

Which autoantibodies announce that lupus nephritis is on the way?

Systemic lupus erythematosus is characterized by a wide assortment of clinical traits, along with a profusion of autoantibodies. In practice, lupus nephritis is rather common, and dsDNA reactivity is crucial. Not only is this antibody a valuable asset for the diagnosis of systemic lupus erythematosus, but it may also contribute to its pathogenesis. Consistent with this view is that titer of anti-dsDNA antibody reflects the presence of lupus nephritis. However, this may rise or drop, before or during a renal flare. Other autoantibodies would thus be of use in monitoring the disease. These include anti- α -actinin, anti-C1q and antinucleosome antibodies. Yet, their prognostic value for lupus nephritis must be further investigated. The affinity of related autoantibodies for DNA should perhaps be taken into account, but more instrumental should be antinucleosome, anti- α -actinin and anti-C1q antibodies. They are associated together, but each may be combined with high-affinity anti-dsDNA, and particularly with antibodies, to glomerular membrane-associated nucleosomes.

KEYWORDS: anti- α -actinin antibody ■ anti-C1q antibody ■ anti-DNA antibody
■ lupus nephritis ■ systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune condition that affects 20 to 70 new patients per 100,000 individuals every year. The disease affects nine females relative to one male, but this disparity between genders fluctuates according to ethnicity and socioeconomic conditions [1]. The presentation of this disease is unique in that its heterogeneity is exceptional. The degree, the number and the site of damaged organs diverge from one patient to another.

However, lupus nephritis (LN) seems to be particularly common across patient groups and proves to be the most life-threatening complication of SLE. The development of renal disease is an important clue to poor outcomes in SLE. One step further, the past few years have witnessed increasing interest in the possibility that early treatment of renal involvement, which is silent at that time, may circumvent its natural worsening. This awareness provided the impetus for a series of analyses aimed at validating variables that would herald forthcoming kidney involvement. Early reports have pointed to the youth of patients, their non-Caucasian origin, the high activity of the disease, its steroid-dependence and inheritance of several haplotypes of HLA alleles [2].

In such a context, biopsy of the kidney is mandatory. The major histopathological finding consists of deposition of autoantibodies, nucleic acid, immune complexes and complement into

the glomeruli. Once accumulated, these components may induce the surrounding inflammatory cells to proliferate. However, assuming that production of the anti-dsDNA antibody is inherent to the immune system, and that just a minority of them behave as nephritogenic, this wide family of autoantibodies warrants scrutiny in order to identify its most pathogenic members. From inconsistencies in the results of these endless studies stems the alternative view that autoantibodies bind directly to glomerular structures. Although a huge quantity and a marked diversity of IgG have been eluted from lupus kidneys, some degree of controversy persists over those antigens liable to drive synthesis of antibodies to hitherto nonimmunogenic structures, thus facilitating their deposition. This would result in built up immune complexes, and complement would be activated locally. The issue is whether some of the autoantibodies are deleterious. If such is the case, detection of nephritogenic autoantibodies may aid the clinician in identifying those patients at risk of developing LN later in life.

The authors of a recent survey of autoantibodies possibly encountered in SLE claimed that their total is likely to exceed 100 [3]. However, the clinical relevance of their majority remains so elusive that only a handful of markers may be reliably associated with LN. To deserve any usage in daily practice, emergence of these

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autoantibodies must antecede the development of LN for sufficient time, as illustrated by the presence of autoantibodies in serum samples collected over 3 years, on average, before the beginning of SLE [4]. In addition, the autoantibody titers must rise or decline, thus averting forthcoming flares [5]. Nonetheless, at the time of referral, autoantibodies exist; these are highly negative predictors for renal complications, and frequently present in this setting.

After a brief description of LN, our review will be restricted to four families of autoantibodies: anti-dsDNA, anti- α -actinin, anti-C1q and anti-nucleosome. These have been selected on the growing consensus that they accompany renal involvement in SLE. From this starting observation, the possibility follows that they might be endowed with pathogenic potential and may be associated with a prognostic significance of the ensuing damages.

Multifaceted lupus nephritis

Approximately one SLE patient in three suffers from LN, of whom 5–10% evolve to renal insufficiency. These complications often develop within the first 3 years after diagnosis [6]. An interesting paradox is that 20% of the 90% of female SLE patients develop LN, compared with 50% of the 10% of males with SLE [7]. Following diagnosis of SLE, men develop LN earlier than women [6–8], and this risk depends on ethnic background [8]. For example, Asian–Americans have a significantly increased probability of developing LN, compared with European–American and non-European–American SLE patients [7].

The clinical symptoms of LN encompass a vast panel of presentations. They include mild-to-severe proteinuria, microscopic hematuria, hypertension and, ultimately, renal failure. The complications combine with each other, and correlate with renal histopathological lesions. Early observations have generated several classifications. However, the International Society of Nephrology (ISN [Brussels, Belgium])/Renal

Pathology Society (RPS [MO, USA]) 2003 classification system (TABLE 1) is the one currently in use [9].

Briefly, histopathological damages designated as Class I correspond to mesangial deposits, but patients may not suffer from renal symptoms. Class II refers to mesangial proliferation and patients present with mild proteinuria, and microscopic hematuria, although the renal prognosis is often excellent. Class III and Class IV imply glomerulus antibody deposition and differ in that less than 50% of the glomeruli are impacted in Class III, and more than 50% in Class IV. In essence, patients with Class III LN manifest hematuria, proteinuria, nephrotic syndrome and, occasionally, hypertension. Class IV characterizes diffuse LN and comprises segmental and global forms, according to the severity of glomerular lesions. Hematuria, massive proteinuria, nephrotic syndrome and acute renal failure occur in 16% of Class IV patients. Class V corresponds to immune-complex-derived membranous nephritis. Again, the lesions display global or segmental distribution, although more than 50% of the capillary basement membrane are involved in either case. Clinical presentations include proteinuria (often at a nephrotic range) with hematuria, but usually without renal insufficiency. LN culminates in Class VI, resulting from the alternance of flares and pauses, leading to overt renal failure, and is substantiated by vascular sclerosis, tubulo-interstitial scarring and glomerular sclerosis. However, these clinical features are not well associated with the classification, since histologically severe LN may be clinically silent.

Besides these well-documented damages, SLE yields a broad variety of vascular lesions, which are neglected in the ISN/RPS classification. Thrombotic microangiopathy is particularly associated with the antiphospholipid antibody syndrome and lupus vasculopathy, and, to a lesser extent, with transmural necrotizing vasculitis.

Table 1. Classification of lupus nephritis according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS, 2003).

Class	Histopathology
Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal lupus nephritis
Class IV	Diffuse segmental or global lupus nephritis
Class V	Membranous lupus nephritis
Class VI	Advanced sclerosing lupus nephritis

Autoantibodies

■ Anti-dsDNA antibodies

There is little doubt that, in some way, anti-dsDNA have nephritogenic potential. However, observation of LN without anti-dsDNA in humans [10] and mice [11] raises the possibility that this autoantibody is not mandatory for renal tissue damage; other autoantibodies also appear to be dispensable. Diseased individuals may [12] or may not have [10] antibodies to nucleosomes, or antibodies to ribosomal P proteins [13,14]. Other provocative data suggest that B lymphocytes and related autoantibodies play a subordinate part in the pathophysiology of the disease. An example is the recent report that rapidly progressive LN did not respond to rituximab, despite depleting B cells and reducing anti-dsDNA [15] (and American College of Rheumatology [GA, USA] 2008, [ISENBERG D, UNIVERSITY COLLEGE OF LONDON, UK & YOUINOU P, UNIVERSITÉ EUROPÉENNE DE BRETAGNE, FRANCE, PERS. COMM.]). Another trial [16] describes a small proportion of individuals with improvement in the LN, notwithstanding the apparent deterioration in anti-dsDNA titer.

The extreme heterogeneity of dsDNA reactivity explains why numerous methods have been set up for revealing different subgroups of autoantibodies. Roughly, they may be distributed into those that bind to dsDNA in a dissociating context (thanks to their high affinity), and those that do not (owing to their low affinity). The methods currently in use are the indirect immunofluorescence test on *Crithidia luciliae* and a variety of ELISA. All these latter assays rely on preparations of dsDNA that are assumed to be pure and coated onto the plates. The *C. luciliae* test identifies antibodies bound to dsDNA-containing kinetoplasts. The Farr radioimmunoassay is no longer used on a routine basis; however, the antibodies detected through this method resisted ammonium sulfate and were thus endowed with high affinity dsDNA in the precipitate.

Studies addressing the usefulness of these methods for the diagnosis of SLE are often based on a subset analysis (TABLE 2). Awareness has generated a tendency to use at least two tests for the diagnosis and monitoring of SLE [17–19].

Investigators eluting IgG obtained at autopsy from glomerular immune deposits in human lupus kidneys first cast doubt on the pathogenicity of anti-dsDNA, realizing that these antibodies displayed multiple different specificities [20]. Further heterogeneity comes from the fact that pathogenicity is restricted to a subpopulation of anti-dsDNA antibodies, even though these specificities represent the most enriched species in the kidneys of lupus-prone mice [21]. Pathogenic autoantibodies attach to mesangial cells, and their passive transfer to normal mice induce proteinuria [22]. This concept has since been documented by experiments using monoclonal antibody (mAb) towards dsDNA. Surprisingly, there were no differences in class, subclass and affinity for dsDNA between pathogenic and nonpathogenic mAb [23]. The binding of aforementioned antibodies to dsDNA accounts for pathogenicity in neither humans [10] nor mice [11]. The target antigens must be accessible to the autoantibody, suggesting that an autoimmune background is required.

Deposit formation of autoantibodies along the glomerular basement membrane (GBM) is an initiating event. However, the mechanisms leading to accumulation of nephritogenic antibodies continue to be debated. Some investigators claim that the antibodies bind directly to GBM through their cross-reactivity with glomerular structures. Among credible candidates are α -actinin, collagen IV, laminin and α -enolase (TABLE 3). Although nucleosomes represent a dominant target structure for nephritogenic autoantibodies in the context of SLE, definitive proof that other candidates predominate over the remaining specificities is lacking.

■ Antinucleosome antibodies

Several mechanisms conspire to render anti-dsDNA antibodies pathogenic. Anti-dsDNA antibodies may be driven in the kidney by unrelated antigens, and bind to them in such a way that complexes of local antigens and anti-dsDNA antibodies bind to renal structures. The nucleosome is by far the most

Table 2. Relevance for the diagnosis of systemic lupus erythematosus of combining the *Crithidia luciliae* test and/or the Farr assay with the ELISA.

<i>C. luciliae</i> and/or Farr	ELISA	Specificity	SLE	Antibody affinity
+	+	+++	Active, flare	High
+	-	++	Mild, inactive	High
-	+	+	Mild, inactive	Low

SLE: Systemic lupus erythematosus.

Table 3. Glomerular structures cross-reacting with anti-dsDNA autoantibodies.

Localization	Renal antigenic target	Proven nephritogenicity
Mesangial cells	α -actinin	Yes
Glomerular basement membrane	Type IV collagen	No
Tubular epithelial cells	Heparan sulfate	No
Ubiquitous	Laminin	Yes
–	α -enolase	No
–	Cardiolipine	No
–	Myosin	No
–	Glutamate receptor	No
–	Elongation factor 2	No
–	Ribosomal protein P1	No

credible candidate [24–26]. This represents the basic unit of chromatin, associating a histone octamer core with a loop of 146 bp dsDNA. The organite can be detected in the blood of SLE patients, owing to a generally enhanced occurrence of apoptosis together with a defect in the clearance of apoptotic bodies' chromatic structures hitherto hidden within the blebs, and is thus abnormally exposed. This sequence was recently and brilliantly discussed by the Rekvig's group. [25]. The serum level of human nucleosomes correlates with disease activity in some [26], but not all studies [27]. Presumably, nucleosome arises from apoptotic nuclei left in the circulation by SLE phagocytes [28]. Interestingly, injection of syngeneic apoptotic cells into normal mice generates immune deposition into their kidneys [29]. They bind to GBM via interactions of their cationic histones with anionic proteins of the GBM, retain anti-dsDNA, trigger activation of complement and favor glomerular injury [30].

Some autoantibodies recognize exclusively the nucleosome, but neither dsDNA nor the histones. Such are the genuine antichromosome antibodies. They are quite rare and, in the context of LN, considered as more pathogenic than anti-dsDNA by some investigators [31] and less pathogenic by others [12]. Of interesting note, the Fritzler's group recently demonstrated that antibodies to chromatin are most highly correlated with the development of LN requiring transplantation [32]. In this seminal study, the odds of progressing to renal transplantation was 16-fold higher in SLE patients testing positive for antinucleosome antibodies compared with those who tested negative.

■ Anti- α -actinin antibodies

α -actinin is a highly-conserved 100-kDa protein belonging to the actin-binding proteins family [33]. α -actinins 1, 2 and 4 are expressed

in podocytes and mesangial cells. Of note, in addition to the regular role of actinins in the organization of cytoskeleton, α -actinin 4 might be involved in the physiology of the kidney. This is suggested by the development of severe glomerulitis in mice with this gene deleted [34]. Conversely, α -actinin 4 is overexpressed at the cell surface of MRL-*lpr/lpr* mesangial cells, compared with normal mice, further substantiating its important role in the development of severe nephritis [35]. Deleterious mutations in the α -actinin 4 gene are associated with an autosomal-dominant form of segmental glomerulosclerosis in humans [36].

α -actinin has been recognized as a potential cross-reactive epitope for anti-dsDNA. Consistent with this view is that those five of seven murine anti-dsDNA mAb that bound as well to α -actinin were pathogenic, whereas the remaining two mAb, which did not recognize α -actinin were not [22]. In addition to high titers of anti- α -actinin antibodies in the serum of lupus-prone mice, these can be eluted from their kidneys [37]. Experimental data support the hypothesis that human anti-dsDNA/anti- α -actinin double-reactive antibodies are particularly pathogenic. For example, ten anti-dsDNA and/or anti- α -actinin mAb have been derived by Epstein-Barr virus-transformation of B lymphocytes from SLE patients [38]. All cross-reactive antibodies were able to bind murine mesangial cells and glomeruli, and mice that received the cell line cells intraperitoneally developed inflammatory features of glomerular damages. Similarly, mice injected with α -actinin mount anti- α -actinin responses, followed by antichromatin, and develop LN thereafter [39]. Some investigators have confirmed the presence of serum antibodies to α -actinin in humans [40], although other investigators deny their nephritogenic potential in mice [41]. Among anti-dsDNA antibodies

deposited in the glomeruli, those double reactive cross-react with histone H1, rather than with α -actinin.

■ Anti-C1q antibodies

C1 is the first complex of the classical pathway of complement activation and C1q is the first component of the C1qrs complex, presenting as a hexameric polypeptide, formed of globular heads linked to a collagen-like tail. These two different parts of the molecule play distinct roles. The heads link the Fc portion of two molecules of IgG, or bind to two monomers within one molecule of IgM. Once the globular heads attach to immune complexes, the collagen-like tail changes conformation and acquires the capacity to activate subsequent enzymatic subunits. Their first physiological function is to facilitate clearance of apoptotic cells and pathogen-containing immune complexes. This is one reason why genetic C1q deficiency is associated with autoimmunity in humans and mice [42].

In general, a solid-phase ELISA serves to detect anti-C1q antibodies. To elude artefactual binding of immune complexes to the plate-coated C1q molecules, one may substitute 1 M NaCl for 0.15 M NaCl to fill the wells, or to eliminate the nonimmunogenic globular heads of C1q before the ELISA. Anti-C1q autoantibodies have been reported in a number of pathologic settings, including autoimmune disorders and infectious diseases [43]. Their prevalence in the normal population is negligible, but as many as 18% of normal individuals older than 70 years of age test positive [44]. Given its presence in 100% of hypocomplementemic urticarial vasculitis cases, its negative predictive value reaches 100% in this context [45].

Several findings fuel the idea that the implication of these autoantibodies in the pathogenesis of LN is indirect. First, the autoantibody can be detected in patients free of renal damages. Second, the autoantibodies are not capable of inducing glomerular lesions on their own in mice. In normal mice, anti-C1q mAb favor the deposition of C1q-containing immune complexes in the kidney, although they do not cause inflammatory lesions and detectable proteinuria [46]. By contrast, mice previously injected with another anti-GBM antibody develop severe glomerular lesions with massive proteinuria. The implication of this finding is that anti-C1q target the collagen-like region of the molecule, provided C1q has previously bound to immune complexes, introduced changes in its conformation and expressed otherwise hidden epitopes [47]. Their pathogenicity is

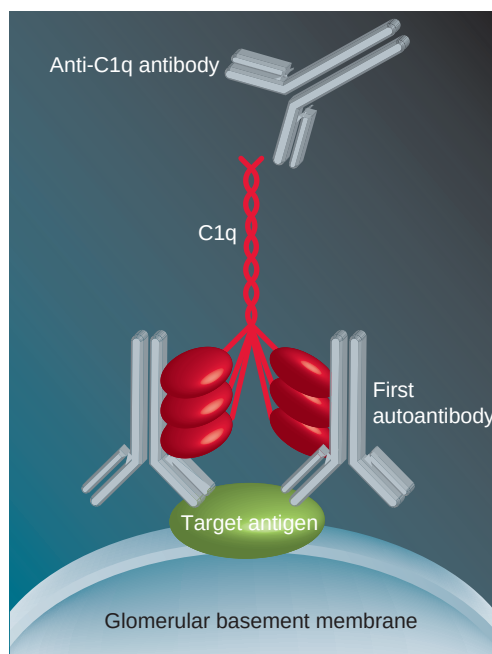


Figure 1. Implication of anti-C1q antibody in the genesis of lupus nephritis. The triggering antigen might be a nucleosome stuck to the glomerular basement membrane or intrinsic components of glomerulus, such as α -actinin or laminin. C1q attaches to glomerulus-bound autoantibody through its globular moiety. Then, anti-C1q antibody recognizes its collagen-like tail and amplifies the inflammatory process.

indirect in LN in that they amplify a process first initiated by an autoantibody that deposits in glomerulus (FIGURE 1).

Clinical usefulness

■ Anti-ds DNA autoantibodies

Not only are anti-dsDNA found in 40–60% of SLE patients, but their emergence in related diseases is exceptional. As a consequence of such sensitivity and specificity, they are still useful in diagnosis. Therefore, they have been selected as a criterion for the classification of the disease [48]. However, less sensitive autoantibodies, such as anti-Smith, have also been selected to be tested for. Anti-dsDNA antibodies are mostly of the IgG1 and IgG3 subclasses [49], and their high affinity results from somatic hypermutation [50]. The data suggest that anti-dsDNA antibodies arise from an antigen-driven immune process. Several studies have shown that the levels strongly correlate with disease activity, most notably in patients with renal disease. However, we must keep in mind that a disproportionate weight of 16 has been allotted to kidney complications in the SLE disease activity index.

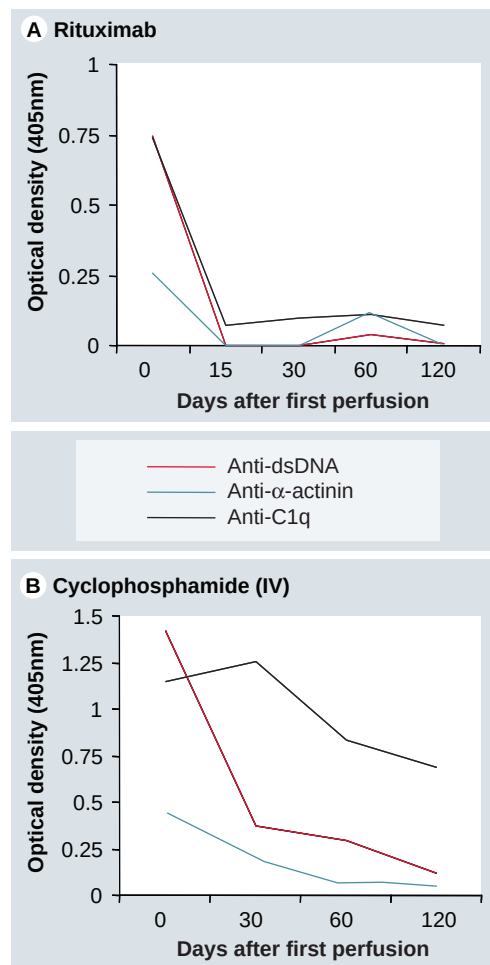


Figure 2. Decrease in autoantibody levels after successful rituximab (A) therapy in one patient with refractory lupus nephritis, and in another patient treated with cyclophosphamide (B).

In practice, there is an increase in the serum level of anti-dsDNA before the flares [51], and, usually, a decrease afterwards (or even during the flare [52]). This is true for IgG isotype (FIGURE 2), whereas anti-dsDNA IgM do not represent a sensitive tool for predicting a relapse

and are not associated with LN [53]. An excess in anti-dsDNA IgG precedes exacerbation such that persistence of high titers of anti-dsDNA antibody reflects a relapse [54]. Repeated determination could be recommended in the monitoring of SLE patients, and particular attention could be given to the early detection of renal flares in those patients with rising anti-dsDNA antibody levels [55].

■ Associated α-actinin & C1q reactivity

There have been claims that α-actinin reactivity is specific for SLE (TABLE 4), most notably in the case of LN [33,56,57]. Again, the majority of anti-α-actinin antibody-positive sera are also reactive for dsDNA, and higher titers characterize those patients with LN [58]. Still, there is a need for longitudinal studies of sizeable cohorts of patients to determine whether the changes in their levels are in fact associated with disease activity.

The extent of anti-C1q appears to be extremely variable [59]. They are strongly linked to LN, since their prevalence in this particular condition can be estimated to be between 60 and 100% [60–64], and the negative predictive value ranges from 30% in one study to 100% in another. In this context, it is worth noting that in 48 patients with biopsy-proven LN, anti-C1q antibody correlated with disease activity with a sensitivity of 87% and a specificity of 92% [61]. The same investigators followed a large cohort of 228 patients with LN to determine the value of each immunological test for monitoring LN activity. They found that anti-C1q had a sensitivity of 81%, a specificity of 71% and a negative predictive value of 94%. Moreover, univariate analysis established that anti-C1q was the best renal flare predictor, with an odds ratio of 12.7 [62]. Detection of anti-C1q should thus be useful in the diagnosis of LN, and even in the identification of SLE patients at risk of developing renal complications.

Table 4. Prevalence of anti-α-actinin antibodies in published cohorts of patients with systemic lupus erythematosus.

Study (year)	SLE no. positive/no. tested (% positive)		Cross-react with dsDNA	Non-SLE controls (%)	Ref.
	LN yes	LN no			
Mason (2004)	6/10 (60%)	2/8 (25%)	Yes	NA	[56]
Croquefer (2005)	NA	23/103 (22%)	Yes	7/283 (2.5%)	[57]
Kalaaji (2006)	7/11 (64%)	4/19 (21%)	Yes	7/62 (11%)	[41]
Renaudineau (2006)	10/24 (42%)	12/76 (16%)	Yes	7/300 (2%)	[40]
Becker-Merok (2006)	6/14 (44%)	14/85 (17%)	Yes	8/153 (5%)	[58]

The disease controls differ between reports (other rheumatic diseases, normal blood donors, non-SLE with antinuclear antibody-positive). The presence and the absence of LN are indicated.

LN: Lupus nephritis; SLE: Systemic lupus erythematosus.

Anti-C1q correlates with histopathology of the kidneys, and their serum levels diminish under efficient therapy. Monitoring anti-C1q in SLE patients could help to predict renal flares. In a randomized controlled trial designed to compare intravenous cyclophosphamide and azathioprine for the treatment of proliferative LN [64], anti-C1q antibody levels decreased as they did in the two therapeutic arms. Further to this observation, a follow-up study revealed that rises in anti-C1q Ab titers often precede renal flares, sometimes by several months. In this cohort [60], 33 of 83 SLE patients without LN history tested positive for anti-C1q. Nine of them developed LN within a median of 9 months, when none of the 50 anti-C1q antibody negative did so.

Thus, four inter-related families of autoantibodies have been identified as plausible predictors of renal involvement in SLE. Some findings support a role for a subpopulation of anti-dsDNA antibodies, as well as for antinucleosome, anti- α -actinin and anti-C1q antibodies. Still, there is a need for longitudinal studies in order to ensure that their emergence anticipates the development of renal complications, but it appears that cytokines, chemokines and lymphocyte subsets [65] offer additional information.

Conclusion & future perspective

Within a few years, the whole battery of tests for nephritis-associated antibodies will be systematically applied to SLE patients from their first referral and monitored on a regular basis. These include anti-dsDNA, antinucleosome, anti- α -actinin and anti-C1q antibodies. However, one is struck by the fact that as many as 90% of kidney-eluted antibodies do not recognize DNA, so additional specificities are about to emerge. Were that to be true, the development of related assays is predictable. Finally, a major breakthrough should occur when the protein microarray technology will be at the disposal of all the medics, and, why not, all the nurses as well.

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Executive summary

- Anti-dsDNA antibodies are not mandatory for renal tissue damage in systemic lupus erythematosus.
- The current tendency is to use at least two anti-dsDNA antibody tests for the diagnosis and the follow-up of systemic lupus erythematosus.
- Nucleosomes bind to glomerular basement membrane and retain anti-dsDNA.
- α -actinin might be a cross-reactive epitope for anti-dsDNA.
- Anti-C1q antibodies are strongly linked to lupus nephritis, and rises in their titers often precede renal flares.

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