Diagnostic performance of serum cystatin C and complement component 1q in lupus nephritis

Bei Xu†, Ya-mei Zhang†, Yu-wei Yang, Yun-shuang Liu and Jia-fu Feng*

Abstract

Background: The information concerning non-invasive, easily obtainable, and accurate biomarkers for diagnosis of lupus nephritis (LN) is extremely limited. The aim of this study was to evaluate the diagnostic performance of cystatin C (CysC) and complement component 1q (C1q) for LN.

Methods: A case-control study that included 905 patients with systemic lupus erythematosus (SLE) without LN (group SLE), 334 patients with active lupus nephritis (group LNA), 255 patients with inactive lupus nephritis (group LNI), and 497 healthy individuals (group HC) was performed in Mianyang Central Hospital from March 2017 to December 2018. The serum levels of CysC, C1q, urea (Urea), and creatinine (Creat) were measured, and 2 estimated glomerular filtration rates (eGFR CysC and eGFR Creat) were calculated by equations which were based on serum CysC established by our group and the modification of diet in renal disease (MDRD), respectively. ANOVA analysis or Kruskal-Wallis test was used for comparing the differences among the groups, and receiver operating characteristic (ROC) curve was applied to identify the diagnostic efficiencies of individual or combined multiple indicators.

Results: Significantly elevated CysC and decreased C1q were observed in the LNA and LNI groups, which was in contrast to their levels in the SLE and HC groups. CysC (AUC = 0.906) or eGFR CysC (AUC = 0.907) assessed the highest diagnostic performance on LNA when detected individually, followed by C1q (AUC = 0.753). Joint utilization of C1q and CysC achieved very good performance (AUC = 0.933) which approximated to the best one observed in the combinations of C1q, Urea, CysC, eGFR Creat, and Creat (AUC = 0.975).

Conclusion: The separately detected CysC (eGFR CysC) and C1q were superior to the conventional biomarkers Urea, Creat, and eGFR Creat in the diagnosis of LNA. Moreover, although the combined detection of Urea, Creat, C1q, CysC, and eGFR Creat had the greatest diagnostic performance, the joint utilization of CysC and C1q could be prioritized for rapid discrimination of LNA if the economic burden is taken into consideration.

Keywords: Lupus nephritis, Biomarker, Cystatin C, Complement component 1q

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the involvement of diverse autoantibodies. The process of SLE pathogenesis can cause multiple tissue and organ damages [1]. When the kidney is involved, this condition can lead to lupus nephritis (LN) [2, 3]. LN was found to occur in 15–30% of the patients with lupus at the time of initial diagnosis and in 30–50% during disease progression [4]. Additionally, it severely negatively affects the SLE patients’ prognosis [5]. Accurate diagnosis and active treatment can preserve the kidney function of LN patients and delay the process of kidney fibrosis, thus postponing the occurrence and development of end-stage kidney disease [2]. Although a few lupus nephritis management guidelines have been published currently, they have been established in the USA and European countries, or are internationally based [6]. There is still little evidence to indicate their relative prediction and monitoring strategies in Asia. Additionally, serum or urine biomarkers such as serum creatinine and its clearance rate...
which are commonly used in the clinical evaluation of the kidney function in LN patients, as well as immune-related molecules, such as anti-double-stranded DNA antibody, anti-cardiolipin antibody, complement component C3b, and anti-C1q antibody, cannot display enough sensitivity and specificity to reflect the real-time renal immunopathological reactions [1, 7]. Since delayed diagnosis is related with a higher occurrence of end-stage kidney disease (ESKD) along with an unsatisfactory prognostication [7], rapid, accurate, and non-invasive diagnosis of kidney impairment is of substantial importance, especially in LN patients [8–11].

Cystatin C (CysC) has received much more attention than Creat as an alternative filtration marker with stronger and more linear risk relationships [12–14], which was also recommended by the KIDGO (2012) Chronic Kidney Disease Clinical Application Guideline. Several studies established that the addition of CysC measurements in the calculation of eGFR significantly improves the risk classification for death, cardiovascular disease, and ESKD [15–17]. Moreover, our group previously developed a glomerular filtration rate (GFR)-estimating equation based on CysC determination (eGFR\text{CysC}_\text{C})\text{CysC}, which further confirmed it could achieve a much better diagnostic performance than that one based on Creat in Chinese patients with chronic kidney disease (CKD) [18]. Unfortunately, the value of eGFR\text{CysC}_\text{CysC} in the diagnosis of kidney impairment in LN patients is still not validated.

Moreover, the complement system is one of the major effector mechanisms of the innate immune system that plays an important role in immune defense [19]. C1q is the initial complement component that activates the classical complement pathway and is critically involved in the clearance of immune complexes and apoptotic cell debris. Impaired clearance leads to exposure of C1 native antigen and development of anti-C1q antibody formation [20]. Hereditary deficiency of C1q is known to be a risk factor for the development of SLE [21]. The anti-C1q antibody has been well studied in SLE and has been proposed as a valuable biological marker in SLE with close association with renal involvement [22]. Conversely, only few published studies focused on serum C1q in SLE patients with LN [19, 23, 24]. Considering this, we designed this study to explore if serum C1q can contribute to the diagnosis of kidney impairment in LN patients as a novel disease biomarker.

Therefore, a case-control study that contained patients with SLE without renal involvement (group SLE), active lupus nephritis (group LNA), and inactive lupus nephritis (group LNI), as well as healthy individuals (group HC), was carried out in the present study. Their serum concentrations of urea, Creat, C1q, and CysC were measured, and Urea/Creat and eGFR (respectively based on CysC and Creat concentrations) were calculated. The main purpose of our study was to identify and establish combined biomarkers for detection from these traditional and new renal function indicators to achieve a more accurate and reliable diagnosis of LN.

**Methods**

**Design**

The present study is a case-control study, in which we analyzed the serum levels of the Urea, Creat (Urea/Creat), CysC (eGFR), and C1q of all subjects to evaluate their effectiveness in diagnosing LN. We included the patients who attended Mianyang Central Hospital (Sichuan, China) from March 2017 to December 2018 for treatment. They were categorized as with only SLE without renal involvement (group SLE), active lupus nephritis (group LNA), and inactive lupus nephritis (group LNI), all of whom fulfilled the inclusion criteria as described below. Otherwise, healthy controls (group HC) without systemic disease activity and with no history of renal disease that visited the hospital during the same period were recruited.

We analyzed the serum levels of the observed indicators of the subjects to evaluate their effectiveness in the diagnosis of LN. Receiver operating characteristic (ROC) curve was conducted to assess the performance of the new indicators in distinguishing LNA patients and healthy subjects in comparison with the frequently used ones such as Urea, Creat, and eGFR\text{Creat}_\text{Creat}.

1. **Inclusion criteria:** SLE was diagnosed according to the systemic lupus erythematosus guidelines by American College of Rheumatology Ad Hoc Committee [25], which was performed by serial or simultaneous presentation of at least 4 of the following 11 criteria: malar rash, discoid rash, photosensitivity, oral ulcers, non-erosive arthritis, serositis, renal dysfunction, neurological derangements (i.e., seizures or psychosis), hematologic disorder (i.e., anemia, leukopenia, thrombocytopenia), immunologic disorder (i.e., anti-DNA antibody, anti-Sm antibody, and false-positive VDRL testing), and presence of antinuclear antibodies. Kidney impairment was defined by a urinary albumin/creatinine ratio (UACR) ≥ 30 mg/g. LN was determined according to the American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis [26], which was defined as clinical and laboratory manifestations that meet the ACR criteria (persistent proteinuria > 0.5 g per day or greater than 3+ by dipstick, and/or cellular casts including red cell, hemoglobin, granular, tubular, or mixed). Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores were calculated to evaluate the renal disease activity [27]. Patients with a SLEDAI score ≥ 4 were considered to have active lupus; otherwise, they were categorized as having inactive lupus.

2. **Exclusion criteria:** patients with primary kidney disease, diabetes mellitus, cardiovascular dysfunction, respiratory...
dysfunction, ongoing infections, pregnancy, and other autoimmune diseases were excluded.

The study protocol was approved by the Medical Ethics Committee of Mianyang Central Hospital, and written informed consent was obtained from each patient before the study (S201400048, S2018085).

**Sample collection**

Venous blood was collected into a BD Vacutainer® SST™ II ADVANCE tube (Becton Dickinson, USA) from all participants in the morning after overnight fasting. Then, the samples were mixed gently upside down eight times and left undisturbed for 30 min. The serum was separated by centrifugation at 3000 rpm for 15 min. All clinical laboratory tests were completed within 2 h after serum separation. Within 30 min after venous blood collection, the patient was instructed to provide approximately 10 mL of clean midstream urine for determination of urinary albumin and creatinine.

**Measurements of serum CysC, C1q, Urea, and Creat**

Serum CysC, C1q, Urea, and Creat were measured using the fully automatic analyzer Labospect™ 008 (Hitachi, Japan). CysC and Urea kits were provided by Sichuan Maccura Biotechnology Co., Ltd. (Sichuan, China). C1q kits were obtained from Shanghai Beijia Chemical Reagent Co., Ltd. (Shanghai, China), and Creat kits were purchased from FUJIFILM Wako Pure Chemical Co., Ltd. (Osaka, Japan). Controls were respectively provided by Bio-Rad (Hercules, CA, USA); Sichuan Maccura Biotechnology Co., Ltd.; and Shanghai Beijia Chemical Reagent Co., Ltd. CysC was measured by turbidimetric immunoassay, and Urea and Creat were detected by the urease method and the sarcosine oxidase assay, respectively. Enzyme-linked immunosorbent assay (ELISA) is the frequently used method for C1q measurement. However, it has poor repeatability for quantitative detection. In this work, serum C1q levels were directly determined by automated immunoturbidimetric analysis. Compared to ELISA assays, its detection speed is much higher, and less interference and low cross-contamination between samples are observed.

**Calculation of estimated GFR**

The eGFR formula based on serum CysC in Chinese patients established by our group was used to calculate the eGFR\textsubscript{CysC} [18]. Otherwise, the modification of diet in renal disease (MDRD) equation developed by a Chinese group was also used to calculate eGFR\textsubscript{Creat} [28]. The following formulas were used for the calculations:

\[
e\text{GFR}\textsubscript{CysC} = 78.64 \times \text{CysC}^{-0.964}
\]

\[
e\text{GFR}\textsubscript{Creat} = 175 \times \text{Cr}^{-1.234} \times \text{Age}^{-0.179} \text{ (if male)}
\]

**Statistical analysis**

All data are expressed as mean ± standard deviation (SD) or as the median (M) and quartile (P25, P75). The normal distribution was tested with the Kolmogorov-Smirnov method and Q-Q chart. Quantitative data with normal distribution were compared by one-way analysis of variance (ANOVA), whereas those with non-normal distribution were subjected to the Kruskal-Wallis non-parametric test. The non-parametric Spearman rank correlation coefficient was used for the statistical analysis of the data. The diagnostic efficiencies of individual or multiple indicators were analyzed with receiver operating characteristic (ROC) curve in which the area under the curve (AUC) was tested with DeLong non-parametric test. According to the maximum Yoden index (Y = sensitivity + specificity − 1), the cutoff value of each observed index was selected. Statistical analysis was performed with SPSS 19.0 (SPSS, Inc., Somers, NY, USA) and MedCalc11.5 (MedCalc Software, Mariakerke, Belgium). A value of \(P < 0.05\) was considered statistically significant.

**Results**

A total number of 1494 patients were recruited, including 125 men and 1369 women with a mean age of 39.2 ± 12.8 years (range 13–82 years). These patients were further divided into 3 groups: 905 SLE patients without renal involvement (group SLE), 334 patients with active lupus nephritis (group LNA), and 255 patients with inactive lupus nephritis (group LNI). Additionally, 497 healthy individuals, including 260 men and 237 women, with a mean age of 46.2 ± 13.8 years (range 11–85 years) served as healthy controls (group HC). Statistical analysis revealed significant differences in gender (\(\chi^2 = 461.777, P < 0.001\)) and age (\(\bar{t} = 9.962, P < 0.001\)) between the patient group and the control group. Urinary albumin and creatinine measurements were employed to calculate UACR to further divide the patients into subgroups. These results had been presented in Table 1.

**Serum levels of CysC, C1q, Urea, and Creat**

The normal distribution test by the Kolmogorov-Smirnov method and Q-Q chart showed that all parameters had a normal or approximately normal distribution, except for CysC, Urea, and Creat in the LNA group. Thus, the Kruskal-Wallis non-parametric test was applied for statistical comparisons between the multiple groups for these parameters, whereas ANOVA was performed for the others. The serum levels of CysC, C1q, Urea, and Creat determined by the laboratory examinations are listed in Table 1; statistically significant differences were present...
among the study groups (all $P < 0.001$). The multiple comparisons (Table 2) showed that significant differences were available in CysC, eGFR$_{CysC}$ and C1q between the healthy subjects and those diagnosed with SLE, LNI, and LNA (all $P < 0.001$), indicating that the elevated CysC and the decreased eGFR$_{CysC}$ and C1q levels might serve as meaningful biomarkers for both SLE and LN diagnoses. To further distinguish the different disease status, multiple comparisons in groups SLE, LNI, and LNA were conducted; significant differences (all $P < 0.001$) of CysC, eGFR$_{CysC}$, and eGFR$_{Creat}$ were observed.

Table 1

| Items                  | HC (n = 497) | SLE (n = 905) | LNA (n = 334) | LNI (n = 255) | F / $\chi^2$ | $P$ |
|------------------------|-------------|-------------|-------------|-------------|----------|------|
| Demographic characteristics                              |             |             |             |             |          |      |
| Male, n (%)            | 260 (52)    | 57 (6)      | 36 (12)     | 32 (13)     |          |      |
| Female, n (%)          | 237 (48)    | 848 (94)    | 298 (88)    | 223 (87)    |          |      |
| Age (years)            | 46.2 (11−85)| 39.2 (13−82)| 38.44 (13−76)| 40.3 (13−80)|          |      |
| Laboratory measurements                               |             |             |             |             |          |      |
| UACR (mg/g), M (P25, P75) | 8.15 (4.52,10.7) | 9.31 (4.43, 12.07) | 1197.00 (557.49,2212.39) | 131.73 (57.01, 220.48) | 217.834 | 0.000 |
| Urinary protein (g/24 h), M (P25, P75) | 0.08 (0.04, 0.11) | 0.14 (0.09, 0.20) | 2.47 (0.95, 3.43) | 0.18 (0.08, 0.27) |          |      |
| Creat (μmol/L)         | 65.2 ± 12.97| 55.1 ± 11.44a| 62.2 (50.7, 87.4)b| 61.2 ± 23.25abc| 217.834 | 0.000 |
| Urea (mmol/L)          | 5.11 ± 1.17 | 4.93 ± 1.58a | 6.15 (4.58, 8.88)ab | 5.74 ± 2.17bc | 131.728 | 0.000 |
| Urea/Creat             | 0.08 ± 0.02 | 0.09 ± 0.03a | 0.10 ± 0.04ab | 0.10 ± 0.03ab | 29.657 | 0.000 |
| CysC (mg/L)            | 0.80 ± 0.13 | 0.96 ± 0.23a | 1.22 (1.01, 1.75)ab | 1.09 ± 0.41abc | 550.624 | 0.000 |
| eGFRCysC [mL/(min·1.73 m2)] | 100.3 ± 16.33 | 85.5 ± 23.92ab | 62.9 ± 23.92abc | 78.5 ± 18.68abc | 283.328 | 0.000 |
| eGFRCreat [mL/(min·1.73 m2)] | 121.0 ± 24.37 | 139.9 ± 36.67a | 112.7 ± 52.76ab | 131.6 ± 40.82abc | 52.886 | 0.000 |
| C1q (mg/L)             | 182.29 ± 28.91 | 170.58 ± 35.52a | 153.24 ± 39.57ab | 170.80 ± 36.16abc | 46.330 | 0.000 |

CysC and C1q were measured by turbidimetric immunoassay, and Urea and Creat were detected by the urease method and sarcosine oxidase assay, respectively. ANOVA analysis or Kruskal-Wallis test was used for comparing the differences among the multiple groups. $F$ / $\chi^2$ and $P$ represented the statistical results of ANOVA analysis or Kruskal-Wallis test among all the study groups.

| Items                  | Comparison pairs | HC to SLE | HC to LNA | HC to LNI | SLE to LNA | SLE to LNI | LNA to LNI |
|------------------------|------------------|----------|-----------|-----------|------------|------------|------------|
| Urea                   | $z$              | 3.117    | −7.605    | −2.612    | −11.12     | −5.293     | 4.051      |
|                        | $P$              | 0.011    | 0.000     | 0.054     | 0.000      | 0.000      | 0.000      |
| Creat                  | $z$              | 13.378   | 1.610     | 6.66      | −9.887     | −3.299     | 4.799      |
|                        | $P$              | 0.000    | 0.645     | 0.000     | 0.000      | 0.006      | 0.000      |
| Urea/Creat             | LSD-t            | −6.412   | −8.119    | −7.346    | −3.381     | −2.932     | 0.104      |
|                        | $P$              | 0.000    | 0.000     | 0.000     | 0.001      | 0.003      | 0.917      |
| CysC                   | $z$              | −13.005  | −22.801   | −13.561   | −13.857    | −4.493     | 6.838      |
|                        | $P$              | 0.000    | 0.000     | 0.000     | 0.000      | 0.000      | 0.000      |
| e-GFR$_{CysC}$         | LSD-t            | 14.340   | 28.624    | 15.292    | 19.128     | 5.322      | −10.189    |
|                        | $P$              | 0.000    | 0.000     | 0.000     | 0.000      | 0.000      | 0.000      |
| eGFRCreat              | LSD-t            | −8.933   | 3.053     | −3.629    | 11.163     | 3.093      | −5.959     |
|                        | $P$              | 0.000    | 0.002     | 0.000     | 0.000      | 0.002      | 0.000      |
| C1q                    | LSD-t            | 6.024    | 11.789    | 4.283     | 7.775      | −0.091     | −6.063     |
|                        | $P$              | 0.000    | 0.000     | 0.000     | 0.000      | 0.028      | 0.000      |

ANOVA analysis or Kruskal-Wallis test was used for comparing the differences among the multiple groups. $z$/LSD-t and $P$ were the statistical results for the comparison between the two groups. LSD-t was the statistics of ANOVA analysis to find the difference between the observed indicators of the normal distribution, and $z$ was the statistics of Kruskal-Wallis non-parametric test to find the difference between the two observed indicators of the non-normal distribution.

Urea urea, Creat creatinine, CysC cystatin C, C1q complement component 1q, UACR urinary albumin to creatinine ratio.

Compared with the HC group, $P < 0.05$
Compared with the SLE group, $P < 0.05$
Compared with the LNA group, $P < 0.05$
eGFR<sub>CysC</sub> and C1q among these groups were found, except for the similar mean concentrations of C1q observed between SLE and LNI groups ($P = 0.928$). These results suggest that using only C1q is not sufficiently precise to differentiate subjects with SLE and LNI, but might be potentially used to reveal renal disease in patients with active and inactive LN. For the other traditional parameters, similar levels of Urea in the HC and LNI groups ($P = 0.054$) were established, whereas significant differences among the other groups were observed in these indicators (all $P < 0.05$). The differences in Creat among the groups were all statistically significant (all $P < 0.05$), except for that between the HC and LNA groups ($P = 0.645$). The ratios of Urea/Creat, had similar values only in the LNA and LNI groups ($P = 0.917$), whereas significant differences in eGFR<sub>Creat</sub> were available among all groups (all $P < 0.05$).

**Analysis of the correlation between the observed indicators**

As can be seen in Table 3, Spearman’s correlation analysis revealed significant correlations between the CysC levels and Urea, Creat, eGFR<sub>CysC</sub>, and eGFR<sub>Creat</sub> in the HC, SLE, LNA, and LNI groups. The CysC levels were positively correlated with the values of Urea and Creat, whereas they were negatively correlated with eGFR<sub>CysC</sub> and eGFR<sub>Creat</sub>. In the HC group, negative correlations were also observed between CysC and Urea/Creat ($P < 0.001$). By contrast, no significant correlations between C1q and other parameters were found among the four groups.

**Table 3** Spearman’s correlation analysis of various parameters

|            | Urea | Creat | Urea/Creat | CysC | eGFR<sub>CysC</sub> | eGFR<sub>Creat</sub> | C1q |
|------------|------|-------|------------|------|---------------------|---------------------|-----|
| HC group   |      |       |            |      |                     |                     |     |
| CysC       | 0.225| 0.561 | −0.223     | 1    | −0.977              | −0.538              | 0.092|
|            | 0.000| 0.000 | 0.000      | −0.000| 0.000              | 0.040               |     |
| C1q        | 0.028| −0.056| 0.086      | 0.092| −0.089              | 0.055               | 1    |
|            | 0.534| 0.217 | 0.055      | 0.040| 0.048              | 0.218               |     |
| SLE group  |      |       |            |      |                     |                     |     |
| CysC       | 0.289| 0.548 | −0.042     | 1    | −0.945              | −0.478              | −0.020|
|            | 0.000| 0.000 | 0.204      | −0.000| 0.000              | 0.556               | 0.556|
| C1q        | −0.012| 0.039 | −0.032     | −0.020| −0.016              | −0.068              | 1    |
|            | 0.721| 0.242 | 0.342      | 0.556| 0.632              | 0.041               |     |
| LNA group  |      |       |            |      |                     |                     |     |
| CysC       | 0.656| 0.716 | −0.099     | 1.000| −1.000              | −0.728              | −0.039|
|            | 0.000| 0.000 | 0.071      | −0.000| 0.000              | 0.473               |     |
| C1q        | 0.000| −0.029| 0.012      | −0.039| 0.039              | −0.011              | 1.000|
|            | 0.996| 0.603 | 0.832      | 0.473| 0.474              | 0.838               |     |
| LNI group  |      |       |            |      |                     |                     |     |
| CysC       | 0.661| 0.819 | −0.037     | 1    | −0.883              | −0.684              | 0.103|
|            | 0.000| 0.000 | 0.552      | −0.000| 0.000              | 0.101               |     |
| C1q        | 0.068| 0.039 | 0.006      | 0.103| −0.076              | −0.109              | 1    |
|            | 0.280| 0.537 | 0.924      | 0.101| 0.228              | 0.082               |     |

Spearman’s correlation analysis was applied to identify the associations between CysC or C1q and other parameters

$r$ correlation coefficient, CysC cystatin C, C1q complement component 1q

**Diagnostic efficiencies of individual or multiple biomarkers for kidney impairment in patients with LN**

ROC analysis was employed to analyze the diagnostic efficiencies of the single and combined detection potential of CysC, eGFR<sub>CysC</sub>, C1q, Creat, eGFR<sub>Creat</sub>, Urea, and Urea/Creat for kidney impairment in LNA patients (Fig. 1). The value of each observed index corresponding to the maximum YI was selected as the cutoff value of the observed index. Creat (76.0%) had the highest individual detection sensitivity, whereas eGFR<sub>Creat</sub> (25.1%) had the lowest sensitivity. Additionally, eGFR<sub>Creat</sub> had the highest specificity (98.0%), whereas the lowest was observed in Creat (30.0%) (Table 4). Further analysis by the DeLong non-parametric test was performed for the AUC of the different parameters. AUC of CysC (AUC = 0.906) or eGFR<sub>CysC</sub> (AUC = 0.907) was significantly higher than that of C1q (AUC = 0.753), Urea (AUC = 0.668), Urea/Creat (AUC = 0.639), eGFR<sub>Creat</sub> (AUC = 0.539), and Creat (AUC = 0.508) (all $P = 0.000$). The finding revealed that CysC or eGFR<sub>CysC</sub> had the best diagnostic performance for LN, followed by C1q when separately detected as a single indicator.

In terms of combined detection, the diagnostic performance of LNA was the highest in its combinations with C1q, Urea, CysC, eGFR<sub>CysC</sub>, and Creat (AUC = 0.975), whereas those of C1q and eGFR<sub>Creat</sub> were the lowest (AUC = 0.756) (Table 5). Statistically significant differences regarding diagnostic efficiency were found between the best combination and the other joint detections (all $P < 0.05$), except the combined utilization of
C1q, CysC, eGFRCreat, and Creat (AUC = 0.974, \( P = 0.310 \)). The joint detection of C1q and CysC achieved the highest diagnostic accuracy (AUC = 0.933) with high sensitivity (82.9%) and specificity (92.6%), which was slightly lower than those of the best three or the combined biomarkers (Table 5). These findings implied that the combined utilization of C1q and CysC could be a potential biomarker for diagnosis in LNA if the economic burden is taken into consideration.

**Discussion**

The identification of new clinical and laboratory biomarkers in the Asian population is crucial for the

### Table 4: Diagnostic efficiency of the individual biomarker of LNA

| Items          | AUC (95% CI) | Z/P\(^{a}\) | Cutoff V | Se (%) | Sp (%) | YI     | Z/P \(^{b}\) |
|----------------|--------------|-------------|----------|--------|--------|--------|-------------|
| Creat         | 0.508 (0.473–0.542) | 0.346/0.729 | 90.1 | 76.0 | 30.0 | 0.209 | 13.816/0.000 |
| eGFRCreat     | 0.539 (0.504–0.573) | 1.731/0.084 | 79.65 | 25.1 | 98.0 | 0.231 | 18.447/0.000 |
| Urea/Creat    | 0.639 (0.605–0.672) | 6.836/0.000 | 0.09 | 55.4 | 68.4 | 0.238 | 11.141/0.000 |
| Urea          | 0.668 (0.634–0.700) | 8.144/0.000 | 6.40 | 46.1 | 86.5 | 0.326 | 11.976/0.000 |
| C1q           | 0.753 (0.723–0.782) | 13.753/0.000 | 144 | 49.1 | 94.4 | 0.435 | 7.015/0.000 |
| CysC          | 0.906 (0.884–0.925) | 35.891/0.000 | 1.00 | 75.7 | 94.6 | 0.703 | –           |
| eGFRCysC      | 0.907 (0.885–0.926) | 36.100/0.000 | 77.89 | 75.7 | 94.6 | 0.703 | –           |

Receiver operating characteristic (ROC) curve was applied to identify the diagnostic efficiencies of individual indicator. \( Z \) was the statistics of DeLong non-parametric test to evaluate the statistical difference between the two AUCs.

Creatinine, Urea, C1q complement component 1q, CysC cystatin C

\(^a\)Z and \( P \) values were the AUC-based statistics of each item

\(^b\)Z and \( P \) values were the AUC-based statistics of each item in comparison with eGFRCysC
accurate determination of the renal involvement in LN diagnosis and supervision.

In this work, we evaluated for the first time the accuracy and suitability of the application of CysC, eGFR CysC, and C1q as biomarkers in the diagnosis of renal impairment in LNA patients from the Chinese population in clinical settings in China.

Our results showed unsatisfactory diagnostic performances of separately detected Creat, Urea, their calculated eGFR creatinine, and Urea/Creat in LNA patients (AUC 0.508–0.668). Hence, we suggest that kidney impairment might precede the changes in Creat and Urea in LNA patients, and the traditional parameters would fail to diagnose early-stage kidney impairment in these populations. Some reasons which might affect the values of Creat were present as follows: (I) many physical factors (such as age, gender, and muscle mass) influence Creat levels much and make the individual difference large; (II) serum Creat is a later biomarker of kidney injury, for that, the rise in serum Creat is only observed when kidney function is decreased by 50% [29]. These various intra-variabilities for Creat might contribute to the different levels between healthy controls and the patient cases in our study. Additionally, the effects of Creat may also be the main reason for the difference between the results of eGFR creatinine (calculated based on Creat) in healthy controls as compared with the cases. Importantly, since eGFR creatinine is influenced by the various factors mentioned above, eGFR CysC (calculated based on CysC) would be more effective than eGFR creatinine to represent the reduced GFR status in the disease groups in our study.

In contrast to the serum Creat concentrations, CysC was not affected by intra-variabilities such as gender and muscle mass, providing a more accurate measure of renal function [30, 31]. Evidence exists of the significant association of higher serum levels of CysC with increased microalbuminuria and lower GFR, which could reflect renal damage and impaired renal function [30]. We obtained similar results with significantly higher CysC but lower eGFR CysC values in SLE, LNA, and LNI groups than those in the healthy controls, which indicates the existence of a close association between CysC/eGFR CysC and SLE, especially in renal function impairment (LN). Additionally, the diagnostic efficiency of Creat in LNA patients was consistently lower than that of CysC, which further implied that Creat is inferior to CysC in the diagnosis of kidney impairment. eGFR is the clinical standard for the assessment of kidney function [32, 33]. Although the two indicators eGFR CysC and eGFR creatinine were both applied in the Chinese group, our findings showed that eGFR CysC had a higher performance in the diagnosis of kidney impairment in LNA. Moreover, the evidence is available that the measurement of Creat to determine the eGFR is limited by the influence of age and sex, as well as the variability in the clinical methodology for Creat determination [34, 35]. As known, the enzyme method is widely used in the majority of laboratories. Thus, the eGFR CysC formula, which was previously established by our group, was confirmed to be more suitable and accurate for the diagnosis of LNA in Chinese SLE patients, revealing that careful and complete consideration is required to choose the most appropriate eGFR equation for the specific clinical application to avoid misdiagnosis and treatment errors. It also should be noted that eGFR is not a measured parameter and is calculated based on the values of Creat and/or

| Items                                      | AUC (95%CI)       | ZP   | Se (%) | Sp (%) | Y1   | ZP   |
|--------------------------------------------|------------------|------|--------|--------|------|------|
| C1q + eGFR creatinine                      | 0.756 (0.725–0.785) | 13.926/0.000 | 51.2   | 92.2   | 0.434 | 12.196/0.000 |
| C1q + Creat                                | 0.765 (0.735–0.794) | 4.587/0.000  | 57.5   | 88.9   | 0.464 | 11.864/0.000 |
| C1q + Urea                                 | 0.805 (0.776–0.831) | 18.108/0.000 | 62.0   | 89.9   | 0.519 | 10.627/0.000 |
| C1q + CysC                                 | 0.933 (0.914–0.949) | 46.787/0.000 | 82.9   | 92.6   | 0.755 | 6.172/0.000  |
| C1q + Urea + eGFR creatinine               | 0.813 (0.785–0.839) | 8.969/0.000  | 70.7   | 81.7   | 0.524 | 10.509/0.000 |
| C1q + Urea + CysC                          | 0.934 (0.915–0.950) | 46.787/0.000 | 82.9   | 92.6   | 0.755 | 6.182/0.000  |
| Urea + CysC + eGFR creatinine              | 0.959 (0.943–0.971) | 67.443/0.000  | 87.7   | 93.2   | 0.809 | 4.178/0.000  |
| C1q + CysC + eGFR creatinine               | 0.969 (0.955–0.980) | 79.306/0.000  | 92.8   | 90.7   | 0.836 | 3.802/0.001  |
| Urea + CysC + eGFR creatinine + Creat      | 0.964 (0.949–0.976) | 73.979/0.000  | 90.4   | 91.8   | 0.822 | 3.053/0.002  |
| C1q + Urea + CysC + eGFR creatinine        | 0.969 (0.955–0.980) | 79.306/0.000  | 92.8   | 90.7   | 0.836 | 3.897/0.001  |
| C1q + CysC + eGFR creatinine + Creat       | 0.974 (0.961–0.984) | 88.822/0.000  | 91.0   | 95.0   | 0.860 | 1.016/0.310  |
| C1q + Urea + CysC + eGFR creatinine + Creat| 0.975 (0.962–0.984) | 88.976/0.000  | 92.5   | 94.4   | 0.869 | —    |

Receiver operating characteristic (ROC) curve was applied to identify the diagnostic efficiencies of combined multiple indicators. Z was the statistics of DeLong non-parametric test to evaluate the statistical difference between the two AUCs. CysC cystatin C, Creat creatinine, Urea urea, C1q complement component 1q.
CysC as well as those of other parameters. Thus, the results may be unavoidably affected by other factors. Hence, a specific threshold should be established in each individual lab, which can contribute to the accurate evaluation of kidney function.

The role and clinical relevance of the serum C1q levels in the renal involvement in LN patients still need to be elucidated. Our findings showed that the levels of C1q were significantly lower in the SLE, LNA, and LNI groups than in the normal controls. The C1q concentrations in the LNA were lower group than those in the LNI and SLE groups. These results are consistent with the ones in previous studies [24, 36], implying that the deficiency of C1q is a risk factor for the development of SLE, and the levels of C1q are closely associated with the development of renal disease in LN. Otherwise, no statistically significant correlations between C1q and other parameters were found among the HC, SLE, LNA, and LNI groups. Therefore, the serum complement C1q might serve as an independent risk factor in SLE and LN. To the best of our knowledge, we for the first time further evaluated the role of serum C1q in the diagnosis of kidney impairment in Chinese LNA patients. ROC curve analysis showed that C1q was superior to the widely used parameters Creat/eGFR_Creat in the diagnosis of kidney impairment in SLE patients with LNA. Therefore, we discovered that the individually detected serum C1q levels can serve as a novel and sensitive non-invasive biomarker for renal damage in LNA.

Because of the heterogeneity of LN, however, the simple use of just one parameter may hardly achieve satisfactory diagnostic performance [3]. The absence of a correlation between C1q and the other biomarkers suggested that the parameters investigated in this study might be related to different aspects of LN. Hence, the combined detection of C1q and other serological biomarkers can significantly improve the diagnostic accuracy. As expected, the diagnostic efficiencies of the combined detection of C1q and other markers were higher than that of the determination of these parameters alone. The diagnostic accuracy of LANA was the highest in the combinations of the five parameters tested, including C1q, Urea, CysC, eGFR_Creat, and Creat. Interestingly, the combined detection of C1q, CysC, and eGFR_Creat could also achieve high diagnostic performance. But when taken the economic burden into consideration, the joint biomarkers of C1q and CysC could be a priority; for that, its diagnostic efficiency was only slightly lower than that of the highest one. In agreement with previous reports [37], our findings confirmed that the determination of a single index cannot accurately be used for disease diagnosis, and the joint detection of a range of indicators is more precise possibly due to their complementary roles.

Overall, compared to the renal biopsy which cannot be routinely conducted serially and unable to reflect the global renal pathological status due to the obtained small-size specimens, and 24 h urine protein quantification which needs to collect the patient’s urine with a long period for 24 h and cumbersome operation, our investigated biomarkers are more convenient, acceptable, and non-invasive with excellent sensitivity and specificity. They would offer an alternative to the rapid discrimination of renal damage in LN.

Although our study revealed that CysC/eGFR_CysC and C1q could be promising biomarkers for the identification of LNA in SLE patients, some limitations have to be acknowledged. First, the scope of our investigation was limited by its small sample size. Second, the data obtained were collected only at a single institution, and no follow-up data were available. Third, LN was determined mainly by laboratory parameters and clinical manifestations rather than by pathogenic analysis of renal biopsies. Moreover, the sex difference had not been taken into consideration, although the recognition and identification of sex-specific biological processes would have led to a better understanding of the parameters’ alterations in the diagnostic performance in LN patients. Therefore, in the future, we plan to conduct a multi-center study with larger sample sizes and rigorous inclusion of incident patients to further investigate the diagnostic values of CysC/eGFR_CysC and C1q in LN patients in China. Moreover, the validity of these observed indicators for the LN diagnosis in comparison with the gold standard tests such as kidney biopsy will be also needed.

**Conclusions**

The significance of the values of CysC/eGFR_CysC and C1q in the diagnosis of renal impairment in LN patients was validated for the first time in China. Moreover, our finding confirmed that the deficiency of serum C1q is a risk factor for the development of SLE, and the levels of C1q are closely associated with the activity of LN. Therefore, C1q could serve as a novel and sensitive non-invasive single biomarker for renal damage. Additionally, apart from the economic burden consideration, we suggest that the joint utilization of CysC and C1q should be prioritized for the rapid detection of LNA, which warrants further investigations in clinical settings. Nevertheless, due to the aforementioned limitations, our findings need to be confirmed in a larger and more specifically selected LN population in the future.

**Abbreviations**

ACR: American College of Rheumatology; ALN: Activity lupus nephritis; Aki: Acute kidney injury; AUC: Area under the curve; ANOVA: One-way analysis of variance; C1q: Component 1q; CysC: Cystatin C; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; ELISA: Enzyme-linked immunosorbent assay; ESKD: End-stage kidney disease; GFR: Glomerular filtration rate; HC: Healthy controls; ILN: Inactivity lupus nephritis; KIDGO: Kidney Disease Improving Global Outcomes; LN: Lupus nephritis; M: Median; MDRD: Modification of diet in renal disease;
ROC: Receiver operating characteristic curve; SD: Standard deviation; SLE: Systemic lupus erythematosus; SLEDAI: SLE disease activity index; UACR: Urinary albumin/creatinine ratio; VDRL: Venereal disease research laboratory test; YI: Younden index

Acknowledgements

None.

Authors’ contributions

BX, YZ, and YL performed the experiments. YZ and YY performed the data analyses. JF participated in the study design and final review of the manuscript. BX and YZ wrote the manuscript. All authors read and approved the manuscript.

Funding

These studies were supported by funding from the National Key Basic Research Development Program of China (973 Program) sub-topic (2015CB755400) and the Science & Technology Department of Sichuan Province (it provided a part of the funds, 2009SC0066).

Availability of data and materials

The data generated and analyzed will be made available to interested readers.

Ethics approval and consent to participate

Informed consent was obtained from all participants, and clinical data, urine, and serum samples were collected and analyzed only for research. The study was approved by the Medical Ethics Committee (Institutional Review Board) and serum samples were collected and analyzed only for research. The study will be made available to interested readers.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 19 August 2019 Accepted: 22 November 2019

Published online: 04 December 2019

References

1. Chia-Li Y, Song-Chou H, Chang-Youh T. Potential serum and urine biomarkers in patients with lupus nephritis and the unsolved problems. Open Access Rheumatol. 2016;8:81–91.
2. Bawazer LA. Current and emerging therapy on lupus nephritis. Acta Med Indones. 2017;49(4):369–77.
3. Dong WX, Zheng ZH, Ding J, Luo X, Li ZQ, Li Y, et al. Combined detection of uMCP-1 and uTWEAK for rapid discrimination of severe lupus nephritis. Lupus. 2018;27:971–81.
4. Imran TF, Yick F, Verma S, Estiverne C, Ogbonnaya-Odor C, Thiruvarudsothy S, et al. Lupus nephritis: an update. Clin Exp Nephrol. 2016;20:1–13.
5. Alimaani S, Meara A, Roivin BH. Update on lupus nephritis. Clin J Am Soc Nephrol. 2017;12:825–35.
6. Wilhelmsen S, Baemna IM, Bertssia GK, Boumpas DT, Gordon C, Lightstone L, et al. Lupus nephritis management guidelines compared. Nephrol Dial Transplant. 2016;31:1004–13.
7. Wang Y, Tao Y, Liu Y, Zhao Y, Song C, Zhou B, et al. Rapid detection of urinary soluble intercellular adhesion molecule-1 for determination of lupus nephritis activity. Medicine (Baltimore). 2018;97(26):e11287.
8. Aziz F, Chaudhary K. Lupus nephritis: a treatment update. Curr Clin Pharmaco. 2018;13(1):4–13.
9. Khalifa A. Role of urinary biomarkers for diagnosis of lupus nephritis. J Clin Exp Pathol. 2018;84.
10. Wagaugi D, Gono T, Kawaguchi Y, Harra M, Koseki Y, Katsumata Y, et al. Frequency of class II and IV nephritis in systemic lupus erythematosus without clinical renal involvement: an analysis of predictive measures. J Rheumatol. 2012;39:79–85.
11. Monori G, Raffletta F, Ponticelli C. Remission and withdrawal of therapy in lupus nephritis. J Nephrol. 2016;29(4):559–65.
12. Loevement-Wendelmuth A, Schaeffer E, Ebert N. Two elderly patients with normal creatinine and elevated cystatin C - a case report. BMC Nephrol. 2017;18(1):87.
13. Medina Arnaudo G. Evaluation of equations using cystatin C for estimation of the glomerular filtration rate in healthy adult population of candidates for kidney donors. Rev Fac Cien Med Univ Nac Cordoba. 2017;74(3):243–50.
14. Wahheed S, Matsuksa K, Sang Y, Hoogevecn R, Ballantyne C, Coresh J, et al. Combined association of albuminuria and cystatin C-based estimated GFR with mortality, coronary heart disease, and heart failure outcomes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Kidney Dis. 2012;60:207–16.
15. Shlipak MG, Matsuhska K, Arlnov J, et al. Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med. 2013;369:932–43.
16. Osaki T, Satoh M, Tanaka F, Tamto K, Takahashi Y, Natsu T, et al. The value of a cystatin C-based estimated glomerular filtration rate for cardiovascular assessment in a general Japanese population: results from the Iwate Tohoku Medical Megabank Project. J Epidemiol. 2019. https://doi.org/10.2188/jea.JE20180274.
17. Peralta CA, Kattz R, Sarnak MJ, Jr, JFried L, De Boer I, et al. Cystatin C identifies chronic kidney disease patients at higher risk for complications. J Am Soc Nephrol. 2011;22:147–55.
18. Feng JF, Qiu L, Zhang L, Li XM, Yang YW, Zeng P, et al. Multicenter study of creatinine and/or cystatin C-based equations for estimation of glomerular filtration rates in Chinese patients with chronic kidney disease. PLoS One. 2013;8:e57240.
19. Trouw LA, Pickering MC, Blom AM. The complement system as a potential therapeutic target in rheumatoid disease. Nat Rev Rheumatol. 2017;13(9):538–47.
20. Kabeerdoss J, Gupta N, Pulukool S, Mohan H, Mahamsathapp G, Danda D. Anti-C1q antibody is associated with renal and cutaneous manifestations in Asian Indian patients with systemic lupus erythematosus. J Clin Diag Res. 2017;11:OC39–42.
21. Stegert M, Bock M, Trendelenburg M. Clinical presentation of human C1q deficiency: how much of a lupus? Mol Immunol. 2015;67:3–11.
22. Chen Z, Wang GS, Wang GH, Li XP. Anti-C1q antibody is a valuable biological marker for prediction of renal pathologic characteristics in lupus nephritis. Clin Rheumatol. 2012;31:1323–9.
23. Song D, Guo WY, Wang FM, Li YZ, Song Y, Yu F, et al. Complement alternative pathway’s activation in patients with lupus nephritis. Am J Med Sci. 2017;353:247–57.
24. Tan Y, Song D, Wu LH, Yu F, Zhao MH. Serum levels and renal deposition of C1q complement component and its antibodies reflect disease activity of lupus nephritis. BMC Nephrol. 2013;14:463.
25. Gladman DD, Urowitz MB, Essdale JM, Hahn BH, Klippep J, Lahita R, et al. Guidelines for referral and management of systemic lupus erythematosus in adults. Arthritis Rheum. 1999;42:785–96.
26. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Dalck DJ, Fitzgerald JD, et al. American College of Rheumatology guidelines for screening, case definition, treatment and management of lupus nephritis. Arthritis Care Res (Hoboken). 2012;64:797–808.
27. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35:630–40.
28. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. J Am Soc Nephrol. 2006;17:2397–44.
29. Gourden Y, Jialal I. Renal function tests. StatPears. Treasure Island: StatPears Publishing; 2019.
30. Garcia-Garcia P, Castejon R, Tutor-Ureta P, Silvestre RA, Mellor-Pita S, Jimenez-Ortiz C, et al. Serum cystatin C is associated with kidney function but not with cardiovascular risk factors or subclinical atherosclerosis in patients with systemic lupus erythematosus. Clin Rheumatol. 2017;36:2097–107.
31. Kar S, Pagliulunga S, Islam R. Cystatin C is a more reliable biomarker for determining eGFR to support drug development studies. J Clin Pharmacol. 2018;58(10):1239–47.
32. Ferguson TW, Komalda P, Tang CL, Ortiz C, et al. Serum cystatin C is associated with kidney function but not with cardiovascular risk factors or subclinical atherosclerosis in patients with systemic lupus erythematosus. Clin Rheumatol. 2017;36:2097–107.
33. Bidin MZ, Shah AM, Stanislav J, Seong CLT. Blood and urine biomarkers in chronic kidney disease: an update. Clin Chin Acta. 2019;495:239–50.
34. Wang D, Feng JF, Qiu L, Zhang L, Li XM, Yang YW, Zeng P, et al. Multicenter study of creatinine and/or cystatin C-based equations for estimation of glomerular filtration rates in Chinese patients with chronic kidney disease. PLoS One. 2013;8:e57240.
36. Chi S, Yu Y, Shi J, Zhang Y, Yang J, Yang L, et al. Antibodies against C1q are a valuable serological marker for identification of systemic lupus erythematosus patients with active lupus nephritis. Dis Markers. 2015;2015:450351.

37. Qu C, Zhang J, Zhang X, Du J, Su B, Li H. Value of combined detection of anti-nuclear antibody, anti-double-stranded DNA antibody and C3, C4 complements in the clinical diagnosis of systemic lupus erythematosus. Exp Ther Med. 2019;17:1390–4.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.