What is the best way to measure renal fibrosis?: A pathologist's perspective

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Interstitial fibrosis is a hallmark structural correlate of progressive and chronic kidney disease. There remain many uncertainties about how to best measure interstitial fibrosis both in research settings and in evaluations of renal biopsies performed for management of individual patients. Areas of uncertainty include determination of the composition of the matrix in a fibrotic parenchyma, the definition of how the interstitium is involved by fibrosing injuries, the choice of histologic stains for evaluation of renal fibrosis, and the reproducibility and robustness of measures currently employed by pathologists, both with and without the assistance of computerized imaging and assessments. In this review, we address some of these issues while citing the key studies that illustrate these difficulties. We point to future approaches that may allow a more accurate and meaningful assessment of renal interstitial fibrosis.

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WHAT IS THE BEST WAY TO MEASURE RENAL FIBROSIS?: A PATHOLOGIST'S PERSPECTIVE

It is commonly accepted that interstitial fibrosis (IF) is a key, and perhaps the key, structural correlate of progressive and chronic kidney disease. It is therefore surprising that there remain many fundamental uncertainties about how to best measure fibrosis and about whether all forms of fibrosis are equally detrimental to the kidney and whether the various approaches available for measurement of fibrosis are robust and reproducible. The review will identify some of the issues underlying these uncertainties, cite some key studies that give us a basis for choosing some approaches over others, and suggest ways in which we may move forward, but regretfully will not resolve the fundamental uncertainties that we will discuss.

Chronic kidney injury is manifested by a variety of structural alterations, including the accumulation of extracellular matrix (ECM). Most of what is considered ECM is colloquially termed IF. Tubular atrophy (TA) often accompanies IF and, when occurring together, IF and TA are collectively termed IFTA.[1–8] Taken in isolation, IF is not necessarily a marker of the degree of intactness or function of nephron units. However, studies have shown that IF quantification can help prognosticate renal outcome in renal allografts and in such native kidney diseases as IgA nephropathy, and may be considered the best available histologic marker of chronic kidney injury.[9–14]

As many investigators and practitioners ascribe a great deal of importance to the issue of IF, accurate IF measurement is often needed in a variety of applications, including research focused on the therapeutic inhibition of IF, comparison of protocol biopsies in studies of renal allografts,[1,15,16] and for clinical prognostication as is the case with IgA nephropathy and lupus nephritis.[14,17–20] However, to do this, one must understand the qualitative and quantitative issues related to the topic of IF. The qualitative issues relate to the actual composition and distribution of the IF (that is, 'what?' and 'where'). The quantitative issues, on the other hand, relate to the amount present (that is, 'how much?'). In addition, one must understand the systems currently used for IFTA assessment and the implications (that is, 'who uses this?' and 'why').

FIBROSIS QUALITY: WHAT IS IN A SCAR?

Composition of matrix

The cortical interstitial volume normally ranges from 5 to 20% with a mean of 12%,[21–24] and this volume reportedly increases with age.[24] The normal cortical interstitial volume is estimated at 5% in the rat.[25] The renal interstitium ECM contains sulfated and non-sulfated glycosaminoglycans,[21,26] such as biglycan and decorin,[27] Types I and III collagen, and fibronectin.[21,28] Type VI collagen is also present, particularly in rodents.[25,29] IF is typically considered to be an excess accumulation of fibrillar collagen, and the role of other matrix molecules such as proteoglycans and other non-collagenous proteins has not been comprehensively investigated. Knowing the composition of a fibrotic matrix is important because matrix components may determine the susceptibility of a matrix to undergo degradation by proteases and possibly undergo regression, and may determine the local tethering and/or activation of growth factors and cytokines that mediate IFTA.

Interstitial cells and their interplay with epithelial cells and vasculature

Fibroblasts constitute a large proportion of renal interstitial cells and are the major cells maintaining constituent ECM, which can be considered the kidney 'skeleton.' Fibroblasts lack a good cell type–specific marker, making their study difficult.[30] Fibroblasts and other cells may acquire a myofibroblastic phenotype, likely a crucial event in expansion of the ECM.[18,30–33] Lymphocytes appear to have important roles in the development of IFTA.[8,34–36] The classes of infiltrating or resident monocyte/macrophages are heterogeneous, displaying a variety of phenotypes.[37–40] Some macrophages may be preferentially pro-fibrotic,[38,41] whereas other classes of monocyte/macrophages may actually attenuate fibrosis.[42] Other cells also contribute to IFTA, including pericytes,[19] dendritic cells,[8,36,43–46] mast cells,[8,47–49] and fibrocytes.[6,37,40,50–54] Measures of IF rarely take into account the cellularity of the fibrotic areas, and how this may reflect the age of the fibrotic process or its potential for reversibility or other biologic features of the fibrotic process.

FIBROSIS DISTRIBUTION: WHERE IS THE FIBROSIS?

Patterns of IF vary and likely do not have identical causes or consequences. For example, the patchy, 'striped' pattern of IF with corresponding TA has been described with calcineurin inhibitor use. It has been proposed that this is because of the apparent preferential involvement of the medullary rays; however, IF also might be the result of toxic injury to discrete segments of small arteries and arterioles with consequent diminished blood supply to those portions of the cortical parenchyma supplied by the injured vessels. Despite the use of this association as a way to identify calcineurin inhibitor effect, this pattern may also be seen with hypertensive kidney disease. This 'striped' fibrosis occurs in addition to the other changes of chronic calcineurin-induced nephrotoxicity, including hyaline arteriopathy, and nonspecific glomerulosclerosis.[55]

Broad scars with the loss of tubules are the sequelae of severe focal injury and destruction of parenchyma, such as in pyelonephritis and infarcts.[8] Chronic obstruction extrinsic to the ureter can lead to IF/TA with relative glomerular sparing, atubular glomeruli, dilated tubules, and intratubular Tamm–Horsfall protein casts with extravasation into the interstitium.[56,57] The IF resulting from the metabolic injuries of diabetic nephropathy is both diffused and more homogeneous in distribution, although modification of the homogeneous distribution may occur as a result of concurrent vascular disease that may be of irregular severity. As kidneys age, there is often a pattern of subcapsular fibrosis, usually attributed to a marginal blood supply that is not replicated in less superficial portions of the renal cortex. Despite these associations, there is often an essentially nonspecific pattern of fibrosis in renal biopsies of patients with chronic kidney disease, including diffuse or patchy fine IF surrounding tubules, which can be either normal or atrophic. This is associated with either diffuse or focal disease of glomeruli, tubules, or vessels.[7,57] Although assessment of cortical IF is often stressed, medullary IF likely parallels cortical IF and epithelial loss, as stressed in studies by Farris *et al.*[58]

What about the interstitial microvasculature?

In allografts, loss of peritubular capillaries (PTCs) occurs following transplantation.[59] One study has shown that PTCs decrease with time in allografts and are inversely related to renal function; decreased PTC density at 3 months predicts later loss of function at 1 year.[59] Loss of PTC presumably results in a diminished supply of nutrients to the tubulointerstitium, and these PTC changes are often thought to parallel the presence of IF. However, it remains unclear whether loss of PTCs is causal in the development of IF and, conversely, whether restoration of the PTC density can lead to reversal of IF. Despite the obvious importance of PTC for a healthy tubulointerstitium, PTC density is rarely measured in preclinical studies of fibrosing injuries and is virtually never measured in clinical practice.

QUANTITATION METHODOLOGY: WHAT DO OUR HISTOLOGIC STAINS STAIN?

Trichrome staining (Figure 1) is often used in addition to other conventional histologic stains (hematoxylin and eosin, PAS, Silver Methenamine) to assess collagen content in the interstitium. Trichrome staining is quite practical for both clinical management of individual patients and for research studies, as it is widely available and inexpensive. For quantitation, visual assessment of trichrome-stained slides is the standard practice at many institutions;[60] however, studies have shown that this approach may have poor reproducibility.[61,62] Part of the reproducibility issue arises from uncertainty as to whether the definition of IF employed is based on total area occupied by the stainable collagen or based on areas containing any amount of stainable collagen (that is, 'fine fibrosis') as discussed further below and illustrated in studies by Furness *et al.*[63] and Farris *et al.*[64]