Systemic lupus erythematosus : from diagnosis to prognosis

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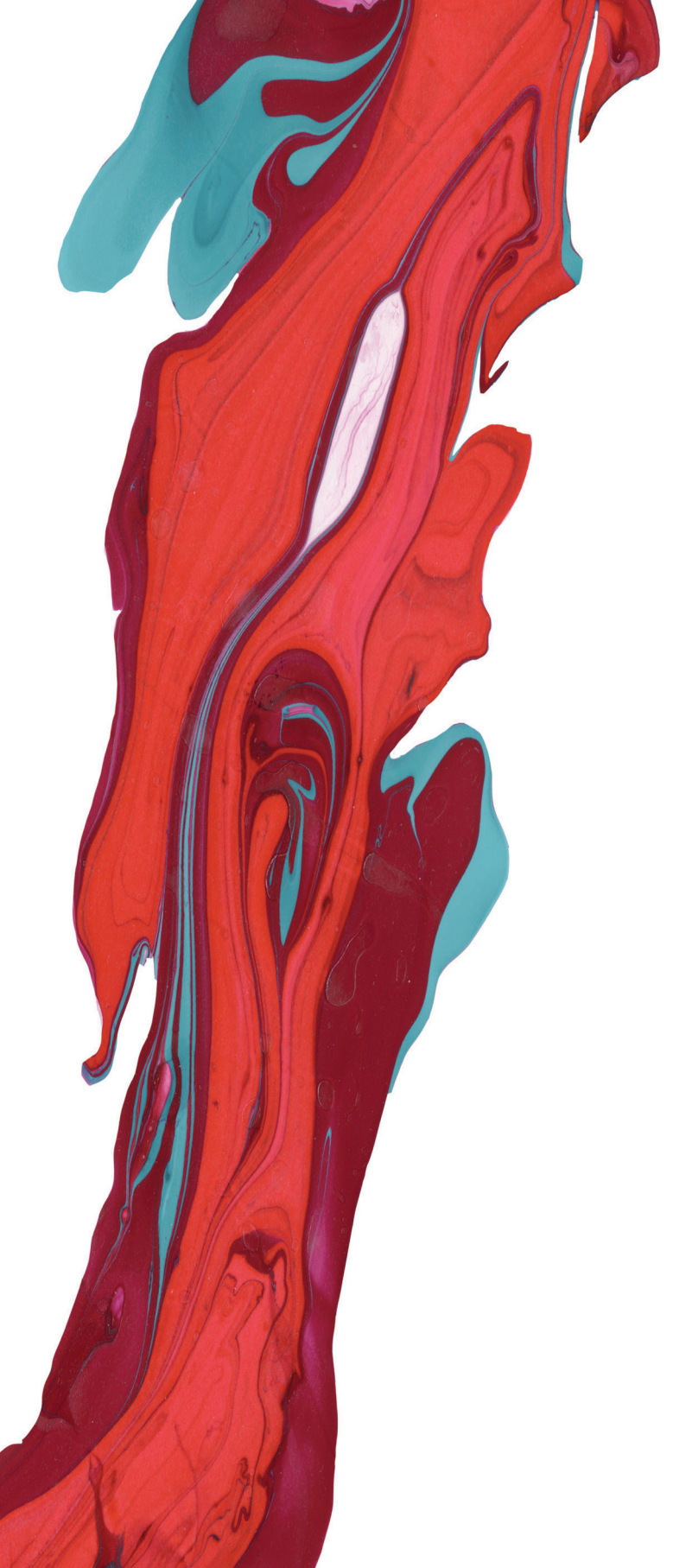

Chapter 2

Validation of the Systemic Lupus International Collaborating Clinics' Classification Criteria in a Cohort of Patients with Full House Glomerular Deposits

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ABSTRACT

Background

In 2012, the Systemic Lupus International Collaborating Clinics (SLICC) presented a new classification for systemic lupus erythematosus (SLE). In this classification, biopsy-confirmed lupus nephritis (LN) with positive antinuclear or anti-double stranded DNA antibodies became a stand-alone criterion. Because of the unknown diagnostic performance among patients from nephrology clinics, we aimed to test the validity of the SLICC classification, compared with the American College of Rheumatology (ACR) classification, in a cohort of patients whose renal biopsies would raise the clinicopathologic suspicion of LN.

Methods

All patients with a renal biopsy showing full house glomerular deposits between 1968–2014 and clinical follow-up in our centre were included and re-evaluated after which clinicians and a pathologist reached a consensus on the reference-standard clinical diagnosis of SLE. The diagnostic performance, and net reclassification improvement (NRI) were assessed. We included 149 patients, 117 of whom had clinical SLE.

Results

Compared with the ACR classification, the SLICC classification had better sensitivity (100 vs. 94%); although, this was at the expense of specificity (91 vs. 100%; NRI −0.03, P=0.56). Excluding the stand-alone renal criterion, the specificity of the SLICC classification reached 100%, with an NRI of 0.06 (P<0.01) compared with the ACR classification.

Conclusions

The SLICC classification performed well in terms of diagnostic sensitivity among patients with full house glomerular deposits; whereas, the stand-alone renal criterion had no additional value and compromised the specificity. Clearly, putative LN patients in nephrology clinics reflect a distinct SLE disease spectrum warranting caution when applying SLE classification criteria.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with diverse clinical manifestations and presenting symptoms that have considerable overlap with other diseases.[1] SLE classification criteria have been designed to create homogeneous groups of SLE patients to conduct collaborative and reproducible research. Although SLE classification criteria have been designed for research purposes, they are often used for the purpose of diagnosis in clinical practice. The focus of SLE classification criteria has traditionally been on patients encountered in rheumatology clinics, although a need for input from non-rheumatology specialists who frequently see lupus patients was recognised.[2] This may be particularly relevant to nephrologists, since patients with renal biopsy findings reminiscent of lupus nephritis (LN) would readily be evaluated in light of these criteria to confirm the diagnosis. The recent descriptions of entities including "renal-limited lupus-like nephritis",[3] and idiopathic[4,5] and secondary "non-lupus full house nephropathy"[4-10] stress the importance of valid SLE classification criteria in the nephrology clinic: to help distinguish LN patients based on clinical and laboratory findings.

The importance of valid SLE classification criteria in the nephrology clinic recently gained attention by the increased weight that was attributed to renal lupus in the Systemic Lupus International Collaborating Clinics (SLICC) 2012 classification.[11] In the SLICC classification,[11] biopsy-confirmed LN in the presence of antinuclear antibodies (ANA) or anti-double stranded DNA (anti-dsDNA) antibodies was introduced as an exception to the conventional requirement of four or more criteria employed in the original[12] and updated[13] American College of Rheumatology (ACR) classifications. Remarkably, the definition of biopsy-confirmed LN was unspecified in both the ACR and the SLICC classification, and firm criteria for biopsy-confirmed LN in the histopathologic classification referred to are missing.[14]

In light of the increased weight of renal lupus in the SLICC classification and the unknown diagnostic performance of the classification in patients from nephrology clinics, we aimed to test the validity of the SLICC classification in a cohort of patients whose renal biopsies would raise the clinicopathologic suspicion of LN. Since the definition of "biopsy-confirmed" LN is left open to interpretation, we selected our cohort based on a biopsy feature characteristic of LN – so as to raise the clinicopathologic suspicion – but concise enough to identify a consistent cohort. From a nephropathologic perspective, the finding of a so-called "full house" pattern of immunofluorescence, with concurrent positive glomerular staining for IgA, IgG, IgM, C3, and C1q, would certainly raise the possibility of SLE as a differential diagnostic consideration warranting the evaluation of clinical criteria. Here, we tested the validity of the SLICC compared with the ACR classification criteria to distinguish SLE patients with full house glomerular deposits. Moreover, we studied additional biopsy findings that may distinguish SLE patients in this setting. This is the first validation study of

the SLICC classification in a cohort selected on the basis of renal biopsy findings raising the clinicopathologic suspicion LN, reflecting a diagnostic problem area encountered in the nephrology clinic.

METHODS

The pathology archives of the Leiden University Medical Center were searched to identify all native renal biopsies between 1968–2014 showing full house immunofluorescence, defined as concurrent positive staining for IgA, IgG, IgM, C3, and C1q with ≥1+ intensity on a 0–3+ scale. Only biopsies showing granular fluorescent staining along capillary walls and/or in the mesangium were included. Patients from our centre accordingly identified entered the study. In accordance with the ethics committee of the Leiden University Medical Center, all patient data were coded and kept anonymously throughout the study. All biopsies were processed for light and immunofluorescence microscopy according to the standard techniques at our centre. Sections for light microscopy were stained with haematoxylin and eosin, periodic acid-Schiff, and methenamine-silver. All biopsies were (re)classified by an experienced pathologist (IMB) according to the ISN/RPS classification of LN,[14] regardless of clinicopathologic diagnosis. For immunofluorescence microscopy, sections were frozen in liquid nitrogen, and cryostat sections were stained with FITC-labelled antisera to human IgA, IgG, IgM, C3, and C1q. Immunofluorescence reports were originally prepared by four experienced nephropathologists who routinely scored the immunofluorescence intensity on a 0–3+ scale. Not all biopsies were sent in for analysis by electron microscopy; but if at hand, tissue was fixed in 1.5% glutaraldehyde and 1% paraformaldehyde and embedded in Epon. Electron microscopy was reviewed if available, and findings were compared to the original report.

Following the methodology of previous validation studies, the reference standard for the clinical diagnosis of SLE was based on clinician's and pathologist's expert opinion.[11, 15-17] Three investigators (ECR, YKOT, TK) independently reviewed medical records in consultation with an experienced nephropathologist (IMB), assessing whether patients had a reference-standard clinicopathologic diagnosis of SLE at the time of renal biopsy by considering biopsy findings and the constellation of presenting clinical features, supportive laboratory studies, and demographics, and by exclusion of alternative diagnoses. Because of the evolving manifestations of SLE, we also confirmed the diagnosis of SLE patients at the time of renal biopsy by considering follow-up (including follow-up biopsies and the post-transplantation course and biopsies) as a separate examination in addition to the diagnosis at the time of biopsy. Patients who were not diagnosed with SLE at the time of biopsy were also studied during follow-up to see if they could be diagnosed with SLE at a later time. Consensus on the clinical diagnosis of SLE at the time of biopsy or during follow-up was achieved by conference.

Furthermore, clinical records were reviewed by the same investigators individually to assess the presence of ACR[12, 13] and SLICC[11] criteria for SLE at any time up to and including the moment of renal biopsy. Qualitative ANA testing was performed routinely at our centre using incubation of HEp 2000 cells (Biomedical Diagnostics, Belgium) with 1:40 diluted serum samples. A positive test result was reported if a clear and distinct immunofluorescence pattern was observed that was more intense than the negative control. Similarly, qualitative anti-dsDNA testing was performed using C. Luciliae kit (Aesku.Diagnostics, Germany) using 1:10 diluted samples. Fulfilment of sufficient criteria according to either classification was assessed. Patients who did not fulfil sufficient ACR or SLICC criteria were studied for the presentation of any of the criteria during available follow-up by the same investigators. Fulfilment of classification criteria was agreed on by conference.

Statistical analyses

Normally distributed data were compared using *t*-tests. Categorical data were compared using Fisher's exact tests, chi-square tests, or linear-by-linear analysis. The number of classification criteria was compared between patients with and without a reference standard diagnosis of SLE by Mann Whitney U test. The sensitivity and specificity of ACR and SLICC classifications were assessed and reported including 95% confidence intervals. Information retrieved from follow-up was not considered when assessing diagnostic performance. Improvement of the SLICC classification compared with the ACR classification was assessed by calculating the net reclassification improvement (NRI) at the time of biopsy.[18] The NRI is based on reclassification tables constructed separately for cases with and without SLE, and quantifies the correct movement in categories, upwards for cases with SLE and downwards for cases without SLE. The null hypothesis NRI=0 was tested using the Z-statistic following McNemar's asymptotic test for correlated proportions. All *P*-values are two-sided and $P<0.05$ was considered significant. All analyses were performed using SPSS 23.0 (IBM, Armonk, New York).

RESULTS

A total of 149 patients with renal biopsies fulfilling our inclusion criteria were identified from the pathology archives between August 1968 and July 2014. Fourteen patients were biopsied before 1980, 32 from 1980–1989, 47 from 1990–1999, 42 from 2000–2009, and 14 from 2010–2014.

Reference standard clinical diagnosis of SLE

According to clinicians' and pathologist's expert opinion, 117/149 patients fulfilled the diagnosis SLE at the time of biopsy. These diagnoses composed the reference standard; these patients will be referred to hereafter as patients with "clinical SLE". Of the patients with clinical SLE, renal involvement first appearing at the time of renal biopsy was the decisive factor establishing the clinical diagnosis in 40 patients. In addition, 75 patients had a clinical

diagnosis of SLE prior to renal biopsy. For two patients, the time since onset of SLE could not be retrieved from the records. The median time between SLE diagnosis and renal biopsy was 1.4 years (interquartile range 0–5.3). For all patients with clinical SLE at the time of renal biopsy, the diagnosis was confirmed by the clinical course during follow-up (median 10.6 years [interquartile range 4.9–18.4]). None of the 32/149 patients without clinical SLE at the time of renal biopsy were clinically diagnosed with SLE during median follow-up of 20.0 years (interquartile range 8.3–33.8). The consensus clinicopathologic diagnoses of these 32 patients were: membranous nephropathy (anti-PLA2R-positive, n=1; cancer-associated, n=3), IgA nephropathy (n=4), infection-related glomerulonephritis (n=2), ANCA-associated glomerulonephritis (n=2), and idiopathic non-lupus full house nephropathy (n=20).[5] Details on the clinical presentation, biopsy findings, and clinical follow-up of these patients are provided elsewhere.[5]

General characteristics of patients with and without clinical SLE

General characteristics of patients in our cohort and the prevalence of individual ACR and SLICC classification criteria are shown in **Table 1**. Patients with clinical SLE were significantly younger and more often female than patients without clinical SLE. For some patients, the absence or presence of cutaneous and/or immunologic criteria was unconvincing, in which cases these criteria were excluded from the comparisons. The 32 patients without clinical SLE less frequently fulfilled individual ACR and SLICC classification criteria than the 117 patients with clinical SLE, except for the criteria oral/nasal ulcers, discoid rash, anti-Sm, and antiphospholipid antibodies.

Biopsy findings in patients with and without clinical SLE

Comparisons of biopsy findings between patients with and without clinical SLE are shown in **Table 2** (see also Rijnink *et al*.[5] for a comparison excluding patients without SLE due to secondary causes). Briefly, the pattern of histopathologic injury by light microscopy was different between patients with and without clinical SLE (P=0.003); with absent lesions or a purely mesangial pattern of injury and a membranous pattern being more prevalent in patients without clinical SLE, and with endocapillary and/or extracapillary lesions being more prevalent in patients with clinical SLE. C1q and IgM immunofluorescence staining was significantly more intense in patients with clinical SLE compared to patients without (both P<0.01). By electron microscopy, patients with clinical SLE more often had subendothelial deposits (P=0.008).

Fulfilment of ACR and SLICC classification criteria

At the time of renal biopsy, 110 patients in our cohort with clinical SLE fulfilled ≥4 ACR and SLICC criteria for the classification of SLE and seven patients fulfilled ≥4 SLICC criteria only (**Figure 1**). Of the 32 patients without clinical SLE, three fulfilled the stand-alone renal criterion of the SLICC classification because of their renal biopsy findings in combination with ANA but had <4 ACR and SLICC criteria at the time of renal biopsy. Twenty-nine patients without clinical SLE did not meet the classification requirements at the time of renal biopsy.

Table 1 General characteristics and prevalence of individual 1997 ACR[a] and 2012 SLICC[b] criteria in patients with and without clinical SLE in the full house cohort.

Characteristic	Clinical SLE (n=117)	No clinical SLE (n=32)	*P*
Age, y ± SD	32.6 ± 14.6	38.7 ± 16.2	0.041
Sex, male:female	30:87	21:11	<0.001
ACR criteria, median (range)	5 (3–9)	1 (1–3)	<0.001
SLICC criteria, median (range)	7 (4–14)	1 (1–3)	<0.001
Clinical criteria	n/total (%)	n/total (%)	
Acute/subacute cutaneous lupus[b]	66/117 (56.4)	0/32 (0)	<0.001
Malar rash[a]	48/112 (42.9)	0/32 (0)	<0.001
Photosensitivity[a]	25/112 (22.3)	0/32 (0)	0.001
Chronic cutaneous lupus[b]	14/117 (12.0)	0/32 (0)	0.041
Discoid rash[a]	11/106 (9.4)	0/32 (0)	0.122
Non-scarring alopecia[b]	21/117 (17.9)	0/32 (0)	0.008
Oral/nasal ulcers[a,b]	25/117 (21.4)	3/32 (9.4)	0.200
Arthritis[a,b]	86/117 (73.5)	1/32 (3.1)	<0.001
Serositis[a,b]	44/117 (37.6)	1/32 (3.1)	<0.001
Neurologic disorder[a,b]	18/117 (15.4)	0/32 (0)	0.013
Haemolytic anaemia[a,b]	20/117 (17.1)	0/32 (0)	0.008
Lympho-/leukopenia[a,b]	36/117 (30.8)	0/32 (0)	<0.001
Thrombocytopenia[a,b]	28/117 (23.9)	0/32 (0)	0.001
Immunologic criteria			
Antinuclear antibody[a,b]	116/117 (99.1)	3/26 (11.5)	<0.001
Anti-dsDNA[a,b]	80/109 (73.4)	0/28 (0)	<0.001
Anti-Sm[a,b]	18/56 (32.1)	0/8 (0)	0.093
Antiphospholipid antibody[a,b]	41/81 (50.6)	0/5 (0)	0.057
Hypocomplementaemia[b]	94/109 (86.2)	2/23 (8.7)	<0.001
Direct Coombs' test[b]	25/75 (33.3)	0/13 (0)	0.016

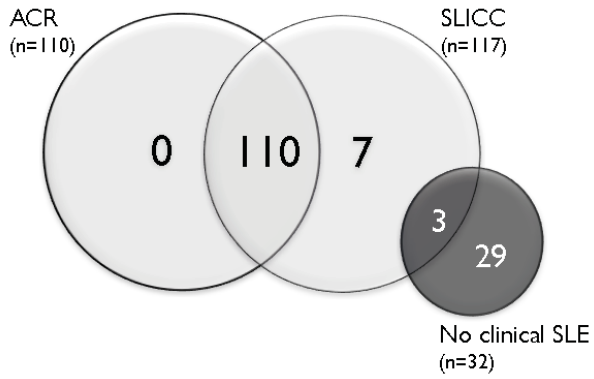
Classification criteria were registered up to and including the time of renal biopsy. Fractions indicate the number of patients with a particular criterion divided by the total number of patients for whom the presence or absence of a criterion could be retrieved. The total number of SLE classification criteria was compared using Mann Whitney U test. The prevalence of individual SLE criteria were compared using Fisher's exact tests. Anti-dsDNA, anti-double stranded DNA antibody; Anti-Sm, anti-Smith antibody.

Table 2 Histopathologic findings in patients with and without clinical SLE.

	Clinical SLE (n=117)	No clinical SLE (n=32)	*P*
Light microscopy			
ISN/RPS 2003 class, n (%)			
I	1 (1)	3 (9)	
II	2 (2)	1 (3)	
III	24 (21)	11 (34)	
IV	79 (68)	8 (25)	
III/IV +V	2 (2)	3 (9)	
V	9 (8)	6 (19)	<0.001
III/IV (+V) A	45 (43)	8 (35)	
III/IV (+V) A/C	59 (56)	11 (48)	
III/IV (+V) C	1 (1)	4 (17)	0.058*
No lesions/ purely mesangial lesions, n (%)	3 (3)	4 (13)	
Endo- and/or extracapillary lesions, n (%)	105 (90)	23 (72)	
Membranous lesions, n (%)	11 (9)	9 (28)	0.003
Immunofluorescence microscopy			
IgA, n (%)			
+	26 (22)	11 (34)	
++	60 (51)	15 (47)	
+++	31 (26)	6 (19)	0.158*
IgM, n (%)			
+	27 (23)	16 (50)	
++	64 (55)	13 (41)	
+++	26 (22)	3 (9)	0.004*
IgG, n (%)			
+	21 (18)	9 (28)	
++	67 (57)	10 (31)	
+++	29 (25)	13 (41)	0.682*
C3, n (%)			
+	8 (7)	7 (22)	
++	64 (55)	14 (44)	
+++	45 (39)	11 (34)	0.131*
C1q, n (%)			
+	9 (8)	11 (34)	
++	67 (57)	14 (44)	
+++	41 (35)	7 (22)	0.002*
Electron microscopy	**(n=29)**	**(n=14)**	
Mesangial deposits, n (%)	10 (35)	5 (36)	1.000
Subendothelial deposits, n (%)	23 (80)	5 (36)	0.008
Subepithelial deposits, n (%)	24 (83)	11 (79)	1.000
Food process effacement, n (%)	24 (83)	11 (79)	1.000
Tubuloreticular inclusions, n (%)	5 (17)	2 (14)	1.000
Number of locations with deposits†, n (%)			
<2	5 (17)	9 (64)	
2	20 (69)	2 (14)	
>2	4 (14)	3 (21)	0.078*

* Linear-by-linear analysis. † Locations include: mesangial, subendothelial and subepithelial.

Figure 1 Venn diagram illustrating the distribution of patients with and without clinical SLE identified by the ACR and SLICC classifications at the time of renal biopsy.



Patients with a reference standard clinical diagnosis of SLE (n=117) are indicated in white, and those without (n=32) in grey.

ACR classification: false negatives

Classification according to the ACR criteria resulted in seven false-negative classifications. These patients had, in addition to renal involvement, hypocomplementaemia and two other criteria (synovitis, neuropsychiatric lupus, lupus anticoagulant, leukopenia, positive ANA, and/or anti-dsDNA). Thus, they were classified according to exactly 4 SLICC criteria. Classification of SLE according to the ACR criteria was not possible, as hypocomplementaemia is not included. During median follow-up of 9.0 years (interquartile range 3.2–19.0), 2 of these 7 patients could also be classified as SLE according to ≥4 ACR criteria after 5 and 6 years. Thus, the sensitivity of the ACR classification increased from 94 to 96% after 6 years of follow-up.

SLICC classification: false positives

Classification according to the SLICC criteria resulted in three false positive classifications. These patients had a renal biopsy with a full house immunofluorescence pattern in combination with lesions by light and electron microscopy consistent with LN as detailed below. Because of a positive ANA, they were classified as SLE based on the stand-alone renal SLICC criterion assuming they had "biopsy-confirmed" LN, but had <4 ACR and SLICC criteria at the time of biopsy and during follow-up (median 7.7 [range 2.9–9.0] years). One patient had crescentic and endocapillary glomerulonephritis with 2+ intensity for IgG, IgA, IgM, C3, and C1q by immunofluorescence, and no material for electron microscopy available. This patient was clinically diagnosed with PR3-ANCA-associated glomerulonephritis and had a positive ANA 4 years prior to renal biopsy, but negative ANA during follow-up. Two other patients had cancer-associated membranous nephropathy; one of them with membranous nephropathy with glomerular sclerosis by light microscopy, IgG 3+, IgA 1+, IgM 1+, C3 1+ and C1q 3+ by immunofluorescence, and subepithelial deposits by electron microscopy; the other with focal endocapillary glomerulonephritis with spikes, IgG 1+, IgA

1+, IgM 1+, C3 3+ and C1q 2+ by immunofluorescence, and subepithelial deposits and tubuloreticular inclusions by electron microscopy. Both had a positive ANA at the time of renal biopsy, and the first also after 4 years of follow-up. A fourth patient without clinical SLE at the time of biopsy and during follow-up fulfilled no additional SLE classification criteria at the time of renal biopsy, but had a persistently positive ANA after 12 years of follow-up. The renal biopsy at baseline showed focal endocapillary glomerulonephritis, IgG 3+, IgA 1+, IgM 1+, C3 3+, C1q 3+ by immunofluorescence, and subepithelial deposits by electron microscopy. Therefore, the specificity of the SLICC classification decreased from 91 to 88% after 12 years follow-up.

Comparing the diagnostic performance of the ACR and SLICC classifications

The performance of the ACR and SLICC classifications in our cohort is shown in **Table 3.** Compared with the ACR classification, the SLICC classification was more sensitive (100 vs. 94%) but less specific (91 vs. 100%). The sensitivity and specificity of the SLICC classification were 100% when excluding the stand-alone renal criterion, based on the finding that three patients were incorrectly classified as SLE based on the stand-alone renal criterion. Compared with the ACR classification, there was no reclassification improvement of the SLICC classification (NRI −0.034, *P*=0.563). Exclusion of the stand-alone renal criterion resulted in 6% of SLE patients being appropriately reclassified in this cohort as compared with the ACR classification, resulting in significant reclassification improvement (NRI 0.060; *P*=0.014). Reclassification tables are shown in **Appendix 2.1**.

Table 3 Performance of the SLICC (2012) and ACR (1997) classifications in the full house cohort.

Classification	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	$NRI_{SLE(+)}$, %	$NRI_{SLE(-)}$, %	NRI	*P*
ACR 1997	94.0 (87.6; 97.4)	100 (86.7; 100)	100 (95.8; 100)	82.1 (65.9; 91.9)	-	-	-	-
SLICC 2012	100 (96.0; 100)	90.6 (73.8; 97.5)	97.5 (92.3; 99.3)	100 (85.4; 100)	6.0	−9.4	−0.034	0.563
SLICC 2012 without stand-alone renal criterion*	100 (96.0; 100)	100 (86.7; 100)	100 (96.0; 100)	100 (86.7; 100)	6.0	0	0.060	0.008

NRI (net reclassification improvement) was calculated relative to the ACR 1997 classification (reference) and was computed separately for clinical SLE cases (SLE(+)) and non-SLE cases (SLE(-)). *P*-values were calculated for the null hypothesis NRI = 0. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value. * Stand-alone renal criterion is defined as a full house renal biopsy in combination with antinuclear or anti-double stranded DNA antibodies.

Comparing our cohort with the SLICC derivation cohort

The prevalence of individual SLICC classification criteria in our cohort was compared to the reported prevalence of these criteria in the SLICC derivation cohort (**Appendix 2.2**). Compared with SLE patients from the SLICC derivation cohort, SLE patients in our cohort had a lower frequency of non-scarring alopecia, oral/nasal ulcers, and leukopenia. Conver-

sely, they had a significantly higher frequency of renal disorder (by selection), neurologic disorder, haemolytic anaemia, thrombocytopenia, anti-dsDNA, and hypocomplementaemia. Compared with control patients without SLE from the SLICC derivation cohort, control patients without SLE in our cohort had significantly less frequent acute/subacute cutaneous lupus and arthritis, while the occurrences of other SLICC classification criteria were similar.

DISCUSSION

We performed the first study to test the validity of the SLICC classification criteria for SLE in a cohort of patients with a clinicopathologic suspicion of SLE based on the finding of full house glomerular deposits in their renal biopsies, a group reflecting a diagnostic problem area in the nephrology clinic. Our findings show that, overall, the SLICC criteria were more sensitive (100 vs. 94%) and enabled earlier classification than the ACR criteria. However, this was at the expense of specificity (91 vs. 100%). We identified three patients with biopsy findings consistent with LN and a positive ANA, who were classified as SLE according to the stand-alone renal SLICC criterion, but who had no further signs or symptoms of SLE. Conversely, no patients with clinical SLE were found who only fulfilled the stand-alone renal criterion. Exclusion of the stand-alone renal criterion from the SLICC classification resulted in significant reclassification improvement compared with the ACR classification in this cohort. We conclude that overall the SLICC classification performed well in our cohort, but the stand-alone renal criterion compromised the specificity. False-positive diagnoses emanating from the adaptation of the stand-alone renal SLICC criterion in clinical practice may have major consequences for patients, given the implications of the diagnoses LN and SLE.

Bayes' theorem states that the odds of disease equal the disease frequency (pretest odds in Bayesian terms) multiplied by the likelihood ratio. In this equation, the likelihood ratio stems from the sensitivity and specificity of the classification criteria set. Both sensitivity and specificity are dependent on the population that is studied. First, the sensitivity of a criterion may vary when studying a different disease spectrum. Patients with SLE in our cohort seemed to represent a different phenotype of SLE than the SLE patients in the SLICC derivation cohort who were selected from rheumatology clinics. Compared with patients in the SLICC derivation cohort, SLE patients with renal involvement in our cohort had a significantly lower frequency of alopecia, oral/nasal ulcers, and leukopenia, and a higher frequency of neurologic disorders, haemolytic anaemia, thrombocytopenia, anti-ds-DNA antibodies, and hypocomplementaemia. These results are in agreement with those from a recently published large inception cohort comparing SLE patients with and without nephritis[19] and support the notion that SLE is a disease with heterogeneous phenotypes.[20] Second, the specificity of a criterion is fully dependent on the control population studied. In designing a classification criteria set, the control population is chosen to represent the diagnostic problem area.[2] By design, renal involvement was less specific in our cohort than

in the SLICC derivation cohort, as the latter did not select patients with renal diseases that would appear in the differential diagnosis of LN. Moreover, the control population used for derivation of the SLICC criteria had more cutaneous and joint manifestations, reflecting typical findings in patients from rheumatology clinics. Thus, the sensitivities, specificities, and emanating likelihood ratios of criteria used in the development of the SLICC classification cannot be unequivocally applied to potential SLE patients in the nephrology clinic.

From our results, the combination of ANA and biopsy-confirmed LN as stand-alone criterion is questionable, as both had suboptimal specificity. Three patients were false-positively classified as SLE according to the stand-alone renal criterion by the SLICC. Although the specificity of ANA in our cohort was 88.5%, ANAs are associated with various other rheumatic and non-rheumatic diseases and can be detected in up to 27% of the general population.[21] Most telling, two patients in our cohort had membranous nephropathy and concurrent malignancy, the latter known for its association with ANA-positivity.[22] A positive ANA itself is very sensitive for SLE, but clearly ANAs are more accurate in ruling out SLE than confirming the diagnosis. Since ANA testing was performed qualitatively using 1:40 diluted serum and titres were unavailable for the patients in our study, it is unknown whether higher cut-offs would have resulted in more patients being appropriately classified using the stand-alone renal criterion. The classification of SLE according to the combination of a renal biopsy consistent with LN and positive anti-dsDNA antibodies in the absence of other criteria for SLE was not observed in our cohort. Possibly, anti-dsDNA antibodies would be more suitable than ANA as part of the stand-alone criterion given their higher specificity for SLE.

In addition to the problems emanating from the autoantibodies included in the stand-alone criterion, there is no consensus among pathologists and clinicians of what defines biopsy-confirmed LN. In our opinion, the finding of a full house pattern of immunofluorescence would certainly raise SLE as a differential diagnostic consideration. However, 32 patients in our cohort without SLE had full house immunofluorescence, affirming that full house immunofluorescence *per se* is a far from optimal indicator for LN and must be interpreted in light of clinical and additional biopsy findings. Clearly, the SLICC criteria excluding the stand-alone renal criterion appeared to be useful to identify SLE cases clinically. Concerning the additional biopsy findings, we found endocapillary and/or extracapillary hypercellularity, relative intensity of IgM and C1q, and subendothelial deposits by electron microscopy to support the diagnosis of SLE. Other biopsy findings, including tubuloreticular inclusions and coexistent mesangial, subendothelial and subepithelial deposits, that have previously been found to be suggestive of LN[3] were not significantly different between patients with and without clinical SLE in our cohort. It may be anticipated that in some cases, these other renal biopsy findings characteristic of LN would lead to the designation of biopsy-confirmed LN.[3] This emphasises that the extent to which the stand-alone criterion by the SLICC may result in false-positive classifications of SLE may be underestimated by this study.

Conversely, the number of false positives may also be overestimated by our study. In the literature, anecdotal reports of patients with "non-lupus full house nephropathy" – similar to the 32 patients without clinical SLE in our study – have shown that the minority of these patients become seropositive and/or develop extrarenal symptoms of SLE during up to 10 years follow-up.[23-27] However, during a median follow-up of 20.0 years (interquartile range 8.3–33.8), none of the 32 patients without clinical SLE in our study were clinically diagnosed with SLE. It cannot be excluded that some patients had latent SLE that would be diagnosed if more long-term follow-up data would be available. However, other studies have shown that non-lupus full house nephropathy can also be associated with atypical presentations of other well-established renal diseases or may occur idiopathically.[3, 5, 7-10, 24, 28-31]

We have shown that the SLICC classification proved to have great sensitivity among patients with a renal biopsy with full house glomerular deposits. This superior sensitivity of the SLICC relative to the ACR classification in our cohort was entirely attributed to the criterion hypocomplementaemia, which is absent in the ACR classification. Indeed, our results underline that complement consumption in the classical pathway is an essential finding in active severe SLE with renal involvement.[32, 33] In our cohort, the specificity of the SLICC classification was lower than the ACR classification, and this was attributed to the introduction of the stand-alone renal criterion to the SLICC classification. Other validation studies similarly showed a higher sensitivity and lower specificity of the SLICC compared with the ACR classification, although none of them elaborated on the stand-alone renal criterion.[11, 15-17, 34, 35] Only Ungprasert *et al*. identified three patients with SLE among 55 who met SLICC criteria who were classified based upon the stand-alone criterion without commenting on their biopsy findings.[35] We conclude that the SLICC classification may perform well as classification and conceivably also as diagnostic criteria for patients with renal biopsy findings consistent with LN, but we suggest re-evaluation of the stand-alone renal criterion for clinical and research purposes.

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