18,723 individuals (aged 12 years and older) between 1988 and 1994, were 5.0 %, 1.9 %, 0.6 % for men, respectively, and 1.6 %, 0.7 % and 0.3 % for women, respectively [7]. The number of people with serum creatinine levels at (or above) 1.5, 1.7, and 2.0 mg/dL is estimated to be 6.2, 2.5, and 0.8 million, respectively. It is obvious from the above elevated serum creatinine level that such reliance on serum creatinine level is not sensitive enough to screen a early CKD. Changes in serum creatinine levels are usually not apparent until there is a 50 percent loss of renal function.

Calculated GFR

The CKD guidelines work group decided to classify CKD as a GFR of less than 60 mL/min/1.73 m^2. It is estimated that the prevalence for CKD is 4.7 % or 8.3 million [8, 9]. By using the different formula (Cockcroft-Gault formula) to calculate creatinine clearance, the prevalence of CKD in Thai population was 9.1 %. Based on the above criteria, it is obvious that the cut off level of GFR of less than 60 mL/min/1.73m^2 for classifying CKD is rather insensitive and does not identify early CKD patients with minimal to moderate kidney damage (CKD stages I -> II, or III). The insensitiveness of creatinine clearance is even further enhanced by the phenomenon of hyperfiltration encountered in a variety of CKD, by which the actual value of creatinine clearance is overestimated [10-12].

Microalbuminuria

Microalbuminuria is another generally accepted biomarker for CKD [13-15]. Of 23,527 individuals 6 years of age or older in NHANESIII, there were 22,244 with both urinary albumin and creatinine data. The prevalence of clinical albuminuria, defined as urine albumin concentration greater than 300 mg/L, was 1.4 % among men, and 1.5 % among women. For an evaluation of microalbuminuria, the estimates were made by using urinary albumin to creatinine ratios, and defined as 30 mg/g or greater but less than 300 mg/g. The overall prevalence of microalbuminuria was 7.8 %. Based on this biomarker of microalbuminuria reflecting kidney damage, the sensitivity of microalbuminuria in screening of early CKD is questioned in diabetic nephropathy as well as in other CKD [16-20].

Fractional excretion of magnesium (FE Mg)

It has been noted that microalbuminuria is less sensitive than FE Mg in detecting early diabetic nephropathy, as shown in **Table 1**. An abnormally elevated FE Mg has been documented in normoalbuminuric type-2 diabetes. FE Mg correlates directly with the magnitude of TIF. Accordingly, an abnormal FE Mg implies diabetic nephropathy which is not yet shown by microalbuminuria.

FE Mg represents a new tubular function that reflects the ability of tubular cells to reabsorb the glomerular filtrate of magnesium and retain the intracellular second most abundant cation magnesium. **Figure 1** shows the relationship between FE Mg and TIF measured in early CKD [18]. It is obvious from the multiple regression analysis that FE Mg directly correlates with the magnitude of TIF.

In essence, a normal value of FE Mg (<2.2 %) indicates an intact tubulointerstitial structure, whereas an abnormally elevated FE Mg implies CKD associated with TIF. Since the presence of tubulointerstitial disease or fibrosis [21, 22] is a biomarker of disease severity and chronicity, FE Mg

Table 1. FE Mg (%) in detecting early diabetic nephropathy.

	Tubular Function FE Mg (%)	P value	Albuminuria (μg/min)
Control	1.45 ± 0.4		< 20
Normoalbuminuric type 2 DM	3.4 ± 0.8	< 0.01	14 ± 5
Albuminuric type 2 DM	6.6 ± 2	< 0.001	3×10^3

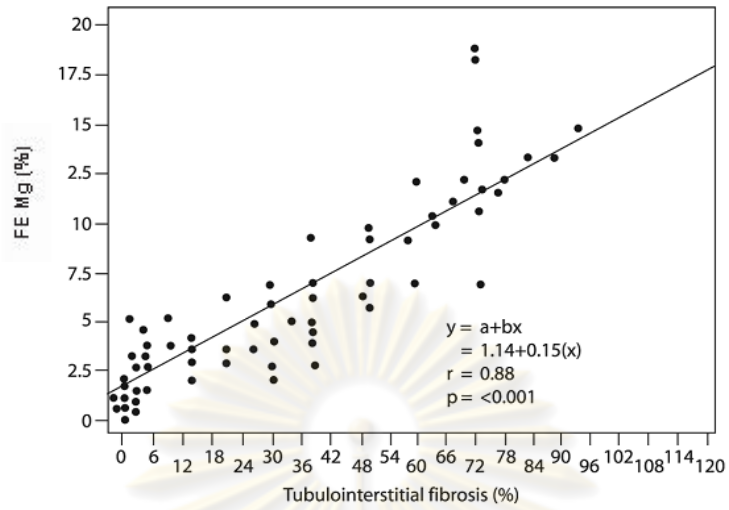

Fig. 1 Correlation between FE Mg and TIF [18].

is a suitable marker to screen early CKD associated with TIF. FE Mg would assist in screening early CKD with minimal renal functional impairment or mild degree of TIF. CKD in diabetic nephropathy is characterized by a long silent period in which substantial morphologic changes occur and then a shorter period of renal functional decline [23]. Clinically, FE Mg may be the most sensitive marker in detecting low-level tubular injury in humans [24]. Non-invasive biomarkers (such as FE Mg) would lead to early initiation of therapy at the early stage of renal disease when there is still an adequate renal functional reserve .

Renal microvascular disease and TIF

The tubulointerstitium is one of the main components of nephrons and receives blood supply via peritubular capillaries extending from the glomerular capillary vasculature. **Figure 2** shows the structure of nephrons and peritubular microvascularature.

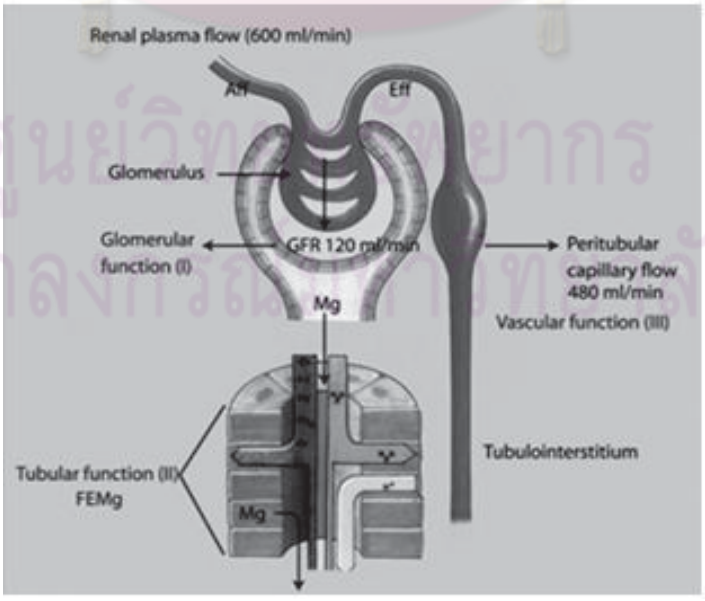

Fig. 2 An illustration of the nephronal structure.

There are a number of factors to influence homeostais of the glomerular endothelial cell. Blood flowing in glomerulus conntains blood elements, a variety of mediators and circulating toxins (namely, oxidative stress) and immunocirculatory imbalance (such as enhanced proinflammatory cytokine tumor necrosis factor alpha), which also induces mechanical shear stress at the interface of the glomerulus. Such mechanical, immunologic and metabolic stresses finely tune homeostasis under normal condition. Under pathologic conditions, the glomerular endothelial cell is disturbed with altered homeostasis [25-36].

The circulating toxins may induce glomerular endothelial injury observed in CKD such as focal segmental glomerulosclerosis, or diabetic nephropathy. These injuries can be assessed by means of endothelial cell cytotoxicity test or circulating endothelial cell count [37-40]. Glomerular endothelial injury is noted early in diabetic nephropathy (such as in the stage of normo-albuminuria) and also early in CKD (such as IgM mesangial nephropathy) with minimal TIF and nephrosis with focal segmental glomerulosclerosis. Recently, we have demonstrated evidence of injuries to both glomerular capillary endothelium and peritubular capillary endothelium by using the diminished staining of endothelial factor VIII staining (a reflection of the endothelial cell loss in the renal microvasculature) in CKD [41]. The loss of glomerular endothelium correlates directly with the development of glomerulosclerosis [42, 43]. Kang et al also documented a loss of the glomerular endothelium in both the aging kidney [44] and in the remnant kidney model [45]. Recently, evidence of correlation between renal microvascular disease and TIF has been accumulated [46-50]. Tubulointerstitial disease is usually encountered in association with renal microvascular rarefaction in CKDs. Bohle et al denoted an inverse correlation between the widening of the interstitium and capillary patency [51]. Recently, we observed an inverse correlation between the reduction in peritubular capillary flow (PTCF) and the increased magnitude of TIF [52] (**Fig. 3**).

A mild and sustained reduction in peritubular capillary flow is, in essence, associated with a low grade TIF. A greater degree of PTCF reduction is associated with a higher magnitude of TIF [53]. The intrarenal hemodynamic study in idiopathic nephrotic syndrome indicates that there is a spatial relationship between the renal perfusion and nephronal structure [53]. As illustrated in **Table 2**, the presence of a normal perfusion (normal PTCF) observed in steroid-sensitive minimal change nephrosis (group 1) or of a slight reduction in PTCF observed in steroid resistant, mesangial proliferative nephrosis (group 2) was associated with an intact structure. However, a further but substantial reduction in PTCF as observed in steroid resistant, mesangial proliferative nephrosis (group 3) correlated with a mild degree of TIF. A greater or severe reduction in PTCF, as observed in nephrosis associated with focal segmental glomerulosclerosis (FSFS) (group 4), also correlated with a greater magnitude of TIF. Such findings support the relationship between renal perfusion and structure, and also address the cause-and-effect relationship since the reduction in PTCF precedes the development of TIF. The result of this intrarenal

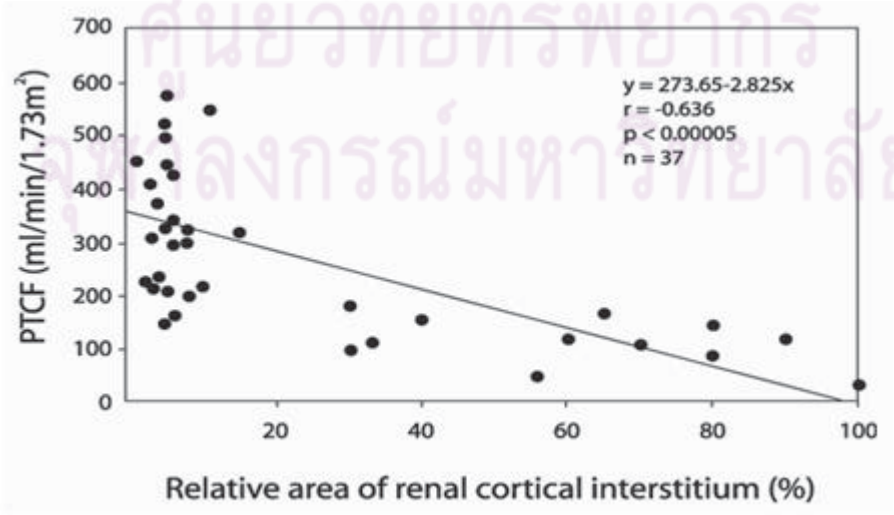

Fig. 3 Relation between PTCF reduction and renal cortical interstitium area with TIF [52].

Table 2. PTCF reduction precedes the development of TIF.

Clinical Setting	PTCF (ml/min/1.73m^2)	FEMg	TIF
Normal	480	Normal	Neg
I SS-NS	483±50	Normal	Neg
II MesP-NS	371±72	Normal	Neg
III MesP-NS-TIF	254±143	Abnormal	+
IV FSGS-NS	104±56	Abnormal	++, +++

SS-NS= steroid-sentitive minimal change nephrosis, MesP-NS=mesangial proferative nephrosis, MesP-NS-TIF=mesangial proliferative nephrosis associated with TIF, FSGF-NS=nepherosis associated with focal segmental glomerulosclerosis.

hemodynamic study appears to be somewhat contradictory to the general belief that the nephronal death such as TIF is the cause rather than the effect of the renal perfusion deficit. A similar observation by Truong et al, who demonstrated in their experimental model of renal ischemia inducing nephronal death, also supports our hypothesis [54].

Glomerular endothelial dysfunction

Injury to the glomerular endothelial cell induces loss of negative surface charge, which enhances-proteinuria and procoagulant activity. Procoagulant activity is characterized by the presence of blood hypercoagulability, shortened platelet half-life and fibrinogen half-life, blood hyperviscosity, fibrin deposit in the kidney, elevated levels of fibrin degradation products in the serum and urine, and altered hemorheology of erythrocytes during active proteinuria [55-65]. In addition, there is defective release of vasodilators in the dysfunctioning glomerular endothelial cell and, at the same time, enhanced release of vasoconstrictors, namely angiotensin II, endothelin, and thromboxane A2 (See **Fig. 4**). The provasoconstrictive stage of glomerular endothelial dysfunction induces a unique pattern of hemodynamic maladjustment characterized by preferentially constricting the efferent arteriole, which is encountered in a variety of CKDs. Such a constriction has three significant hemodynamic impacts. First, proximal to the efferent arteriolar constriction, it induces an overestimated glomerular filtration rate due to hyperfiltration. Second, it causes an elevated intraglomerular hydrostatic pressure (PG) due to the higher blood inflow and lower blood outflow. Intraglomerular hypertension in conjunction with defective blood quality, altered hemorheology, enhanced proinflammatory activity, and renal hypoperfusion culminate in the development of

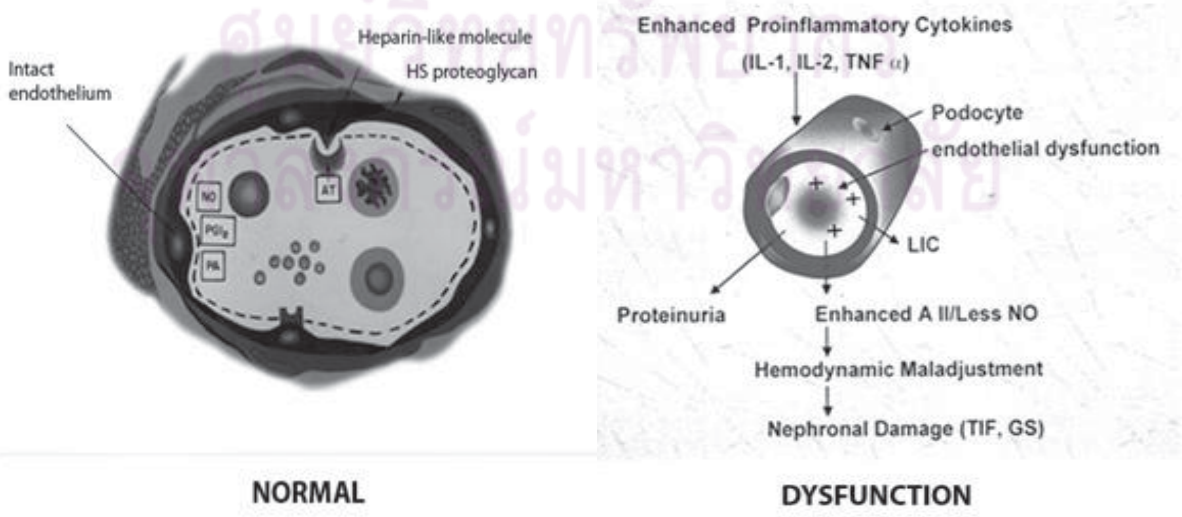

Fig. 4 An illustration of comparison between normal and dysfunctioning endothelium.

glomerulosclerosis. Third, distal to the efferent arteriolar constriction, it exaggratedly reduces the PTCF supplying the tubulointerstitium (**Fig. 5)**. Increased PG distends the glomerular capillary loop, thereby detaching the podocyte from the basement membrane. Increased podocyte injury in this manner, in conjunction with toxic injury from reactive oxygen species and proinflammatory cytokines, decreases the production of vascular endothelial growth factor (VEGF), which is essential to the survival and regeneration of the endothelial cell. Both Kriz et al [66] and Rennke [67] elegantly demonstrated podocyte injury by detachment from the basement membrane secondary to ballooning of the capillary loop in nephrosis with FSGS. Mild reduction in the level of VEGF mRNA and its receptor (flk-1) mRNA was transiently documented in the first week following injection of puromycin aminonucleoside to induce nephrosis [68]. However, permanent loss of podocyte function, with impaired production and release of VEGF, is substantiated in nephrosis with FSGS and in other CKDs [69, 70]. Podocyte injury and defective release of VEGF, leading to impaired angiogenesis, correlate with progressive renal disease [44-46, 71]. A decrease in VEGF and its receptor renders the endothelial cell apoptotic or cytotoxic. Thus, injury to the glomerular endothelial cell is enhanced and aggravates hemodynamic maladjustment and injury to podocyte, eventually causing further injury to the glomerular endothelial cell and postglomerular endothelial cell in the renal microcirculation, in a vicious cycle. Finally, sustained ischemic injury to the PTCF, in conjunction with the activation of the profibrotic pathway induced by toxic mediators such as angiotensin II, cytokines, reactive oxygen species, culminate in the development of TIF [72-81].

The significance of reduction in PTCF observed in a variety of CKDs in relation to the development of TIF has been demonstrated by the multiple regression analysis, which revealed that the reductions in PTCF correlate inversely with the development of tubulointerstitial fibrosis [82-84] (**Fig. 6**). Interestingly, the lower the reduction in PTCF, the greater the magnitude of TIF.

In addition to the hemodynamic factor inducing chronic ischemia and TIF, chronic hypoxia has recently been proposed as a crucial mechanism for the progression of different types of renal disease [85]. Improvement in renal cortical microvascular pO_2 by angiotensin II blockade, results in renoprotection [86]. In accordance with the glomerular endothelial cell dysfunction (induced by oxidative stress and immunocirculatory disturbance), such toxic triggers affect the podocyte and induce podocyte dysfunction or injury. Podocyte injury with structural damage such as podocytopenia and nephron loss is believed to be associated with proteinuria [87]. A synergistic interaction between injury to the glomerular endothelium and podocyte amplifies the viscious cycle that eventually leads to progressive reduction in renal perfusion and PTCF, and increases in the magnitude of proteinuria. Our sequential studies in intrarenal hemodynamics reveal a progressive reduction in PTCF as the disease severity progresses [36].

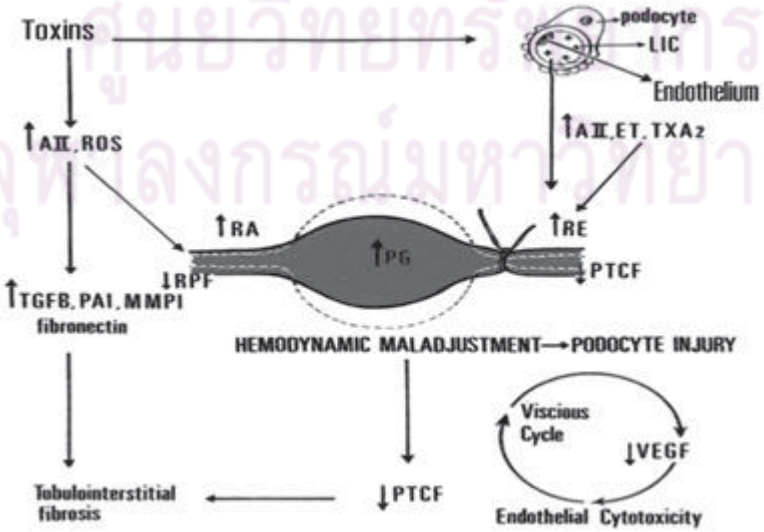

Fig. 5 An illustration of hemodynamic maladjustment inducing TIF.

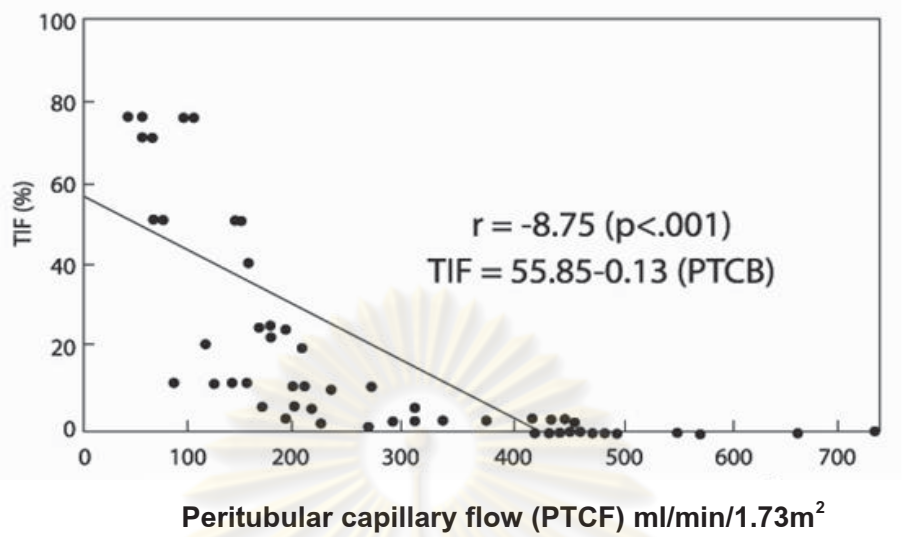

Fig. 6 An inverse correlation between the PTCF reduction and TIF [53].

Therapeutic strategy to restore renal function

The current therapeutic strategies to prevent renal disease progression aim at: i) several surrogate end points, namely, the suppression of proteinuria; ii) blockade of the renin-angiotensin aldosterone system (RAS) to correct the hemodynamic maladjustment, and iii) optimal goal of restoration of renal function. Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT1) antagonists can reduce proteinuria and decelerate the renal disease progression. Their common action is to reduce the stimulation of the AT1 receptor by its ligand angiotensin II (Ang II) as follows. ACE inhibitors block ACE, and thus limits the amount of Ang II available for binding to the AT1 receptor. On the other hand, AT1 antagonists can directly inhibit the binding of Ang II to AT1. Both RAS blockers induce a compensatory upregulation of renin, with more Ang II being formed. In addition to Ang II, there are several other products of Ang II that exert physiologic actions. High levels of angiotensin (1-7) may contribute to vasodilation. According to De Gasparo et al [88], AT1 antagonists (but not ACE inhibitors) lead to an exaggerated stimulation or the AT2 receptor. Accumulative evidence supports that either ACE inhibitor or AT1 receptor antagonist, when used as monodrug therapy, is not effective in halting renal disease progression. Such monodrug therapy simply slows the renal disease progression, but it cannot restore renal function [89-91]. This view is supported by the continuous increment of CKD patients that enter end-stage renal disease under the current strategies (preventive and therapeutic). Such failures may be due to the two crucial issues as follow: 1) Insensitive methodology that cannot screen early CKD. No or absence of sensitive methodology delays diagnosis, leading to late initiation of preventive strategy. 2) Monodrug therapy that has been commonly practiced in the last decade. This therapy is insufficient to correct the hemodynamic maladjustment that is associated with progressive decline in peritubular capillary flow and chronic ischemia of the tubulointerstitium. In CKD patients with limited renal functional reserve or with moderately to severely advanced renal impairment, efferent arteriolar resistance is progressively elevated significantly, as shown in **Table 3**.

Table 3. The level of efferent arteriolar resistance in early and late CKD patients.

	Efferent arteriolar resistance (dyne.s/cm^5)	**GFR (ml/min/1.73m^2)**
Normal Control	< 3000	120
Early CKD	3,000$^+$-8,000	60-100$^+$
Late CKD	8,001-30,000	< 60

Based upon the above information, most effective strategies (preventive and therapeutic) to restore renal function require: i) better screening markers that can detect early CKD patients at the suitable time when adequate functional renal reserve for renal regeneration exists, and ii) combined drug therapy of vasodilators to appropriately correct the hemodynamic maladjustment. According to this conceptual view, we have recently succeded, in detecting early diabetic nephropathy at the stage of normoalbuminuria by adopting FE Mg to screen early CKD patients with TIF. At present, we can detect CKD patients with minimal renal functional impairment such as nephrotic patients associated with FSGS, and mesangial proliferative nephrosis with TIF. Noticably, early detection of CKD patients with early renal functional impairment leads to early initiation of appropriate therapy for CKD patients at the time that preventive strategy might be able to halt progression. For this purpose, we have used multidrug therapy using 4 vasodilators (ACE inhibitor, AT1 receptor antagonist, calcium channel blocker, antiplatelet) so as to appropriately relax the efferent arteriolar constriction and also correct the hemodynamic maladjustment. Such therapeutic approach can certainly restore renal function in normoalbuminuric type-2 diabetic nephropathy, albuminuric type-2 diabetic nephropathy, nephrosis associated with focal segmental glomerulosclerosis, mesangial proliferative nephrosis, and CKD patients with severely impaired renal function [38, 92, 93]. Recently, Campbell et al proposed combination of ACE inhibitors and AT1 receptor antagonists, demonstrating that this combination could restore renal function in human chronic nephropathies [94]. However, it is still controversial whether dual blockade of renin-angiotensin system is successful for restoration of renal function in CKD patient [95-98].

Despite the good suppression of proteinuria, the best dual blockades simply slow down the renal disease progression in most cases. These failures in restoring renal function may be due to the following: i) target end point of therapy aimed at the suppression of proteinuria or blood pressure control and ii) delayed treatment in CKD patients with limited functional reserve. In i), correction of hemodynamic maladjustment is not aimed and thus relaxation of very high efferent arteriolar resistance is insufficent without increment in peritubular capillary flow. In ii), at such advanced stages of CKD, a restoration of renal function or renal regeneration is defective. Multiple defects are: 1) depleted endothelial progenitor cells, 2) depleted vascular endothelial growth factor, and 3) a depleted nitric oxide (NO) substrate due to enhanced arginase or a to a depletion of NO cofactor tetra hydrobiopterin.

To accomplish conditions in favor of restoring renal function, early initiation of appropriate preventive strategy in early CKD patients with an adequate renal functional reservers is encouraged. The aim is correction of hemodynamic maladjustment. The successful therapeutic strategy is reflected by three surrogate end points following treatment as: a rising creatinine clearance, a decline in FE Mg, and a decline in proteinuria.

Our therapeutic accomplishment has fulfilled the above criteria. The decline in FE Mg following therapy implies a regression of renal inflammation. This view of renal regression is supported by experimental studies in animals that dual RAS blockages reduce the proinflammatory cytokine in kidney tissue [97, 99]. Thus, an effective strategy (preventive and therapeutic) would have a significant outcome in minimizing the number of CKD patients entering ESRD as well as in reducing the medical expenses relating to renal replacement therapy.

List of abbreviations

ACE= angiotensin-converting enzyme,
Ang II=angiotensin II,
AT1=angiotensin II type 1 receptor,
CKD=chronic kidney disease,
ESRD=end-stage renal disease,
FE Mg=fractional excetion of magnesium,
FSGS=focal segmental glomerulosclerosis,
GFR=glomerular filtration rate,
PTCF=peritubular capillary flow,
RAS=renin-angiotensin aldosterone system,
TIF=tubulointerstitial fibriosis,
VEGF=vascular endothelial growth factor.

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