Collagen Remodeling Biomarkers in Lupus Nephritis

Dawn J. Caster and Michael L. Merchant

The extracellular matrix (ECM) is a complex network of collagens, elastin, and interconnecting glycoproteins and proteoglycans that surround cells and provide structural support. The ECM is a dynamic structure, undergoing constant remodeling, and providing services beyond a simple barrier or scaffold (1). Recent studies have demonstrated that the ECM and the broad complement of interacting proteins (e.g. the matrisome) play significant roles in inflammation and cell-to-cell interactions (2). Low-grade inflammation, a common feature of many kidney diseases, often contributes to aberrant ECM deposition and fibrosis. Significantly, renal fibrosis is a hallmark of renal dysfunction progression and CKD, leading to end-organ failure.

Many of the proteins making up the renal ECM are spatially resolved into the glomerular (glomerular basement membrane, the mesangial matrix, and Bowman’s capsule) and tubulointerstitial (tubular basement membrane capillaries, and interstitial space) compartments. The most abundant proteins in the ECM are collagens, accounting for almost one third of ECM proteins (3). Type IV collagen is the most well described ECM protein in terms of kidney disease. Type IV collagen has six alpha chains that form three different trimer combinations, and the α3α4α5 heterotrimer is the most predominant in the glomerular basement membrane (3). Mutations in COL4A3, COL4A4, or COL4A5 genes are associated with Alport Syndrome, thin basement membrane disease, and familial FSGS (4,5). Proteomic analysis has identified collagens I, III, VI, VII, XII, XV, and XVIII within the glomerular ECM (6,7). Collagens I, II, III, V, VI, VII, and XV are normally expressed in the tubulointerstitium and increased expression have been associated with fibrosis (3). A recent proteomic analysis of kidneys across different age groups demonstrated consistent increases in collagen VI with aging kidneys (8).

Lupus nephritis (LN) is one of the most serious complications of systemic lupus erythematosus (SLE), leading to increased morbidity and mortality. LN is classified on the basis of the location of immune complex deposition and is associated with inflammation and chronic changes to the kidney. Inflammatory lesions (endocapillary hypercellularity, neutrophil infiltration, fibrinoid necrosis, hyaline deposits, cellular/fibrocellular crescents, interstitial inflammation) contribute to the activity index, whereas chronic lesions (global and segmental sclerosis, fibrous crescents, tubular atrophy, interstitial fibrosis) contribute to the chronicity index (9). Current noninvasive clinical markers for LN include proteinuria, hematuria, serum creatinine, complement levels, and antibodies to dsDNA and C1Q (10). However, these markers do not reliably predict the degree of active versus chronic lesions, and kidney biopsy remains the gold standard for diagnosis and assessment of disease activity.

There is a growing body of literature on novel biomarkers in LN, but lack of standardization and validation have limited their clinical use (10,11). The ideal LN biomarker would prognostically identify those at risk for developing disease, distinguish between active and chronic lesions, determine risk of progression, and help stratify therapeutic choice and duration of therapy (10). No single biomarker has been able to achieve this, and a much more likely scenario will be the use of multiple biomarkers, which may include panels of genomic, serum, urine, and tissue biomarkers (10). One proposed biomarker panel is the Renal Activity Index for Lupus (RAIL) score, which combined six urinary biomarkers (Neutrophil gelatinase-associated lipocalin [NGAL], Monocyte Chemoattractant Protein-1 [MCP-1], ceruloplasmin, adiponectin, hemopexin, and Kidney Injury Molecule-1 [KIM-1]) and predicted LN activity (12). The RAIL score highly correlated with renal histology (National Institutes of Health [NIH] LN activity index) with an area under the curve (AUC) of 0.92 in a pediatric cohort (12). The results were not as robust, when the same algorithm was applied adult patients with LN (AUC 0.62) (13). By adjusting the weight of the individual biomarkers within the pediatric RAIL score, researchers were able to improve the adult RAIL AUC to 0.88, but this needs external validation and the adjustment of the original score risks overfitting (13). Thus, there is a great potential for biomarkers to predict LN activity, but also a great need to optimize and validate biomarkers in different cohorts.

In this issue, Genovese et al. evaluated markers of collagen metabolism as markers of disease activity in LN. Targeted analyses for type III and VI formation, PRO-C3 and PRO-C6, were used as profibrotic disease activity markers in a large, single-center, LN cohort. To ascertain a net aspect of collagen metabolism they also evaluated C3M, a marker of collagen type III degradation. For controls, they evaluated patients with SLE without LN, patients with other glomerular diseases (biopsy controls), and normal subjects.
Differences were detected in PRO-C3 (serum) and PRO-C6 (urine and serum) levels between healthy controls and LN, but not between SLE with and without LN. Additionally, biopsy controls also had elevated levels of PRO-C3 (serum) and PRO-C6 (serum and urine). PRO-C3 urine levels were even higher in SLE and biopsy controls than in LN subjects. Given prior studies correlating increased collagen III and VI levels in CKD, it is not surprising to see elevated levels in biopsy controls. However, the elevated levels in patients with SLE without nephritis requires further exploration. It would be helpful to follow these patients longitudinally and see if any go on to develop nephritis or CKD. Recent studies suggest that ECM remodeling may occur before histologic or clinical evidence of disease (8,14).

Serum and urine PRO-C6 inversely correlated with eGFR, which is consistent with prior findings correlating PRO-C6 to CKD (15). Serum PRO-C6 was increased in patients with LN with high NIH Activity Index on kidney biopsy, whereas urine PRO-C6 was decreased in this cohort. There was no difference in biomarker levels comparing high versus low NIH Chronicity Index, but there were correlations with interstitial fibrosis and tubular atrophy, which are components of the chronicity index. Urine C3M inversely correlated with interstitial fibrosis and tubular atrophy. Serum PRO-C6 was associated with interstitial fibrosis and interstitial mononuclear cell infiltration.

This study highlights the potential for markers of collagen turnover to predict disease activity and chronicity in LN. This study supports PRO-C6 and PRO-C3 levels as markers of disease activity and fibrosis in LN. However, these findings do not appear to be specific to LN because similar elevations were observed in glomerular disease controls. The lack of specificity to LN is not surprising, given multiple prior studies linking markers of collagen III and collagen VI to fibrosis and CKD in a spectrum of kidney diseases, including GN (summarized in Table 1). These include a study by Genovese et al. evaluating type III collagen turnover in immunoglobulin A nephropathy (IgAN), which showed PRO-C3 levels increasing and C3M levels decreasing as CKD became more advanced (16). Sparding et al. (17) found urine and serum PRO-C6 levels correlated with interstitial fibrosis and decreased kidney function in patients with IgAN and antineutrophil cytoplasmic antibody–associated vasculitis.

Multiple LN guidelines have been suggested by governing organizations including Kidney Disease: Improving Global Outcomes, American College of Rheumatology, and the European League Against Rheumatism/European Renal Association–European Dialysis and Transplant Association. These guidelines all use end-point assessments on the basis of changes in proteinuria and eGFR to define response to treatment (18). Proteinuria at 12 months is currently the single best predictor of long-term kidney outcome (19,20). If validated as markers of fibrosis, PRO-C3 and PRO-C6 levels may help predict CKD in LN. Further, interventions that can decrease collagen III and collagen VI production may be important targets in CKD prevention, including LN.

Attenuation of interstitial fibrosis has been achieved in LN models. A recent study demonstrated the administration of neutralizing antibodies to tumor necrosis factor (TNF)-like weak inducer of apoptosis in the MRL/lpr LN mouse model diminished interstitial fibrosis through reductions in type 1 collagen and fibronectin expression (21). Biomarkers for fibrosis are attractive, given potential emerging therapies, which include neutralizing antibodies to inhibit fibrotic signaling pathways. Ongoing trials focusing either on interferon (IFN) or interleukin (IL) include: the Safety and Efficacy of Two Doses of Anifrolumab Compared to Placebo in Adult Subjects With Active Proliferative Lupus

| Biomarker       | Serum Biomarker                                                                 | Urine Biomarker                                                                 |
|-----------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| **Collagen III-related proteins** | Positively associated with CKD and tubulointerstitial fibrosis (25). | Increased in IgAN (16)                                                         |
| PRO-C3          | Associated with microinflammation in IgAN (16)                                  | Positively associated with CKD and tubulointerstitial fibrosis Decreased in IgAN (16) |
|                 | Negatively associated with eGFR in T1DN (26)                                    | Positively associated with eGFR in T1DN (26)                                    |
| C3m             |                                                                                 | Negatively associated with CKD and tubulointerstitial fibrosis (25)              |
| **Collagen VI-related proteins** | Negatively associated with eGFR in T1DN (26)                                    |                                                                                 |
| PRO-C6          | Increased with CKD status and degree of tubulointerstitial fibrosis in IgAN and ANCA-associated vasculitis (17) | Increased with CKD status and degree of tubulointerstitial fibrosis in IgAN and ANCA-associated vasculitis (17) |
|                 | Positively correlated with Banff interstitial fibrosis/tubular atrophy score at 6 and 24 months post-transplant; positively correlated with tubulointerstitial inflammation score at 24 months (27) |                                        |

IgAN, IgA nephropathy; T1DN, type-1 diabetic nephropathy; ANCA, antineutrophil cytoplasmic antibodies.
Nephritis (TULIP-LN) trial (AstraZeneca, anifrolumab, NCT02547922) using an IFN-α receptor blocker, the Study of Safety, Efficacy and Tolerability of Secukinumab Versus Placebo, in Combination With SoC Therapy, in Patients With Active Lupus Nephritis (SELUNE) trial (Novartis, secukinumab, NCT04181762) targeting IL-17A, and the A Study of Guselkumab in Participants With Active Lupus Nephritis (ORCHID-LN) trial (Janssen, guselkumab, NCT04376827) targeting IL-23. In this study, hydroxychloroquine was the only concomitant treatment that was associated with lower PRO-C3 and PRO-C6 levels. Hydroxychloroquine use is associated with improved long-term outcomes in patients with LN (22). Hydroxychloroquine is also a potential therapeutic for IgAN (23). Hydroxychloroquine may have anti-inflammatory effects and tubular atrophy. They also found a positive correlation of kidney diseases. However, C3M, a marker of collagen III and collagen VI production may predict single-center clinical application.

Disclosures

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Author Contributions

D.J. Caster and M.L. Merchant wrote the original draft, and reviewed and edited the manuscript.

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