

# Potential serum and urine biomarkers in patients with lupus nephritis and the unsolved problems

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**Abstract:** Lupus nephritis (LN) is one of the most frequent and serious complications in the patients with systemic lupus erythematosus. Autoimmune-mediated inflammation in both renal glomerular and tubulointerstitial tissues is the major pathological finding of LN. In clinical practice, the elevated anti-dsDNA antibody titer concomitant with reduced complement C3 and C4 levels has become the predictive and disease-activity surrogate biomarkers in LN. However, more and more evidences suggest that autoantibodies other than anti-dsDNA antibodies, such as anti-nucleosome, anti-C1q, anti-C3b, anti-cardiolipin, anti-endothelial cell, anti-ribonuclear proteins, and anti-glomerular matrix (anti-actinin) antibodies, may also involve in LN. Researchers have demonstrated that the circulating preformed and in situ-formed immune complexes as well as the direct cytotoxic effects by those cross-reactive autoantibodies mediated kidney damage. On the other hand, many efforts had been made to find useful urine biomarkers for LN activity via measurement of immune-related mediators, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry proteomic signature, and assessment of mRNA and exosomal-derived microRNA from urine sediment cell. Our group had also devoted to this field with some novel findings. In this review, we briefly discuss the possible mechanisms of LN and try to figure out the potential serum and urine biomarkers in LN. Finally, some of the unsolved problems in this field are discussed.

**Keywords:** anti-dsDNA antibodies, serum biomarkers, urine biomarkers, THP

## Introduction

Systemic lupus erythematosus (SLE) is an archetype of systemic autoimmune disease characterized by the presence of diverse autoantibodies and self-reactive T lymphocytes that cause multiple tissue and organ damage. Lupus nephritis (LN) is one of the most important and devastating complications in patients with SLE. Despite remarkable progression in treatment, up to 25% of SLE patients progress to end-stage renal failure 10 years after the onset of renal damage.<sup>1</sup> Nowadays, renal biopsy remains the gold standard for establishing the tissue diagnosis, prognosis, and guidance of the therapeutic decision in LN. However, renal biopsy cannot be routinely conducted serially, and the obtained small-size specimens are unable to reflect the global renal pathological status of the LN.<sup>2</sup> In contrast, the clinically available routine tests such as measurement of 24-hour urine protein, the cell composition of urine sediments, and the fluctuation of serum anti-dsDNA antibodies concomitant with reduced complement C3 and C4 levels have long been applied in monitoring LN activity in daily practice.<sup>3,4</sup> However, these clinical parameters lack enough sensitivity and

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specificity to reflect the real-time renal immunopathological activity and the extent of tissue damage. Particularly, these situations would be further confounded by the preexisting chronic inflammation. It is believed that urine is an ideal specimen for finding potential biomarkers of LN due to easy accessibility and can directly reflect the real-time status of the kidney inflammation and tissue damage. In addition, LN is considered an immune-mediated inflammation in both glomerular and tubulointerstitial tissues due to aberrant systemic and intrarenal immunity.<sup>5–9</sup> Accordingly, a bunch of immune products including protein molecules, mRNAs, and microRNAs related to cytokines/chemokines/growth factors and their soluble receptors, adhesion molecules, enzymes, and activated endothelial/epithelial products have been successively discovered as surrogate urine biomarkers in LN.<sup>10–20</sup> Unfortunately, none of these urine immune-related molecules has been validated hitherto in clinical practice.

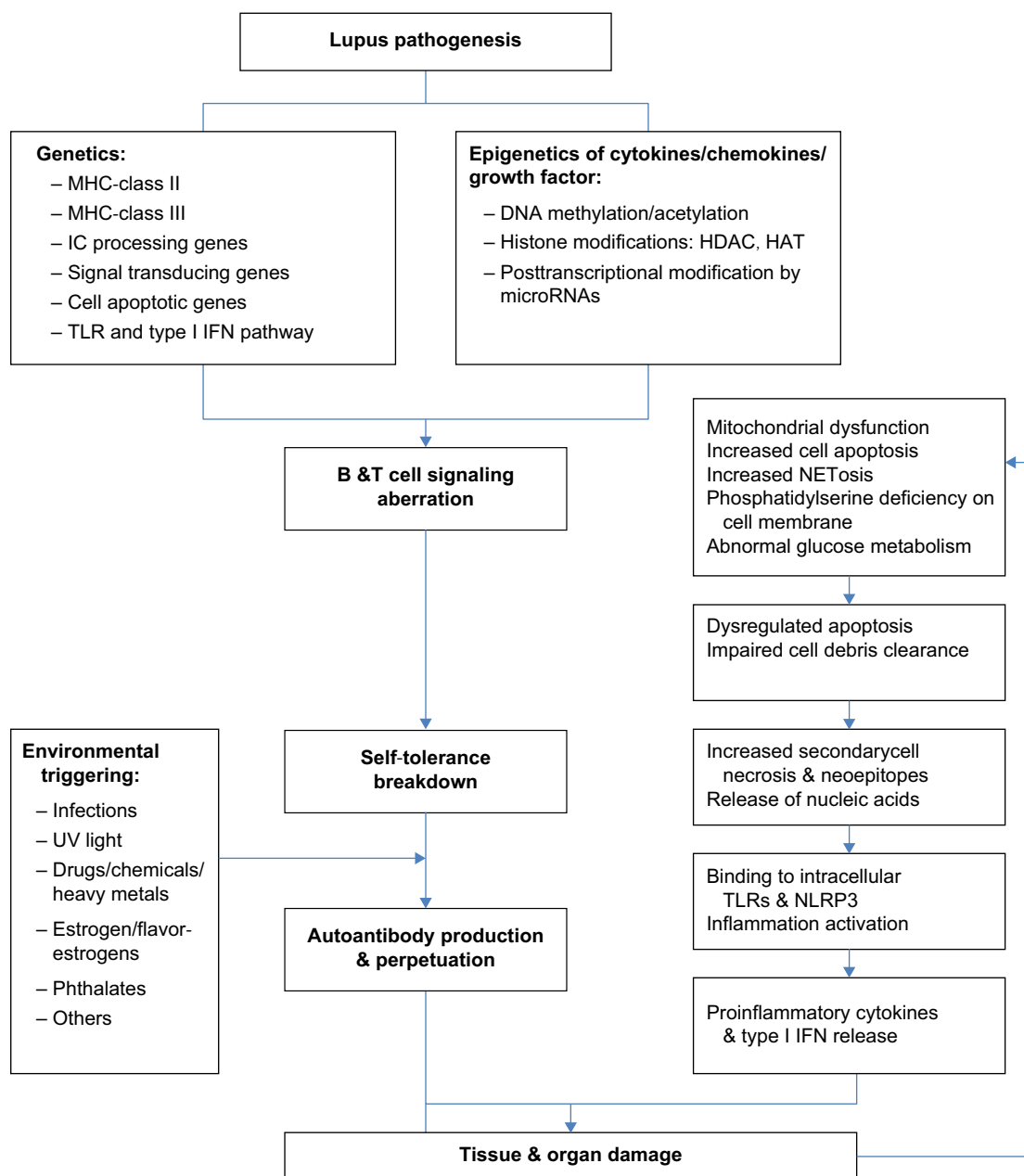
## Possible immunological mechanisms for lupus pathogenesis

It is conceivable that “breakdown of self-tolerance” is the hallmark of autoimmune diseases.<sup>21</sup> The genetic and epigenetic predispositions would be the upstream causes for aberrant T and B cell signaling.<sup>22–28</sup> As illustrated in Figure 1, the genetic predisposing loci for SLE include MHC-class II (HLA-DR2, HLA-DR3, HLA-DQ6, etc), MHC-class III (C4A null gene), and other extra-MHC loci that involve in immune complex (IC) process, signal transduction, cell apoptosis and its clearance, and the signaling pathways of Toll-like receptors, NOD-like receptors, and type I interferon expression.<sup>29–34</sup> Of equal importance is the abnormal epigenetic regulations of cytokines/chemokines/growth factors including DNA methylation (DNA methyltransferase)/demethylation (activation-induced cytidine deaminase), and histone modifications (histone acetyl- and deacetyltransferase).<sup>35–39</sup> Recently, deranged posttranscriptional regulation of mRNAs by microRNAs was found involved in LN.<sup>39–46</sup> In addition, certain cell membrane defects (low phosphatidyl-serine content),<sup>47,48</sup> low enzyme activity (low serum DNase 1 activity),<sup>49</sup> aberrant T cell signaling,<sup>50,51</sup> poor bioenergetics,<sup>52–54</sup> excessive oxidative stress due to mitochondrial dysfunction,<sup>54–60</sup> and exacerbated polymorphonuclear neutrophil (PMN) NETosis<sup>61–64</sup> may also involve in lupus pathogenesis. These multiple abnormalities would increase cell apoptosis in patients with SLE. The low complements and C-reactive protein production may further impair necrotic cell debris clearance. It is conceivable that complement system is

deeply involved in the pathogenesis of SLE in multiple ways. Complements are implicated in phagocytosis and clearance of apoptotic cells.<sup>65</sup> Hereditary homogenous deficiency of the early components of the complement classical pathway, especially C1q, is strongly associated with susceptibility to SLE.<sup>66–68</sup> Furthermore, antibodies against some complement components, C3b and C1q, are found part of autoantibody responses association with development of LN.<sup>69,70</sup> As a result, increased nucleic acids released from excessive cell necrosis by delayed apoptotic cell clearance provide neoepitopes or act as pathogen-associated molecular pattern-like or danger-associated molecular pattern-like molecules to stimulate intracellular TLRs and NLRP-3 inflammasomes. These activations subsequently enhance production of proinflammatory cytokines IL-1 $\beta$ , IL-6, IL-8, IL-17, TNF- $\alpha$ , and type I interferon from innate immune system.<sup>71–74</sup> Besides these endogenous defects in SLE, some environmental factors such as infections, chemicals, heavy metals, or drugs can boost, initiate and sustain the overt autoimmune reactions.<sup>75,76</sup> Finally, autoantibodies from B cells and proinflammatory cytokines from T and innate immune cells elicit diverse tissue/organ damage.

## Potential serum biomarkers specific for tissue/organ damage in SLE

Classically, deposition of serum preformed antigen–antibody IC in glomerular basement membrane activates complements and inflammation that is traditionally regarded as an important extrarenal mechanism for LN. As listed in Table 1, recent studies reveal that intricate serum multiple autoantibodies and immune factors may involve in a particular tissue/organ damage. Alternatively, one specific autoantibody may cause multiorgan damage. It is worthy to notice that many pathological autoantibodies including anti-dsDNA, anti-cardiolipin, anti-ribosomal P, anti-SSA/Ro, anti-Sm, anti-endothelial cell, anti-epithelial cell, anti-glomerular matrix, and anti-glomerular basement membrane antibodies concomitant with reduced serum complement C<sub>3</sub> and C<sub>4</sub> levels have been found correlated with LN.<sup>77–79</sup> It is believed that the cross-reactive property of these diverse pathological autoantibodies exhibits redundant and pleiotropic cytotoxic effects on different tissues/organs quite similar to those of cytokines/chemokines on cellular functions. Besides, elevated serum concentration of TNF- $\alpha$ /soluble TNF-RII, IL-6/IL-6R, and soluble VCAM-I has also been proved to be correlated with lupus nephropathy.<sup>20,80</sup> By contrast, the presence of anti-SSB/La autoantibodies can ameliorate and prevent renal damage in patients with SLE by an unknown mechanism.



**Figure 1** The etiopathogenetic factors contributing to breakdown of self-tolerance and autoimmune reactions that cause tissue/organ damage in patients with systemic lupus erythematosus.

**Abbreviations:** IC, immune complex; UV, ultraviolet.

## Immunopathological mechanisms in LN

Renal pathology reveals that autoimmune reactions occur in both glomerular and tubulointerstitial tissues in LN.<sup>9</sup> As shown in Table 2, immunofluorescence and electron microscopic studies have showed the deposition of immunoglobulins and complements on both sides of basement membrane of glomeruli. These intrarenal IC depositions may derive from either circulating preformed or in situ-formed ICs in glomeruli.<sup>9</sup> Further investigations have explored that two mechanisms of cytotoxic autoantibodies can cause

glomerular damage via charge–charge interaction<sup>81–83</sup> and direct binding to cross-reactive glomerular autoantigens to form in situ IC.<sup>84–86</sup> On the other hand, the infiltration of both innate immune cells (monocytes/macrophages/dendritic cells and PMNs) and adaptive immune cells (Th1, Th2, and Th17) into glomerular parenchymal tissues indicates cellular autoimmunity occurrence in lupus glomeruli (Table 2).

On the part of tubulointerstitial involvement in LN, several distinctive pathological findings are noted in Table 2: 1) activation of peritubular endothelial cells and tubular epithelial cells by proinflammatory cytokine stimulation,

**Table 1** Potential serum autoantibody and protein biomarkers specific for tissue and cell damage in systemic lupus erythematosus

Cell/tissue damage	Serum autoantibodies
Nephropathy	IC deposition, anti-dsDNA, anti-cardiolipin, antigen-antibody, anti-nucleosome, anti-C1q, anti-C3b, anti-SSA/Ro, anti-ribosomal P, anti-Sm, anti-endothelial cell, anti-epithelial cell, anti-glomerular matrix, TNF- $\alpha$ /sTNF-RII, IL-6/sIL-6R, sVCAM-I
Neuropsychiatric lesions	Anti-NR2, anti-ribosomal P, anti-cardiolipin, anti-endothelial cell, anti-dsDNA
Dermatitis	Anti-SSA/Ro, anti-SSB/La, anti-ribosomal P
Vasculitis	Anti-a-enolase, anti-cardiolipin, ICs
Hemolytic anemia	Anti-RBC, anti-cardiolipin
Neutropenia	Anti-neutrophil, anti-cardiolipin, anti-SSB/La, anti-dsDNA
Lymphopenia	Anti-lymphocyte, anti-cardiolipin, anti-SSB/La, anti-dsDNA, anti-ribosomal P
Thrombocytopenia	Anti-platelet protein 3, anti-cardiolipin
Thromboembolism	Anti-cardiolipin
Habitual abortion and fetal loss	Anti-cardiolipin

**Abbreviations:** IC, immune complex; UV, ultraviolet; NR2, N-methyl-D-aspartate receptor subtype 2a/2b.

**Table 2** Immunopathology and possible immunopathogenesis of lupus nephritis

Tissue inflammation	Immunopathogenesis
Glomerulonephritis	Intrarenal IC deposition Circulating IC deposition In situ IC deposition Cytotoxic autoantibody-mediated damage Direct binding to autoantigens Charge-charge interactions between autoantibody and glomerular matrix Cellular autoimmunity elicited by Monocytes/macrophages/DCs PMNs Th1 cells Th17 cells
Tubulointerstitial nephritis	Activation of peritubular endothelial cells and tubular epithelial cells by proinflammatory cytokines Peritubular vascular injury by ICs Amplification of inflammation via release of cytokines and cytotoxic factors Cellular autoimmunity: Tertiary lymphoid organ formation and immunoglobulin production Anti-matrix and anti-epithelial cell autoantibodies from systemic circulation

**Abbreviation:** IC, immune complex.

2) peritubular vascular injury by IC deposition or cytotoxic autoantibodies, 3) amplification of inflammation via release of cytokines and cytotoxic factors by activated immune cells, 4) cellular autoimmunity manifested by tertiary lymphoid organ formation leading to immunoglobulin production, and 5) effects by anti-matrix and anti-epithelial cell autoantibodies from systemic circulation. In conclusion, lupus tubulointerstitial nephritis is caused mainly by autoantibodies against matrix, endothelial and epithelial cells, and proinflammatory cytokines from systemic circulation as well as local production.<sup>9</sup> However, more investigations are required to confirm it.

## Anti-dsDNA antibodies cross-react with different autoantigens other than dsDNA expressed on renal tissues to mediate cytostatic effects

Although a number of autoantibodies exist in SLE serum, anti-dsDNA antibody is considered the most important and unique one in mediating LN. Sun et al<sup>84,85</sup> and Tsai et al<sup>87</sup> demonstrated that mouse monoclonal antibody against dsDNA cross-reacted with acidic ribosomal P proteins P0, P1, and P2, expressed on the cell surface of glomerular mesangial cells, liver, spleen, brain tissues, and different blood cells. After binding with glomerular mesangial cells, the cytostatic effect was found correlated with the titer of anti-dsDNA. This finding is consistent with the observation that serum titer of anti-dsDNA can reflect the global lupus disease activity and particularly the LN. Further investigations suggest that both cross-reactivity and charge-charge interactions render anti-dsDNA antibodies binding with cross-reactive antigens and negatively charged molecules other than dsDNAs.<sup>82-86</sup> As shown in Table 3, widely distributed molecules on glomerular mesangial cells, epithelial cells, endothelial cells, basement membranes, glomerular matrix, or extracellular proteins are the targets of anti-dsDNA. Despite these, the serum titer of anti-dsDNA is neither specific nor sensitive enough to predict, monitor, or prognosticate the severity of LN in the case of acute renal inflammation superimposing on chronic renal damage. Accordingly, searching for more reliable biomarkers is mandatory for clinical practice. Since cellular autoimmune reactions occur in LN (Table 2), the immune-related molecules excreted from inflamed renal tissues to urine may probably become the potential urine surrogate biomarkers in LN other than the serum autoantibodies or renal immunopathological findings.<sup>7-9</sup>

**Table 3** Anti-dsDNA antibodies cross-react with different surface-expressed molecules or directly bind to negatively charged extracellular matrical proteins via electrostatic force

Mode of interaction	Molecules on cells/tissues
<b>Cross reactivity</b>	
Surface-expressed proteins	
Ribosomal P <sub>0</sub> , P <sub>1</sub> , and P <sub>2</sub>	Glomerular mesangial cells
$\alpha$ -Actinin	Glomerular mesangial cells
$\alpha$ -Enolase	Glomerular mesangial cells and epithelial cells
Annexin A2	Glomerular mesangial cells and epithelial cells
Phospholipids	Glomerular mesangial cells and epithelial cells
Basement membrane	Glomeruli and endothelial cells
Matrix-expressed proteins	
Collagen	Glomerular matrix
Laminin	Glomerular matrix
Fibronectin	Glomerular matrix
Fibrin	Glomerular matrix
Extracellular proteins	
HSP70	Intracellular stress protein
$\beta_2$ GPI	Serum protein
<b>Charge-charge interactions</b>	
Heparan sulfate	Glomerular matrix
Chondroitin sulfate	Glomerular matrix

**Abbreviations:** HSP70, human heat shock protein 70;  $\beta_2$ GPI,  $\beta_2$ -glycoprotein I.

## The excreted protein molecules in the urine of normal and LN patients

Normal human urine contains scant amount of albumin, certain proteolytic enzymes, corticosteroid metabolites, hormones and metabolites, modulators of urinary stone formation, and THP (which acts as a binding molecule for urinary tract microbial pathogens and different cytokines/chemokines) as shown in Table 4. Proteinuria, increased urine sediments with different cell components, and the presence of cellular/hyaline casts are considered the abnormal urine findings in LN. However, these abnormal findings are totally nonspecific and cannot be used for differentiating acute inflammation from acute inflammation superimposing on chronic renal damage. Among the excreted protein molecules in normal urine, it is noteworthy to realize that THP is synthesized only by the tubular epithelial cells in the thick ascending limb of Henle's loop and proximal convoluted tubules. The daily excreted amount of THP in normal urine is ~50–150 mg/day.<sup>88,89</sup> The major physiological function of THP is preventing the attachment of pathological microbes and then penetrating into urinary tract interstitium.<sup>90–93</sup> Another important physiological function of THP relies on its capacity to nonspecifically bind with a variety of circulating protein molecules, cytokines, and chemokines in the kidneys.<sup>94,95</sup> Decreased excretion or deranged functions of THP molecule in urine may reflect renal tubular cell damage by any cause.

**Table 4** The excreted protein molecules in normal human urine

Categories of bioactivity	Protein molecules
Proteolytic enzymes	Urokinase-related molecules Urinary kallikrein molecules
Corticosteroid metabolites	22 corticosteroid hormone metabolites
Hormones and metabolites	Growth hormone-releasing peptide and their major metabolites Estrogens and their metabolites Testosterones and their metabolites Thrombomodulins
Modulators of urinary stone formation	Nephrocalcin Osteopontin Prothrombin fragment I Bikaverin THP
Microbial-defensing molecule	THP
Cytokine/chemokine modulatory molecules	THP

## Potential urine biomarkers in LN

From the clinical point of view, urine is considered the ideal source for finding the potential biomarkers in LN due to its easy accessibility and can directly reflect the real-time pathological status of kidneys. Taking into consideration the autoimmune nature of LN, many immune-related molecules are excreted into urine from the inflamed and damaged kidneys of LN. Recent investigations demonstrated that glomerular mesangial cells work as the intrarenal fixed tissue macrophage-like contractile cells and are the first affected cells in LN.<sup>96,97</sup> Many authors have showed that not only innate and adaptive immune cells but also renal parenchymal cells are activated to proliferate by immunological reactions in both glomerular and tubulointerstitial tissues in LN.<sup>98,99</sup> In chronic LN, tissue damage, tissue atrophy, and eventually fibrosis lead to endstage kidney disease.

Tsai et al<sup>10</sup> reported increased excretion of soluble IL-2 receptors and free light-chain immunoglobulins in the urine of patients with active LN. These findings suggest that both T and B cells in LN are engaged in cell proliferation, differentiation, and maturation by autoimmune mechanism. Then, Tsai et al<sup>13</sup> and Boenisch et al<sup>100</sup> further demonstrated increased excretion of  $\beta_2$ -microglobulin, IL-6, and IL-8 and decreased excretion of THP in the urine of patients with active LN. These results further suggest that the proinflammatory cytokines produced from activated monocytes/macrophages, neutrophils, and even renal parenchymal cells can reflect the degree of inflammation in LN. The increased urine  $\beta_2$ -microglobulin excretion also reflects both immune cell activation and tissue destruction in LN. However, the most unique finding of this study indicates that decreased THP excretion can be considered a potential biomarker for



tubulointerstitial inflammation/damage in LN. Later, Yang et al<sup>101</sup> further reported increased 24-hour urine NGAL in active LN. NGAL, a 25 kDa protein molecule capable of binding and transporting small hydrophobic molecules (such as iron), is secreted by many cell populations including PMNs, macrophages, and epithelial and endothelial cells.<sup>102,103</sup> Increased urine NGAL excretion was reported in both acute and chronic LN.<sup>104,105</sup> As listed in Table 5, not only a number of serum protein molecules are excreted in the LN urine but also a vast amount of in situ-produced immune-related molecules in renal tissues such as proinflammatory/anti-inflammatory cytokines, chemokines, soluble cytokine/chemokine receptors, soluble adhesion molecules, and tissue growth factors have been successively discovered in LN urine. Besides, many authors have found that specific mRNAs and exosomal microRNAs are obtained from urinary sediment cells of patients with LN.<sup>14,15,17,46,106</sup> The urine proteomic signature analysis by using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry has also been successfully applied for urinary biomarker identification in LN.<sup>107</sup> The enzyme-linked immunosorbent assay measurement of NAG has been found as another

biomarker of tubulointerstitial nephritis as well as THP.<sup>108–110</sup> Unfortunately, none of these florid urine biomarkers has been validated to date in clinical application.

## Comparison of sensitivity and specificity of part of the potential serum and urine biomarkers in LN

To become an ideal and useful disease biomarker in clinical practice, both sensitivity and specificity should be high enough (>80%). Table 6 lists the comparison of sensitivity and specificity of different published serum<sup>80,111–113</sup> and urine<sup>19,114–117</sup> biomarkers related to LN. However, only serum anti-dsDNA antibodies fulfill the aforementioned criteria as a useful disease biomarker in patients with LN. Urine NGAL may probably fulfill the criteria of an LN disease activity biomarker, but it is not the routine test. Regarding miR-29c, only one report demonstrated in the literatures despite high sensitivity and specificity.<sup>115</sup> Further investigation is necessary to confirm this possibility.

## THP, a unique urine glycoprotein, acts as a nonspecific binder for modulating immune reaction in urinary system

THP was discovered by Tamm and Horsfall in 1950.<sup>92</sup> This unique glycoprotein containing 25%–30% carbohydrate moieties is synthesized only in the thick ascending limb of Henle's

**Table 5** Potential urinary biomarkers in lupus nephritis

Categories of biomarkers	Urinary molecules
<b>Lupus glomerulonephritis</b>	
Protein molecules	Albumin Transferrin LFABP NGAL TWEAK Hepcidin, ceruloplasmin $\beta_2$ -Microglobulin, $\alpha_1$ -acid glycoprotein THP
Inflammatory cytokines/chemokines	IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IP-10, CXCL MCP-1 (CCL2), MIP-1 $\alpha$ (CCL3) Fractalkine (CX3CL1), CXCL6
Anti-inflammatory cytokines	IL-10, TGF- $\beta$
Soluble cytokine/chemokine receptors	IL-2R, TNF-R1, CXCR3, VCAM-1, P-selectin
Growth factor	VEGF
Urine proteomic signature	2.7, 22, 23, 44, 56, 79, 100, and 103 kDa (by SELDI-TOF-MS)
Exosomal microRNAs	( $\uparrow$ ) MiR-125a, MiR-150, MiR-155, MiR-146 ( $\downarrow$ ) MiR-141, MiR-192, MiR-200a, MiR-200c, MiR-221, MiR-222, MiR-429
Urine sediment mRNA	IP-10, CXCR3, TGF- $\beta$ , VEGF, FOXP-3
<b>Tubulointerstitial involvement</b>	
	$\beta_2$ -Microglobulin, IL-6, IL-8 NAG THP $\downarrow$

**Abbreviation:** SELDI-TOF-MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry.

**Table 6** The reported sensitivity and specificity of some potential serum and urine biomarkers in lupus nephritis

Category of specimens	Sensitivity (%)	Specificity (%)
<b>Serum biomarkers</b>		
Anti-dsDNA IgG (range)	80.0–88.6	92.3–97.7
Anti-nucleosome IgG	74.7	96.0
Anti-histone IgG	28.2	97.7
Anti-Sm IgG	15.4	100
Anti-C1q IgG	63.0	71.0
Anti-C3b IgG	36.0	98.0
Decreased C3c	64.1	88.4
Decreased C4	51.3	95.3
<b>Urine biomarkers</b>		
NGAL	79.49	80.0
VCAM	98.2	66.7
TGF- $\beta$ I	64.1	68.0
MCP-1	76.92	80.0
IL-17	66.67	72.0
IL-8	34.0	55.7
OPG	74.0	78.3
TWEAK	89.0	56.0
miR-29c	94.0	82.0

loop and the early distal convoluted tubule of the kidney.<sup>90,91</sup> The major physiological function of THP is initially identified as a potent inhibition of viral hemagglutination in urine against New Castle, mumps, and influenza viruses for preventing pathogen attachment and then invasion into urinary interstitial tissues.<sup>92,93</sup> Recent studies revealed that THP is a natural nonspecific binder of cytokines and chemokines with different binding affinities for modulating the fate of these immune factors in circulation or their in situ production in the kidneys.<sup>94,95</sup> Table 7 summarizes the capacity of THP to bind with different protein molecules including serum proteins, neutrophil granular enzymes, and various cytokines/chemokines. In physiological and certain pathological conditions, the sugar moieties in THP side chains are altered, or the amount in urine excretion is reduced.<sup>118–123</sup> Evidence suggests that mutations in THP gene may lead to congenital cystic kidney diseases or familial juvenile hyperuricemic nephropathy.<sup>124–127</sup> Table 8 lists the alterations in THP glycosylation or defective production in different pathological conditions. Although we found

reduced urine excretion of THP in active lupus tubulointerstitial nephritis,<sup>100</sup> it is still not elucidated whether the molecular structure or the functions of THP are altered or not in LN. In addition, THP had been reported as a ligand for TLR4<sup>128</sup> and scavenger receptors on macrophages/dendritic cells,<sup>129,130</sup> and SREC1 on endothelial cells.<sup>130,131</sup> The THP receptor binding activates these phagocytes to increase uptake and clearance of the engulfed bacteria and CpG DNAs. Accordingly, it is possible that reduced THP production in patients with LN would delay CpG DNAs clearance in the damaged kidney. This delayed clearance of CpG DNAs facilitates anti-nucleic acid antibody production that may further exacerbate kidney damage in SLE patients. However, the cause–effect relationships among less THP production, increased CpG DNA levels, facilitation of anti-dsDNA antibodies production, and occurrence of tubulointerstitial LN are not clear at the present time.

## Unsolved problems in identifying useful urine biomarkers in LN

To become the useful biomarkers for prediction, monitoring, and prognosis of a particular disease in clinical practice, one should consider the accessibility, specificity and sensitivity of the test, and the popularity of the specimen should be considered. The reported urine biomarkers in LN mostly are immune-related molecules produced by innate and adaptive immune reactions.<sup>131</sup> The dilemma in selecting the ideal urine biomarkers of LN in clinical practice remains unless the following problems are solved:

1. Which urine specimen is better for detecting the specific biomarker? Spot urine vs 24-hour urine.
2. Which biomarker can accurately reflect the real-time kidney inflammation and tissue damage in acute vs chronic inflammation vs acute inflammation superimposing on chronic kidney damage.
3. What is the specific urine biomarker for acute fulminant renal inflammation such as rapidly progressive glomerulonephritis?
4. Does THP per se play a role in modulating balance of proinflammatory/anti-inflammatory cytokines in LN? Is the glycosylation of THP in LN altered or not?
5. Is it necessary to reevaluate the roles of different nephritogenic autoantibodies in LN?
6. How to design a laboratory screen strip for rapid evaluation of the renal inflammation by a drop of urine?
7. What is the crucial role of glomerular mesangial cells in the pathogenesis of LN?
8. What are the roles of Th17 cells and PMNs in initiating and sustaining the progression of LN?

**Table 7** Capacity of THP to bind with different molecules

Binding capacity	Molecules
High affinity	TNF- $\alpha$ , human IgGs, C1q, BSA, cathepsin G
Medium affinity	IL-8
Low affinity	IL-6, IFN- $\gamma$ , lactoferrin
None affinity	Proteinase 3

**Table 8** Changes of THP structure in physiology and different pathological conditions

Different conditions	Structure change in THP molecule
<b>Pregnancy</b>	Increased glycosylation
<b>Familial juvenile hyperuricemic nephropathy</b>	Mutations in THP genes
<b>Autosomal medullary cystic kidney disease 2</b>	Mutations in THP genes
<b>Tubulointerstitial nephritis in SLE</b>	Decrease in THP excretion
<b>Allograft renal rejection</b>	Reduced mannose residues Reduced Sia $\alpha$ [2,3]Gal/Gal NAc and $\beta$ [1,4]Glc NAc
<b>Glomerulonephritis</b>	Increased IgG, IgM, and IgA depositions in urine casts
<b>Interstitial cystitis</b>	Reduced high-molecular weight sialylated oligosaccharides
<b>Different urinary diseases</b>	
Urinary tract infections, glomerulonephritis, or interstitial nephritis	Decreased N-acetylglucosamine and N-acetylgalactosamine
Bartter's syndrome	Decreased N-acetylglucosamine and less-sialylated chains

**Abbreviation:** SLE, systemic lupus erythematosus.

9. What is the role and acting mechanism of renal parenchymal cells in LN?

## Conclusion

In clinical practice, biomarkers can be classified into predictive, diagnostic, and prognostic categories. SLE, a complex polygenic autoimmune disease, can be triggered by environmental factors leading to chronic immune dysregulation. LN is a serious complication with high mortality and morbidity in SLE patients. Currently reported serum or urine biomarkers for LN are no less than immune-related molecules that fail to specifically reflect the real-time disease activity of kidneys. Since the upstream etiological mechanisms for LN are the deranged genetic and epigenetic regulations, the genome-wise singlenucleotide polymorphism/mutant analysis in well-defined LN subpopulations with different disease severity will be the best strategy for searching the predictive and prognostic biomarkers in LN.

## Disclosure

The authors declare no conflicts of interest in this work.

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