

Diagnosis and assessment of renal fibrosis: the state of the art

Lena Berchtold^{a,b}, Iris Friedli^c, Jean-Paul Vallée^c, Solange Moll^d, Pierre-Yves Martin^b, Sophie de Seigneux^b

^a Service of Internal Medicine, Department of Internal Medicine, University Hospital of Geneva, Switzerland

^b Service of Nephrology, Department of Internal Medicine Specialties, University Hospital of Geneva, Switzerland.

^c Service of Radiology, Department of Radiology and Medical Informatics, University Hospital of Geneva, Switzerland.

^d Institute of Clinical Pathology, University Hospital Geneva, Switzerland

Summary

Chronic kidney disease (CKD) is defined as an alteration of kidney function and/or structure lasting for more than 3 months and is a major public health issue. Histologically, the severity of CKD correlates with the magnitude of kidney cortical interstitial fibrosis. Estimation of kidney fibrosis is crucial to assess prognosis and guide therapy in both native and allograft kidneys. Biopsy is currently the gold standard for assessing fibrosis with histological techniques. Although this procedure has become safer over recent years, complications and limitations remain. Given these restrictions, new, noninvasive techniques are necessary for the evaluation and follow-up of CKD patients. Radiological methods such as ultrasound and magnetic resonance imaging are emerging for assessment kidney fibrosis. These two techniques have advantages but also limitations. In addition to radiological assessment of fibrosis, urinary and plasma biomarkers are being developed and tested as predictive tools for histological lesions in the kidney. This article reviews the current evidence for these novel techniques in the evaluation of kidney interstitial fibrosis.

Key words: kidney fibrosis; biomarkers; chronic kidney disease; MRI; histology

Abbreviations

ADC: apparent diffusion coefficient
BMP-7: bone morphogenetic protein-7
CCL2: chemokine (C-C motif) ligand 2
CKD: chronic kidney disease

eGFR: estimated glomerular filtration rate
MCP1: monocyte chemoattractant protein-1
MDA: malondialdehyde
MicroRNA: microribonucleic acid
MMP: matrix metalloproteinase
MRI: magnetic resonance imaging
PIIINP: procollagen type III amino-terminal propeptide
RBP: retinol binding protein
TGF-beta: transforming growth factor beta
TIMP: tissue inhibitor of metalloproteinase
TNF-alpha: tumour necrosis factor-alpha
VDBP: vitamin D binding protein

Introduction

Chronic kidney disease (CKD) is defined as an alteration of kidney function and/or structure lasting for more than 3 months. It is a major public health issue since it affects 11% of the US and Swiss populations [1, 2]. CKD is also recognised as a condition that increases cardiovascular disease risk and is associated with a significant increase in mortality compared with the general population [3]. Fibrosis is the hallmark of CKD and the severity of CKD usually correlates with the magnitude of kidney cortical fibrosis.

Kidney fibrosis is characterised by increased synthesis and deposition of extracellular matrix components within the tubulointerstitial space (interstitial fibrosis) and glomeruli (glomerulosclerosis). Tubular atrophy often goes with interstitial fibrosis. Cor-

tical interstitial fibrosis is better correlated with renal function loss than is glomerulosclerosis, and is a process common to a wide variety of kidney injuries [4]. The emergence of renal fibrosis is the consequence of maladaptive wound-healing processes after an injury [5]. Interstitial fibrosis remains the main factor contributing to kidney structural deterioration and loss of function. Therefore it is a crucial target for therapies since a wide variety of diseases converge into this single process [6]. The pathogenesis of fibrosis is very complex, and as yet not completely understood. It will not be detailed here but has been reviewed in depth elsewhere [7].

Evaluation of interstitial fibrosis is crucial to the assessment of prognosis and to guide therapy for most kidney diseases. However, how best to measure kidney fibrosis remains uncertain. Therefore, new approaches that allow a more accurate and reproducible assessment of renal interstitial fibrosis are required. Here, we review recent advances in the diagnosis and follow-up of renal fibrosis.

Clinical aspects

The histopathological findings associated with CKD are glomerulosclerosis, tubulointerstitial fibrosis and inflammation with loss of renal parenchyma characterised by tubular atrophy, capillary loss and podocyte depletion.

Renal interstitial fibrosis correlates with and predicts renal function loss in all experimental models of CKD [4]. In addition, the presence of interstitial inflammation in combination with kidney fibrosis is associated to an increased risk of kidney function decline in the long term [8–10]. Several studies have demonstrated that quantification of interstitial fibrosis and inflammation gives a more accurate prognosis of renal outcome in renal allografts [11] and native kidney diseases [12–14], including in lupus and immunoglobulin (Ig) A nephropathies.

Currently, several research protocols are aiming at reverting fibrosis, in both animals and human studies [15–20]. Although spontaneous regression of fibrosis has been documented in some renal pathologies [21], worsening of renal fibrosis is usually observed together with deterioration of kidney function [13]. The follow-up of fibrosis is therefore important for evaluation of the spontaneous evolution of CKD, and for the assessment of response to specific therapies, including emerging drugs [6, 18, 22].

Histopathological assessment

Kidney fibrosis and inflammation are currently being evaluated by means of needle core biopsy followed by histopathological assessment. Percutaneous kidney biopsies are usually performed with ultrasonic guidance under local anaesthesia. After a renal biopsy, patients are observed on bed rest for a few hours to watch for possible complications (see below).

Kidney biopsy has several limitations. This procedure has become safer over recent years; however, complications still occur, including minor and major bleedings, pain, arteriovenous fistulas and perirenal soft tissue infections. These may lead to prolonged hospitalisation, loss of renal function and even death [23, 24]. Furthermore, the financial cost of the procedure is significant. In addition, the amount of tissue obtained from a needle core biopsy on some occasions may not be sufficient to assess the severity of a disease. Histological changes are usually scored semiquantitatively, with an element of subjectivity. Objective quantitative parameters such as Sirius red staining coupled with morphometric analysis are rarely used routinely [6]. The time needed for tissue preparation before examination may also be a limitation in some urgent circumstances, and serial kidney biopsies are difficult. Finally, one of the main intrinsic limitations to the assessment of fibrosis via biopsy is the sampling bias related to the technique.

Routine histological evaluation of a renal biopsy involves examination of the tissue under light, immunofluorescence, and electron microscopy. Conventional histological stains such as haematoxylin and eosin, periodic acid Schiff, silver methenamine and trichrome are routinely used (fig. 1). Fibrosis quantification by visual assessment of trichrome-stained slides is the standard practice in most pathology departments [25]. There are some limitations to this technique, since it has been reported to be only moderately reproducible [26]. Part of the poor reproducibility arises from subjective scores assigned by pathologists, whereas Masson trichrome may not be sensitive for mild fibrosis [26, 27]. Sirius red, viewed in both polarised and unpolarised light, may be used for fibrosis assessment [28].

Quantification by use of morphometric techniques of staining such as trichrome, polarised and unpolarised Sirius red and immunohistochemistry (collagen III) has been developed with various results [29–32]. Correlation between specific stains and estimated glomerular filtration rate (eGFR) remains variable. More complex and objective techniques

are being tested, with for example the use of a combination of different colourations [33]. Currently, the closest correlation to eGFR, best efficiency and best reproducibility are still with trichrome-stained and collagen III morphometry as shown by Farris et al., whereas unpolarised Sirius red also appears valuable [33]. A combination of whole section imaging and automated image analysis with Sirius red polarisation seems to be complementary to Masson trichrome [34].

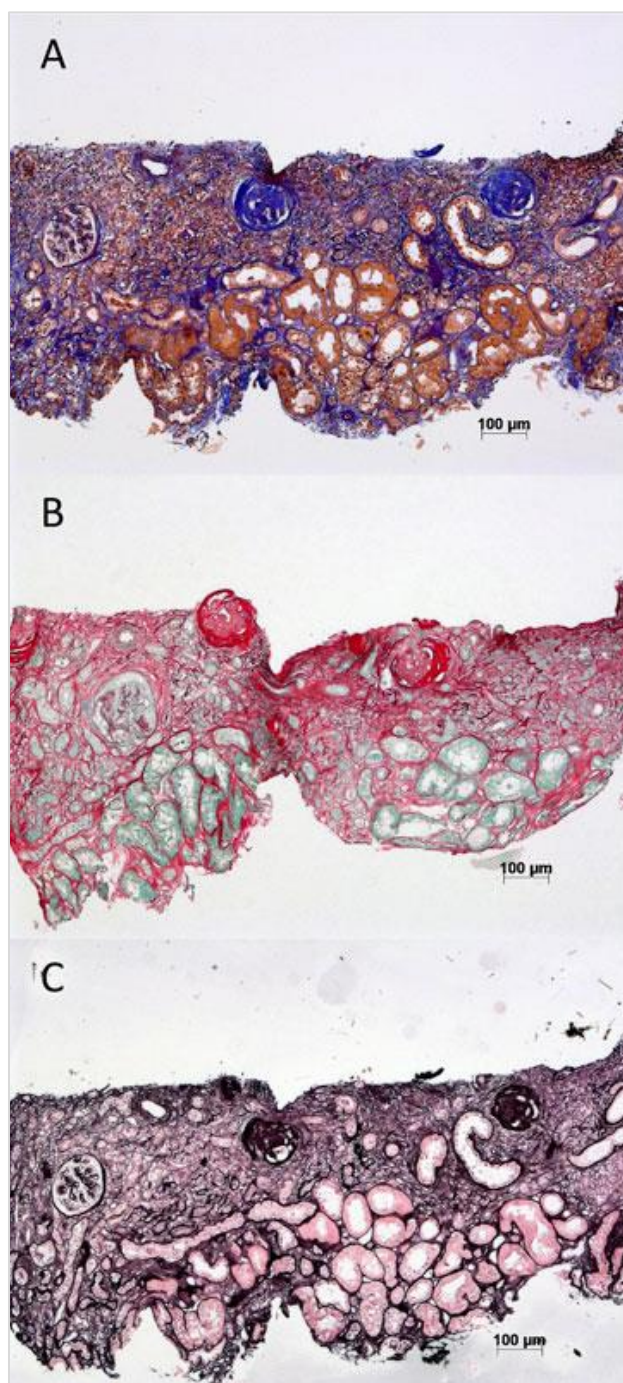


Figure 1: Examples of commonly used stains for histological assessment of renal interstitial fibrosis. (A) Trichrome. (B) Sirius red. (C) Silver methanamine.

Altogether, there is a lack of standardised assessment of interstitial fibrosis by pathologists, and morphometric methods are scarcely used routinely. Current assessment relies mainly on visual assessment of trichrome staining, a method that, although validated, is still subject to the interpretation of the pathologist involved. Given these limitations, although biopsy is currently the gold standard for the diagnosis of kidney fibrosis, new noninvasive techniques might help in the evaluation and follow-up of CKD patients.

Radiological assessment

There is no recognised radiological method to assess kidney interstitial fibrosis, unlike other organs such as the liver [35, 36].

Ultrasound

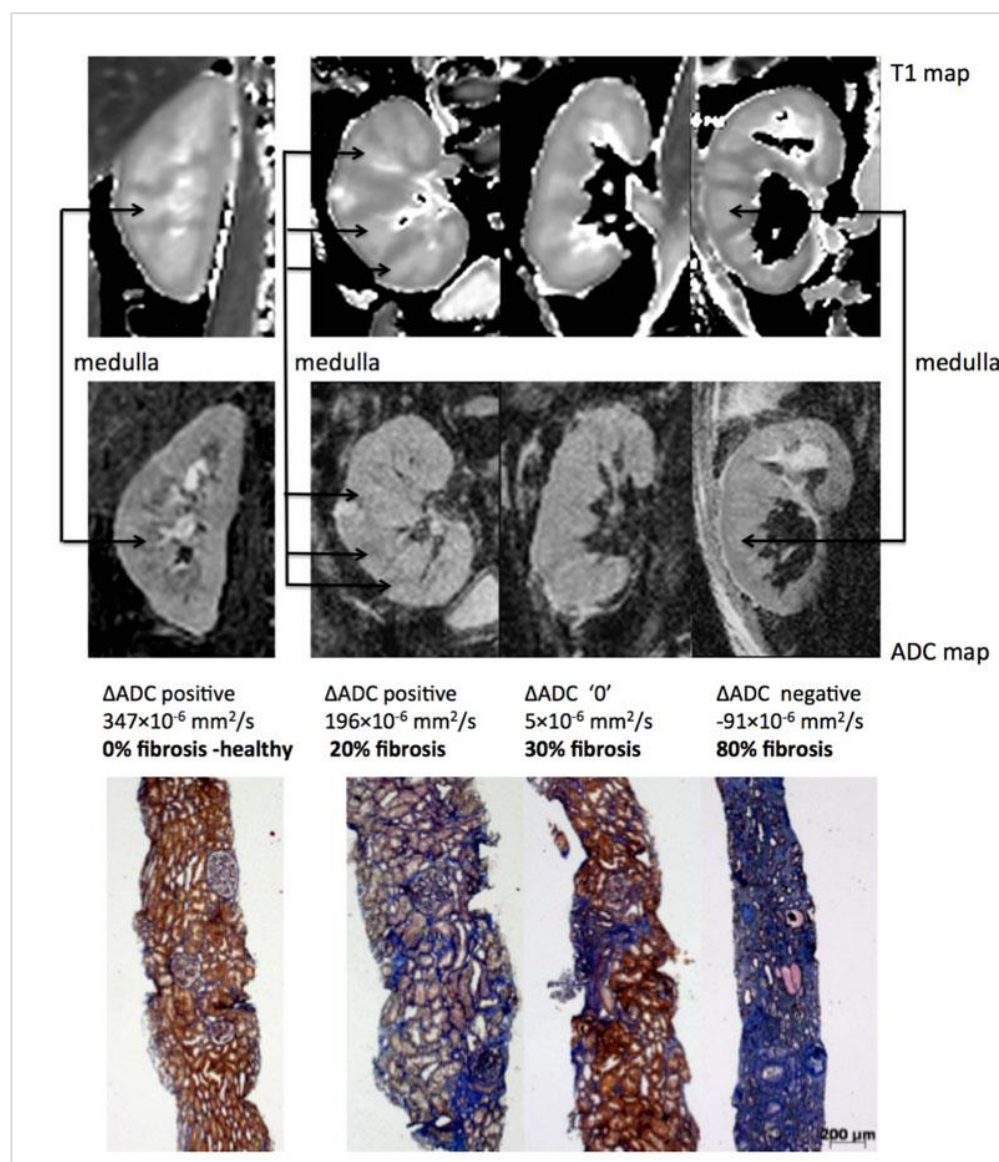
Ultrasound has shown some promising results in providing a noninvasive means of measuring renal cortical stiffness resulting from pathological damage. Shear wave velocity imaging [37–42], transient elastography [43–46], real-time elastography [47], Doppler sonography [48, 49] and ultrasound corticomedullary strain [47, 48], among others, appear partially relevant in measuring renal cortical fibrosis. However, results are heterogeneous, with positive and negative studies. Furthermore, these methods are strongly dependent on external factors, such as blood pressure, kidney weight, body weight and the applied transducer force, not to mention also high intra- and interobserver variability.

The use of ultrasound in the assessment of renal interstitial fibrosis appears promising. However, its main limitation is the heterogeneity of renal parenchyma for elastography evaluation as well as the depth of native kidneys. This technique is therefore interesting but currently not as reliable as for the liver.

Magnetic resonance imaging

Magnetic resonance imaging (MRI), with use of various sequences including T1 mapping, blood oxygenation level dependent and diffusion-weighted imaging, is emerging as a promising non-invasive tool to assess kidney interstitial fibrosis.

T1 mapping creates a parametric map where each pixel represents the T1 spin-lattice relaxation time of the probed tissue. T1 relaxation time varies depending on the tissue physiopathology status, such as inflammation, oedema, fat or fibrosis. The vast majority of native T1 mapping applications are related to cardiac fibrosis, with increased T1 values



recorded in diffuse myocardial fibrosis [50]. In the kidney, small animal studies have found increased T1 values in mice models of acute kidney injury [51] and of renal transplantation [52]. Similar associations were measured in human kidney: cortical T1 was negatively correlated with renal function in native and transplanted kidneys, suggesting that this sequence could be used to assess kidney interstitial fibrosis [53, 54]. Nevertheless, a strong correlation with fibrosis has not been confirmed by other studies, and T1 may rather be a marker of inflammation or vascularisation changes [55].

Renal blood oxygenation level dependent (BOLD) MRI (T2 sequence)* allows probing renal tissue oxygenation in different pathophysiological conditions [56]. In mice, changes of renal T2* were associated with the severity of acute kidney injury and interstitial fibrosis [57]. In human, the effective

transverse relaxation time T2* values of BOLD-MRI correlated with renal function, but not with histology in diabetic nephropathy, suggesting that other factors than kidney fibrosis, such as impairment of oxygenation, could contribute to image modifications in this MRI sequence [58].

Diffusion-weighted imaging (DWI) is another sequence investigated for quantification of interstitial fibrosis. DWI is sensitive to Brownian motion of water molecules within a given tissue. Currently DWI is quantified as the apparent diffusion coefficient (ADC). The emerging importance of ADC values for monitoring renal fibrosis was first validated in a small animal model of unilateral ureteral obstruction [59]. In CKD patients, a significant decrease of ADC was related to increased interstitial fibrosis obtained histopathologically in different studies [58, 60, 61]. Zhao et al. demonstrated that

cortical and medullary ADC values correlated with interstitial fibrosis, as assessed with histology, in native kidney disease [61]. The major limitations of renal diffusion MRI in clinical practice are the absence of a standardised protocol, artefact linked to image acquisition [62] and large interindividual variation of ADC values due to intrinsic factors, such as hydration status, and extrinsic factors such as magnetic field strength, MR sequence type, MRI manufacturer.

Currently, sequence optimisation is performed to overcome these limitations and decrease diffusion MRI variability. For example, we recently optimised diffusion MRI sequences for the kidney in healthy volunteers. We could demonstrate that a novel sequence (called RESOLVE) largely improved kidney image resolution in healthy volunteers [63]. This enhanced image resolution allowed better differentiation of the cortex from the medulla, and correction of absolute cortical ADC values to medullary values by deriving the difference between cortical and medullary ADC (delta ADC). This correction based on the delta ADC significantly decreased interindividual variability and improved discrimination for cortical fibrosis in patients [64]. Delta ADC correlated well with fibrosis in both experimental models of fibrosis in animals and kidney allograft recipients undergoing biopsies ($R^2 = 0.64$, $p < 0.001$) [64]. Extensive (>40%) fibrosis could be identified by negative delta ADC values (ADC values higher in the medulla than the cortex) (fig. 2). Although more work is needed to validate this index before application in the clinical setting, this study presents encouraging results for diffusion MRI as a novel tool for kidney fibrosis evaluation. Altogether, MRI is a promising tool to evaluate kidney fibrosis. However, the methods remain to be confirmed by larger scale studies [65], because of the absence of consensus regarding MRI protocols, absence of reference values and the presence of artefacts, including respiratory motion and image distortion [62]. MRI, in particular with diffusion sequences, is one of the most promising tools to evaluate kidney fibrosis, but its use in the clinical setting still requires adaptation.

Biomarkers of kidney fibrosis

Several plasma or urinary biomarkers are currently being evaluated as indicators of fibrosis. The following list is not exhaustive, but includes some of the most promising markers to date. We have chosen to classify them according to their biological function.

Profibrotic and structural proteins

Renal fibrosis is characterised by the accumulation of an excessive amount of extracellular matrix. Type III collagen is part of this extracellular matrix. It is synthesised as procollagen and the amino-terminal propeptide is cleaved during deposition. This procollagen type III amino-terminal propeptide (PIIINP) is then released from the extracellular matrix into the blood and urine. The urinary PIIINP/creatinine ratio was shown to be closely correlated with the extent of interstitial fibrosis in biopsies from CKD [66] and kidney allograft patients [67]. Circulating procollagen III correlated with interstitial fibrosis in 40 patients with subacute and chronic nephropathies [68]. These markers may be useful indicators of the extent of renal fibrosis. In the study by Ix et al., urine PIIINP was also associated with CKD progression and incident end-stage renal disease. These results suggest that higher urine and/or plasma PIIINP levels are associated (independently of eGFR) with interstitial fibrosis, CKD progression, risk of end-stage renal disease and death [69].

Periostin is an extracellular protein highly expressed during development and is overexpressed in various experimental types of CKD, where it appears to play an important profibrotic role [70]. In murine models, inhibition of periostin expression protected against the development of renal inflammation and fibrosis [71]. Tissue levels of periostin may correlate with kidney disease severity and might be used as an early marker of injury [72]. More recently, urinary periostin levels were shown to be elevated in chronic allograft nephropathy [73]. This protein may therefore emerge as a good predictor of fibrosis.

Transforming growth factor beta (TGF-beta) is a profibrotic cytokine central to the pathogenesis of kidney fibrosis [3, 74]. BMP-7 (bone morphogenetic protein-7) is a natural antagonist to TGF-beta1 with antifibrotic and anti-inflammatory properties. BMP-7 is being evaluated in mice for treatment of kidney fibrosis [75, 76]. None of these cytokines has been clearly demonstrated to correlate with the extent of kidney fibrosis. However, urinary TGF-beta1 increased in diabetic nephropathy and in membranous glomerulonephritis and was related to disease severity [77, 78]. Serum TGF-beta1 also increases in African-American subjects with end-stage kidney disease [79]. Finally, Wong and al. showed recently that the levels of circulating active TGF-beta1 or BMP-7 and their ratio were good predictors of renal function evolution in diabetic pa-

tients [80]. Adding these two biomarkers to the conventional predictors (urinary albumin-to-creatinine ratio, eGFR and clinical factors) provided a higher predictive value for predefined renal endpoints. Altogether, although more studies are needed and histological correlations are lacking, these two factors may be useful in assessing the risk of renal disease progression.

Proinflammatory and oxidative markers

There is a link between macrophage infiltration and excessive extracellular matrix protein deposition. Chemokine (C-C motif) ligand 2 (CCL2), also called monocyte chemoattractant protein-1 (MCP1), is a receptor chemokine with chemoattractant properties for monocytes/macrophages and other inflammatory cells [81]. The majority of clinical studies have investigated its potential role as biomarker in renal allografts. Urinary CCL2 was tested in a multicentre cohort at 6 months after transplantation and was an independent predictor for the development of interstitial fibrosis at 24 months [82–84].

Renal cells produce matrix metalloproteinases (MMPs) which are secreted zinc-dependent endopeptidases that play a role in extracellular matrix remodelling. MMPs are important in the initiation of fibrogenesis in various organs, including the kidney. Moreover, MMPs are regulated by endogenous inhibitors known as tissue inhibitors of metalloproteinase (TIMPs), and dysregulation of the MMP/TIMP system is an additional contributor to the formation of interstitial fibrosis [85]. Observation shows that MMP-7 levels were elevated in serum and biopsies from patients with interstitial fibrosis [86]. Another study showed that proMMP-9 intragraft expression correlated with fibrotic change, whereas circulating proMMP2 and MMP9 did not differ between allograft patients with and without interstitial fibrosis [87]. MMP-2 expression decreased while TIMP-1 and serum creatinine increased with the rise of interstitial fibrosis grade in kidney transplant recipients [88]. However, in 40 allograft patients, urinary/serum MMPs/TIMPs did not clearly correlate with interstitial fibrosis [89]. Finally, in a recent study of 102 CKD patients, urinary MMP-7 levels were elevated as compared with control subjects and did correlate with kidney fibrosis [90].

Plasma levels of tumour necrosis factor alpha (TNF-alpha) increase during CKD. This biomarker is a proinflammatory mediator, which binds to soluble TNF receptors. The TNF receptor is a biomarker of inflammation in kidney disease. A high level of

TNF receptor correlated with renal function decline in patients with type 1 and type 2 diabetes mellitus [91, 92]. In CKD, higher levels of TNF receptor were independently associated with faster rates of kidney function decline [93]. More recently, Sodona et al. have shown that, in IgA nephropathy, patients in the highest tertile of serum TNF receptor levels displayed more severe renal interstitial fibrosis than did those in the lowest or second tertiles [94].

Filtered proteins

Vitamin D binding protein (VDBP) transports vitamin D metabolites through the circulation. VDBP is filtered by the glomerulus and subsequently taken up by proximal tubular cells through receptor-mediated endocytosis. Since receptor-mediated uptake of proteins is energy-consuming, tubular injury may result in urinary VDBP (uVDBP) loss [95]. Mirkovic et al. investigated the value of uVDBP as a marker of tubulointerstitial inflammation and fibrosis in adriamycin rats, and of renal damage in humans. In rats, uVDBP was associated, independently of albuminuria, with macrophage accumulation and collagen III expression in the interstitium. In humans, uVDBP increased in albuminuric compared to normoalbuminuric subjects. Moreover, uVDBP was associated with tubular and inflammatory damage markers (kidney injury molecule-1, beta2-microglobulin, cystatin C, MCP-1 and neutrophil gelatinase-associated lipocalin) independently of albuminuria [96].

Urinary excretion of retinol binding protein (RBP) is a sensitive marker of allograft fibrosis and can predict long-term graft loss independent of histology and urinary albumin. Indeed, the urinary protein profile correlated significantly with Banff scores (i, t, ci, ct) in 221 individuals 1 year after renal transplantation [97]. In 162 CKD patients, there was a significant correlation between the degree of interstitial fibrosis and the RBP/creatinine ratio. This correlation remained significant after adjustment for the estimated glomerular filtration rate, age, body mass index, alpha1-microglobulin, beta 2-microglobulin [98]. Thus, measurement of urinary VDBP and RBP may offer a potential for non-invasive evaluation of fibrosis.

Microribonucleic acids

MicroRNAs are a class of noncoding RNA that play a critical role in various cellular and physiological processes by modulating the degradation of messenger RNAs, therefore altering their expression levels

Table 1: Urinary and serum biomarkers of interstitial kidney fibrosis.

	Biomarker	Source	Key point	Ref
Profibrotic and structural proteins	PIIINP	Blood Urine	Positive association with fibrosis and CKD progression	Ghoul et al [62], Teppo et al [63], Soylemezuglu et al [64], Ix et al [65]
	Periostin	Urine	Association with eGFR	Guerrot et al [66], Mael-Ainin et al [67] Sen et al [68] Satirapoj et al [69]
	TGF-beta BMP-7	Blood Urine	Association with renal function. Prediction of function evolution	Basile et al [70] Wang et al [71] Wang et al [72] Sato et al [73] Honkanen et al [74] Suthanthiran et al [75] Wong et al [76]
	CCL2	Urine	Predictor of fibrosis progression and eGFR decline in kidney allograft	Carr et al [77] Ho et al [78] Ho et al [79] Hirt-Minkowski et al [80]
	MMPs	Blood	Higher serum MMP7 with fibrosis	Tan et al [81] Rodder et al [82] Racca et al [83] Yan et al [84] Hirt-Minkowski [85] Zhou [86]
	TNF receptor	Blood	Higher TNF receptor associated to more extensive fibrosis and renal function decline	Niewczas et al [87] Gohda et al [88] Tonelli et al [89] Carlsson et al [90]
Filtered proteins	VDBP	Urine	Correlation to collagen deposition in rats. Association to albuminuria and tubular damage markers in humans	Doorenbos et al [91] Mirkovic et al [92]
	RBP	Urine	Higher urinary RBP is associated to fibrosis in CKD and kidney allografts	Amer et al [93] Pellet et al [94]
miRNAs	miRNAs	Urine Blood	High serum miR-21 and urinary miR-29 are associated to fibrosis but some are influence by renal function. Results to follow	Maluf et al [102] Anglicheau et al [103] Lv et al [104] Glowacki et al [105] Neal et al [106]

BMP = bone morphogenetic protein; CCL2 = chemokine (C-C motif) ligand 2; CKD = chronic kidney disease; miRNAs = microribonucleic acids; MMPs = matrix metalloproteinases; PIIINP = procollagen type III amino-terminal propeptide; RBP = retinol binding protein; TGF = transforming growth factor; TIMPs = tissue inhibitors of metalloproteinases; TNF = tumour necrosis factor; VDBP = vitamin D binding protein

[99]. MicroRNAs may play a key pathogenic role in kidney disease progression [100]. Some *in vitro* and *in vivo* models have shown a role of microRNA in the development and progression of diabetic nephropathy, as well as in other models of experimental CKD [101–103]. Urinary microRNAs may be considered noninvasive biomarkers for monitoring the progression of renal damage [104]. Muthukumar et al. have validated urinary microRNAs as diagnostic and prognostic biomarkers of acute cellular rejection, interstitial fibrosis and tubular atrophy [105]. Moreover, urine microRNA profiling was identified as a potential tool for monitoring graft function and anticipating progression to chronic allograft dysfunction in kidney transplant [106, 107]. MicroRNA-29c urinary levels corre-

lated with glomerular filtration rate and tubulointerstitial fibrosis in 32 CKD patients and could predict the degree of tubulointerstitial fibrosis with area under the curve of 0.883 [108]. Furthermore, circulating microRNA-21 levels are associated with renal fibrosis in mice with unilateral ureteral obstruction and in transplant recipients [109].

Urinary miRNA may represent a promising novel noninvasive biomarker of renal fibrosis and a potential therapeutic approach to suppress renal fibrosis. Some limitations remain, because even if microRNAs appear stable in body fluids, a recent study suggested that circulating levels of some microRNAs are reduced in patients with severe kidney failure [110]. These very promising markers need to be better validated in clinical studies.

In summary, both plasma and urinary biomarkers to evaluate renal fibrosis are emerging. Several mark-

ers show great promise, among which urinary microRNAs and procollagens are probably the most studied and validated (table 1).

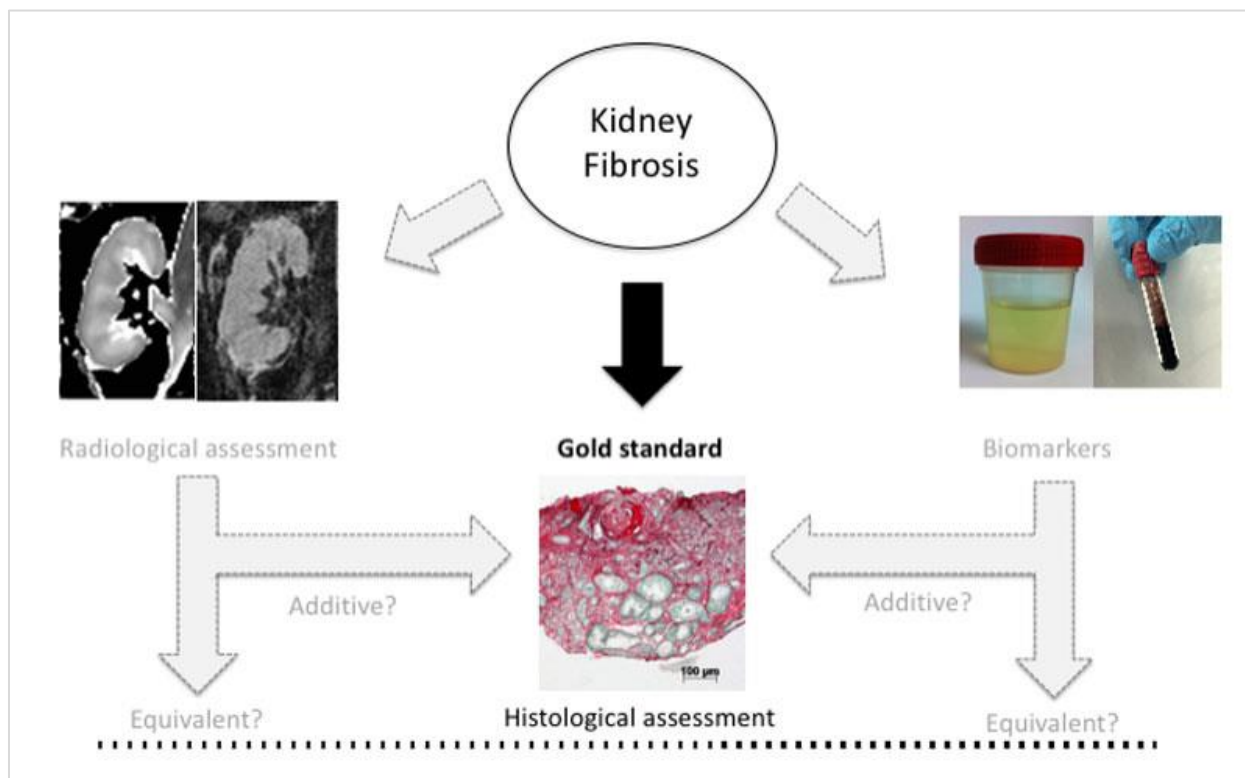


Figure 3: Kidney fibrosis is currently evaluated by histological assessment. Novel tools such as biomarkers or MRI are emerging. The place of these tools in the assessment process has yet to be determined as compared with the gold standard.

Conclusion

In recent years, several novel tools for the diagnosis of renal fibrosis have been identified. With advancing ultrasound and MRI techniques and emergence of urine and serum biomarkers, noninvasive monitoring of renal disease processes may become feasible in the near future (fig. 3). Early noninvasive markers identifying patients at highest risk of fibrosis would be useful to stratify patients for more or less intensive monitoring or therapy. Of importance, even if kidney biopsy remains the gold standard to diagnose kidney disease and to determine its severity, these tools could avoid unnecessary biopsies in the case of extensive fibrosis. However, because declining kidney function may modify biomarker expression, reproducibility and prospective validations remain major challenges for the future.

Disclosure statement

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Author's contribution

LB wrote and revised the manuscript; SdS wrote and revised the manuscript, JPV revised and approved final manuscript, SM revised and approved final manuscript, IF wrote and revised the manuscript, PYM read, edited and approved final manuscript.

Correspondence:

Professor Pierre-Yves Martin, MD
Hopitaux Universitaires Genève
Rue Gabrielle Perret-Gentil 4
CH - 1205 Genève
pierre-yves.martin[at]hcuge.ch

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