Pathology (December 2019) 51(7), pp. 727–732

IMMUNOPATHOLOGY

The importance of tubuloreticular inclusions in lupus nephritis

Johannes Nossent[1,2], Warren Raymond[1], Milica Ognjenovic[1], Alexandra Kang[3], Aron Chakera[1,4], Daniel Wong[3]

[1]School of Medicine, Faculty of Health Science, University of Western Australia, Crawley, WA, Australia; [2]Department of Rheumatology, Sir Charles Gairdner Hospital, Perth, WA, Australia; [3]PathWest Laboratory Medicine, Anatomical Pathology, QEII Medical Centre, Perth, WA, Australia; [4]Department of Renal Medicine, Sir Charles Gairdner Hospital, Perth, WA, Australia

Summary

Tubuloreticular inclusions (TRI) are distinctive cytoplasmic structures of unknown origin that typically associate with autoimmune and viral diseases. We investigated the clinical and prognostic relevance of TRI detection in patients with lupus nephritis (LN).

We conducted a single centre study of patients (n=84) with biopsy evidence of LN. Clinical variables included demographics, SLEDAI score, and autoantibody profiling; while histological evaluation included TRI presence, International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification with NIH activity and chronicity indices, immunofluorescence, and other EM findings. Patients with and without TRI were compared by non-parametric statistical methods and survival analysis for the endpoints of death and renal failure.

TRI were detected in 37 patients (44%) that were younger (28.4 vs 34.3 years, p=0.02) and more often from Asian background (37.8% vs 19.1%, p=0.04) compared to patients without TRI. SLEDAI score (11 vs 12 units, p=0.36) and amount of proteinuria (370 vs 340 mg/mmol, p=0.71) were similar in both groups; however, TRI positive patients had increased frequency of anti-SSB antibodies (16% vs 2%, p=0.02), 'full house' immune complex deposition (85% vs 58%, p=0.04) and subendothelial electron dense deposits (83% vs 65%, p=0.07), but were less often anti-dsDNA Ab positive (62% vs 85%, p=0.02). Patient and renal survival were not influenced by TRI status.

TRI were observed in nearly half of all LN patients and TRI positive patients more often carried anti-SSB antibodies. However, TRI had little bearing on disease presentation or outcome in LN.

Key words: Lupus nephritis; tubuloreticular inclusions; autoantibodies; prognosis.

Received 8 March, revised 23 June, accepted 29 July 2019
Available online 24 October 2019

INTRODUCTION

Lupus nephritis (LN) is one of the most serious complications in patients with systemic lupus erythematosus (SLE). It increases the risk of both renal failure and premature mortality, due to the associated comorbidities and therapeutic efforts.[1,2] The renal prognosis is largely dependent on the severity of LN as currently determined by International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification class and the degree of chronic irreversible glomerular as well as tubulointerstitial changes.[3] Tubuloreticular inclusions (TRI) are distinctive structures found by electron microscopy (EM) that consist of reticular aggregates of branching membranous tubules within the cisternae of endoplasmic reticulum (ER), usually found within endothelial cells.[4] TRI were first observed 50 years ago in the glomerular endothelium of SLE patients and were originally termed lupus inclusions,[5] but have since been demonstrated in other cell types, such as, lymphocytes, monocytes, Schwann cells, fibroblasts, and epithelial cells.[4,6] TRI were originally suspected to be of viral aetiology as they mimic the nucleocapsids of paramyxoviruses, but subsequent investigations could not confirm a viral origin.[5,7] While the precise nature of TRI remains elusive, overproduction of alpha-interferon and interferon induced proteins have been linked to the appearance of TRI.[4,8,9] Although TRI can been seen in a range of other conditions, such as, lymphoma and viral infections,[10,11] TRI are useful in establishing a diagnosis of SLE,[12] with a few studies showing an association between the number of cells with TRI and clinical and renal disease activity in SLE.[13,14]

Given the limited information on the clinical correlates and prognostic value of TRI in SLE, we investigated the presence, serological associations and prognostic value of TRI in a group of SLE patients with biopsy proven LN.

METHODS

Participants were patients attending Sir Charles Gairdner Hospital since 1995, who fulfilled classification criteria for SLE and had renal biopsy findings compatible with LN.[15] Patient data at time of biopsy and at last follow-up visit were recorded retrospectively using a predefined form that included details on gender, date of birth, ethnicity, date of SLE diagnosis, i.e., date of cumulatively fulfilling American College of Rheumatology (ACR) up to 2010 or Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria for Systemic Lupus Erythematosus after 2010,[16,17] number and type of SLE criteria, SLEDAI-2K score,[18] renal function and other routine laboratory data, autoantibody profiles, status at last follow-up including start of chronic (>3 months) dialysis or renal transplantation, and date of death.

The main indication for biopsy was evaluation of new or increasing proteinuria >0.5 g/24 h (n=77) and/or haematuria (n=56). Renal specimens were

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2019.07.007

processed and assessed in an accredited renal histopathology laboratory in accordance with guidelines (see Supplementary Methods, Appendix A).[19,20] Findings by light microscopy (LM) were classified according to the 2003 Society of Nephrology/Renal Pathology Society classification (ISN/RPS) pathological classification[21] with semiquantitative National Institutes of Health (NIH) scores for active and chronic lesions.[22] TRI presence was defined as the renal pathologist recording the presence of cytoplasmatic tubular structures on electron microscopy (EM) without further requirement for quantification of TRI (Supplementary Fig. 1, Appendix A). For the purpose of this analysis, mixed ISN/RPS Class III+V and Class IV+V lesions were classified as Class III and Class IV. We used Standard Australian Classification of Countries to classify patients as 'Indigenous', 'Caucasian' or 'Asian' with 'Indigenous' referring to persons identifying as Aboriginal, Torres Strait Islander or both. The remaining patients, one of Maori descent and six from Sub-Saharan African background, were grouped as the 'other' cohort. The Human Research Ethics Committee (HREC) of our institution approved this study (SCGOPHCG – HREC # 8841).

Statistical analysis

Continuous data are given as mean values and were analysed by Kruskal–Wallis test and categorical data with Fisher's exact and Chi-square for proportions and rates. For this analysis we combined classes I and II, and III and IV based on the current prevailing opinion that prognostic differences between mesangial, proliferative, and membranous forms of LN guide treatment.[23] Survival curves used the date of the renal biopsy as a time zero (baseline) with date of death and start of chronic renal replacement therapy (RRT) as endpoints. Kaplan–Meier life-table estimates for subgroups were compared by log-rank test with non-survivors censored from the renal survival curves from the date of death. IBM SPSS v.24 software was used for data analysis with resulting two-sided p values <0.05 as the definition of statistical significance.

RESULTS

EM data were unavailable for 11 patients leaving a study sample of 84 patients. TRI positive patients (n=37, 44%) were younger at diagnosis, more frequently of Asian descent and had lower serum albumin levels compared to patients without TRI (Table 1). Both groups had similar measures of renal function, the amount of proteinuria, accrued number of ACR/SLICC classification criteria (Table 1), overall SLE disease activity (SLEDAI-2K), and proportion with extra renal disease features, including arthritis, malar rash, and serositis [all p>0.10 (data not shown)]. TRI positive patients were less frequently anti-dsDNA Ab seropositive, but had significantly more anti-SSB Ab seropositivity compared to TRI negative patients, while the groups were similar for levels of C3 and C4, and the presence of other antibodies against extractable nuclear antigens.

Renal biopsy findings showed slightly more class II and less class V lesions in TRI positive patients (p=0.08) with a lower NIH Chronicity Index (CI) (p=0.05) but no difference in NIH Activity Index (AI) scores (Table 2). TRI positive patients had proportionally more 'full house' renal immune complex deposition (86% vs 52%, p=0.06) and subendothelial electron dense deposits (EDD) (81% vs 66 %, p=0.08).

Longitudinal findings

During a mean follow-up time of 10.2 (±7.2) years, nine patients (10.7%) developed end stage renal failure with no difference in the frequency (13.5% vs 8.5%, p=0.47) or time to start of chronic dialysis between TRI positive and negative patients (p=0.88) (Fig. 1A). Another four patients developed doubling of their baseline serum creatinine levels, with equal distribution between TRI positive and negative patients (p=0.7). Six patients (7.1%) died with no difference in the frequency (13.5% vs 8.5%, p=0.49) or time to death between TRI positive and negative patients (p=0.21) (Fig. 1B).

Table 1 Baseline clinical and serological characteristics of patients with biopsy proven lupus nephritis (n=84) according to presence of TRI

	All (n=84)	TRI+ (n=37)	TRI– (n=47)	p value
Age onset of SLE, years	30.4	26.9	33.1	0.03
Onset LN after SLE, months	13.5	15.1	12.3	0.82
Female gender	75 (89.2%)	35 (4.6%)	40 (85.1%)	0.28
Ethnic background				0.06
Caucasian	36 (42.9%)	12 (32.4%)	24 (51.1%)	
Asian	23 (27.4%)	14 (37.8%)	9 (19.1%)	
Indigenous	11 (13.1%)	6 (16.2%)	5 (10.6%)	
Other (Pacific, African)	14 (13.1%)	5 (13.5%)	9 (18.9)	
Weight, kg	69.1	68.6	69.1	0.82
SLEDAI-2K score	11.2	12.1	10.9	0.36
Accrued ACR/SLICC criteria	5.4	5.2	5.5	0.19
Haemoglobin, g/L	115.2	119.5	111.8	0.25
Lymphocytes, $\times 10^9$/L	1.65	1.39	1.84	0.68
Erythrocyte sedimentation rate, mm/H	46.3	34.9	53.3	0.11
C-reactive protein, mg/L	35.1	33.2	65.7	0.07
Creatinine, mmol/L	95.1	108.3	78.9	0.62
Proteinuria, mg/mmol	381	397	361	0.71
Albumin, g/L	29.6	26.4	31.2	0.02
Anti-dsDNA Ab +	63 (75%)	23 (62.2%)	40 (85.1%)	0.02
Anti-Sm Ab +	13 (15.5%)	5 (13.5%)	8 (17%)	0.76
Antiphospholipid Ab +	28 (33%)	9 (24.3%)	19 (40.4%)	0.16
Anti-SSA Ab +	32 (38.1%)	14 (37.8%)	18 (38.3%)	0.93
Anti-SSB Ab +	7 (8.3%)	6 (16.2%)	1 (2.1%)	0.04
C3, g/L	0.78	0.81	0.75	0.49
C4, g/L	0.18	0.17	0.20	0.61

Figures are mean values or numbers.
ACR/SLICC, American College of Rheumatology/Systemic Lupus International Collaborating Clinics; Anti-dsDNA Ab, antibodies against double stranded DNA; Anti-Sm, antibodies to Smith antigen; LN, lupus nephritis; SLE, systemic lupus erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index-2000; TRI, tubuloreticular inclusions.

Table 2 Renal biopsy findings in patients with lupus nephritis (n=84) according to the presence of TRI

	All (n=84)	TRI+ (n=37)	TRI– (n=47)	p value
No. glomeruli assessed	21 (11.1)	20.5 (10.5)	21.5 (11.8)	0.89
Mesangial LN (Class 1+2)	17 (20%)	11 (29.7%)	6 (12.7%)	0.08
Proliferative LN (Class 3+4)	52 (62%)	22 (59.5%)	30 (63.8%)	
Membranous LN (Class 5)	15 (18%)	4 (10.8%)	11 (23.4%)	
NIH Activity Index (0–24)	4.7 (3.6)	4.5 (3.66)	4.9 (3.56)	0.61
NIH Chronicity Index (0–12)	1.2 (1.5)	0.86 (1.3)	1.38 (1.7)	0.05
Full house IF (n=49)		17 (85.6%)	17 (58.2 %)	0.06
EDD present	79 (95.2%)	36 (97%)	43 (91.4%)	0.13
Subepithelial EDD	62 (73.8%)	29 (78.1%)	33 (70.2%)	0.31
Membranous EDD	54 (64.2%)	25 (77.7%)	29 (61.7%)	0.49
Subendothelial EDD	61 (72.6%)	30 (81.1%)	31 (65.9%)	0.08
Vasculitis	1 (1.2%)	–	1 (2.1%)	0.95
Thrombosis	2 (2.4%)	–	2 (4.2%)	0.3

Figures are mean (SD) or numbers (%).
EDD, electron dense deposits; IF, immunofluorescence; LN, lupus nephritis; NIH, National Institutes of Health; TRI, tubuloreticular inclusions.

A total of 22 patients (23%) underwent a second biopsy after a median disease course of 40.5 months, mainly due to concerns about disease persistence or flare. Class switching occurred in six patients (27%) (Table 3). There was no significant change in AI and a borderline significant (p=0.08) increase in CI on repeat biopsies with TRI detected in 10/22 patients (45.5%). TRI was seen on the second biopsy of 7/13 (54%) patients with TRI on first biopsy, but also in 3/9 (33%) of patients with no TRI on initial biopsy.

DISCUSSION

In this comprehensive study, TRI were present in the renal biopsies of nearly half of the LN patients, but did not clearly associate with clinical or histological disease activity. Furthermore, we show for the first time that TRI have no obvious prognostic impact in SLE, while our findings also indicate that TRI can resolve or occur over time. Finally, we demonstrate a very tentative association between TRI and antibodies against SS/B-La protein.

The utility of renal biopsies in the assessment of disease severity and subsequent management of patients with LN is well established.[24] TRI have long been considered a helpful diagnostic tool with reported TRI frequencies in LN ranging from 40% to 88%.[25–27] The incidence in our cohort falls within this range and we found TRI to be more frequent in younger and Asian patients. The reasons for this are not obvious as overall SLEDAI scores, renal function and urinary and non-renal manifestations were similar to TRI negative patients. Thus, similar to the findings of Tisher *et al.*,[25] we could not confirm a connection between TRI and overall SLE disease activity.[13] We were also unable to detect an association between TRI and renal or patient survival but being the first study to investigate the long-term prognostic value of TRI, so far there are no comparative data available.

In terms of renal pathology, we found a borderline significant trend of skewing of ISN/RPS classification towards non-proliferative LN in TRI positive patients, despite an increased prevalence of 'full House' immune deposits and subendothelial EDD. Although based on low numbers, this may suggest that the underlying immune process in TRI positive patients is less likely to induce endothelial proliferation. However, this would be in contrast with findings by Venkataseshan *et al.*, who found cylindric confronting cisternae as a subtype of inclusion more prominent in patients with active glomerular and tubulointerstitial disease compared to patients with inactive disease.[14] Renal inflammation in LN is thought to be mediated by immune complexes, especially those that contain subsets of nephritogenic anti-dsDNA antibodies.[28,29] We observed a moderate but interesting discrepancy in serological markers for TRI positive patients, who had lower CRP levels, were less likely to be anti-dsDNA Ab positive and more often carried anti-SSB antibodies. This serological profile would not only fit with the lower inflammatory burden in TRI positive patients with proliferative LN (Table 2), but could also point towards a role for anti-SSB antibodies in the development of TRI. Since a viral origin for TRI has become less likely,[7,30] TRI is considered to represent an ultrastructural modification within the endoplasmic reticulum resulting from cellular injury or the expression of a neoantigen. Ultra-cytochemical studies by Rich *et al.* provided evidence that TRI are composed of protein and lipids and do not contain DNA but closely associate with the presence of RNAs.[4,8] Protein La, also known as autoantigen La or Sjögren's syndrome antigen B (SSB) is evolutionarily conserved and abundantly present in both the nucleus and cytoplasm, where it plays fundamental roles in RNA metabolism by associating with RNA polymerase III transcripts and binding to pre-tRNAs to prevent exonuclease digestion.[31,32] La/SSB protein is a common target for autoantibodies in patients with Sjögren's syndrome and SLE and inactivation of La/SSB by autoantibodies has been suggested to interfere with normal[31] and viral RNA metabolism.[32] Although the presented data cannot not be considered concrete evidence, our observations of an increased although moderate prevalence of anti-SSB antibodies in patients with TRI could support the idea that RNA accumulation contributes to the formation of TRI.

The results of this study need to be considered in light of its limitations. This includes the usual restraints of retrospective data collection as well as the possibility that glomeruli with TRI may have been missed on EM due to sampling as TRI induction by SLE sera occurs in 25–30% of cells only.[13] We applied 2003 ISN/RPS classification criteria which have recently been updated during a consensus meeting and now include activity and chronicity indices.[33] Also, subgroup analyses had limited statistical power given the relatively

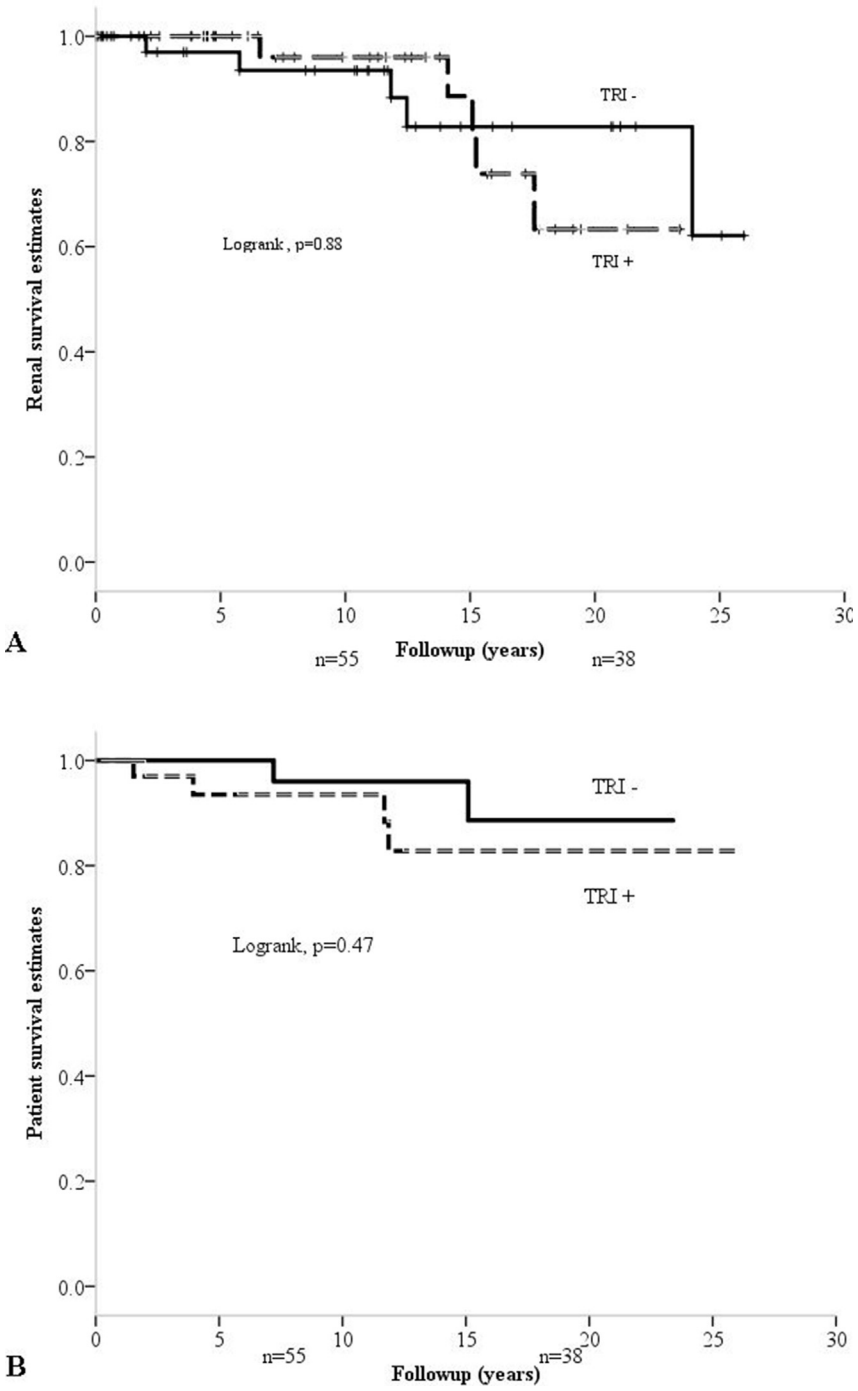

Fig. 1 (A) Renal survival (endpoint chronic renal replacement therapy) and (B) patient survival by the presence of tubuloreticular inclusions (TRI) in patients with lupus nephritis (*n*=84).

Table 3 Changes in histological findings over time (n=22)

Initial biopsy	n	Second biopsy			p value
		TRI+	TRI–		
TRI+	13	7	6		0.58
TRI–	9	3	6		
		ISN/RPS class 2	ISN/RPS class 3+4	ISN/RPS class 5	
ISN/RPS class 2	6	3	2	1	0.52
ISN/RPS class 3+4	15	2	13	–	
ISN/RPS class 5	1	–	1	–	
	Median	AI	CI		
AI (IQR)	4 (2–11)	4 (1–17)	–		0.15
CI (IQR)	1 (0–6)	–	2 (0–5)		0.08

AI, National Institutes of Health Activity Index score; CI, National Institutes of Health Chronicity Index score; IQR, interquartile range; ISN/RPS, International Society of Nephrology/Renal Pathology Society classification; TRI, tubuloreticular inclusions.

small numbers and may have missed relevant associations. In spite of the limitations, this represents currently the largest and most comprehensive study on TRI in LN combining clinical, serological and histological characteristics and longitudinal data.

In conclusion, TRI was detected in nearly half of renal biopsies in LN patients. TRI presence was not static over time and there was a trend towards increased frequency of mesangial lesions and anti-SSB antibodies in TRI positive patients. Most importantly, TRI did not impact on renal or patient survival.

Acknowledgements: The authors wish to acknowledge the assistance provided by Brandon Wong, Kimberly Minas and the staff at the Departments of Renal Medicine and Rheumatology at Sir Charles Gairdner Hospital in data collection.

Conflicts of interest and sources of funding: This study was supported through an unrestricted grant by the Arthritis Foundation of Western Australia, which had no further role in data collection, analysis or manuscript writing. The authors state that there are no conflicts of interest to disclose.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.07.007.

Address for correspondence: Prof Dr Johannes Nossent, School of Medicine and Pharmacology, The University of Western Australia, 35 Stirling Hwy (M503), Crawley, WA, 6009, Australia. E-mail: Johannes.nossent@uwa.edu.au

References

1. Parikh SV, Rovin BH. Current and emerging therapies for lupus nephritis. *J Am Soc Nephrol* 2016; 27: 2929–39.
2. Imran TF, Yick F, Verma S, *et al*. Lupus nephritis: an update. *Clin Exp Nephrol* 2016; 20: 1–13.
3. Davidson A. What is damaging the kidney in lupus nephritis? *Nat Rev Rheumatol* 2016; 12: 143–53.
4. Rich SA, Gibbons WE. Nature and origin of lupus-type inclusions. *Hum Pathol* 1990; 21: 1191–2.
5. Pincus T, Blacklow NR, Grimley PM, *et al*. Glomerular microtubules of systemic lupus erythematosus. *Lancet* 1970; 2: 1058–61.
6. Klippel JH, Carette S, Preble OT, *et al*. Serum alpha interferon and lymphocyte inclusions in systemic lupus erythematosus. *Ann Rheum Dis* 1985; 44: 104–8.
7. Gyorkey F, Min KW, Sincovics JG, *et al*. Systemic lupus erythematosus and myxovirus. *N Engl J Med* 1969; 280: 333.
8. Rich SA, Reilly AA. Evidence that the interferon-induced Daudi cell human lupus inclusions are de novo synthesized complexes of ribonucleoprotein and membrane. *J Biol Chem* 1989; 264: 3529–37.
9. Rich SA, Bose M, Tempst P, *et al*. Purification, microsequencing, and immunolocalization of p36, a new interferon-alpha-induced protein that is associated with human lupus inclusions. *J Biol Chem* 1996; 271: 1118–26.
10. Elmaghrabi A, Brown E, Khin E, *et al*. Tubuloreticular inclusions in the absence of systemic lupus erythematosus and HIV infection: a report of three pediatric cases. *Case Rep Nephrol Dial* 2017; 7: 91–101.
11. Vakharia DD, Szebenyi SE, Gutterman JU, *et al*. Interferon-alpha-induced human lupus inclusions and p36 protein in cancer and AIDS. *J Interferon Cytokine Res* 1996; 16: 709–15.
12. Grausz H, Earley LE, Stephens BG, *et al*. Diagnostic import of virus-like particles in the glomerular endothelium of patients with systemic lupus erythematosus. *N Engl J Med* 1970; 283: 506–11.
13. Rich SA, Owens TR, Anzola MC, *et al*. Induction of lupus inclusions by sera from patients with systemic lupus erythematosus. *Arthritis Rheum* 1986; 29: 501–7.
14. Venkataseshan VS, Marquet E, Grishman E. Significance of cytoplasmic inclusions in lupus nephritis. *Ultrastruct Pathol* 1991; 15: 1–14.
15. Nossent J, Raymond W, Kang A, *et al*. The current role for clinical and renal histological findings as predictor for outcome in Australian patients with lupus nephritis. *Lupus* 2018; 27: 1838–46.
16. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
17. Petri M, Orbai AM, Alarcon GS, *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677–86.
18. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288–91.
19. Walker PD. The renal biopsy. *Arch Pathol Lab Med* 2009; 133: 181–8.
20. Amann K, Haas CS. What you should know about the work-up of a renal biopsy. *Nephrol Dial Transplant* 2006; 21: 1157–61.
21. Weening JJ, D'Agati VD, Schwartz MM, *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004; 65: 521–30.
22. Austin 3rd HA, Muenz LR, Joyce KM, *et al*. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int* 1984; 25: 689–95.
23. Almaani S, Meara A, Rovin BH. Update on lupus nephritis. *Clin J Am Soc Nephrol* 2017; 12: 825–35.
24. Moroni G, Vercelloni PG, Quaglini S, *et al*. Changing patterns in clinical-histological presentation and renal outcome over the last five decades in a cohort of 499 patients with lupus nephritis. *Ann Rheum Dis* 2018; 77: 1318–25.
25. Tisher CC, Kelso HB, Robinson RR, *et al*. Intraendothelial inclusions in kidneys of patients with systemic lupus erythematosus. *Ann Intern Med* 1971; 75: 537–47.
26. Bariety J, Richer D, Appay MD, *et al*. Frequency of intraendothelial 'virus-like' particles: an electron microscopy study of 376 human renal biopsies. *J Clin Pathol* 1973; 26: 21–4.
27. Lee C-J, Suh K-S, Kim K-H, *et al*. The clinicopathologic significance of endothelial tubuloreticular inclusions in glomerular diseases. *Ultrastruct Pathol* 2013; 37: 386–94.

28. Nossent HC, Rekvig OP. Is closer linkage between systemic lupus erythematosus and anti-double-stranded DNA antibodies a desirable and attainable goal? *Arthritis Res Ther* 2005; 7: 85–7.
29. Rekvig OP. The anti-DNA antibody: origin and impact, dogmas and controversies. *Nat Rev Rheumatol* 2015; 11: 530–40.
30. Kawano K, Miller L, Kimmelstiel P. Virus-like structures in lupus erythematosus. *N Engl J Med* 1969; 281: 1228–9.
31. Liang C, Xiong K, Szulwach KE, *et al*. Sjogren syndrome antigen B (SSB)/La promotes global microRNA expression by binding microRNA precursors through stem-loop recognition. *J Biol Chem* 2013; 288: 723–36.
32. Hasler D, Lehmann G, Murakawa Y, *et al*. The lupus autoantigen La prevents mis-channeling of tRNA fragments into the human microRNA pathway. *Mol Cell* 2016; 63: 110–24.
33. Bajema IM, Wilhelmus S, Alpers CE, *et al*. Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. *Kidney Int* 2018; 93: 789–96.