Original Paper

Detection of Renal Hypoxia in Lupus Nephritis Using Blood Oxygen Level-Dependent MR Imaging: A Multiple Correspondence Analysis

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Key Words

Lupus nephritis • Blood oxygen level dependent • Renal pathology • Multiple correspondence analysis

Abstract

Background/Aims: Nephrologists have pursued ideal, dynamic and noninvasive methods for assessing renal function and disease progression. Blood oxygen level dependent (BOLD) imaging is a useful technique for assessing renal disease. This current study was performed to explore the correlation between the hypoxia iconographical index and renal pathological features in lupus nephritis. **Methods:** Adult patients with lupus nephritis (LN) and healthy volunteers were recruited for this study. Renal biopsy tissues were characterized based on the LNISN/RPS 2003 classification. R2* values of functional magnetic resonance parameters were acquired using the BOLD technique. The data characteristics of R2* values of different pathological patterns were calculated. Multiple correspondence analysis (MCA) was performed to explore the correlation between R2* values and clinical or pathological features. **Results:** A total of twenty-three patients and eighteen healthy volunteers were examined with BOLD MRI. Renal pathological patterns included five class III (including 3 class III+V), eight class IV (including 4 class IV+V) and five class IV. The mean renal R2* values in LN patients were higher than those in healthy volunteers. R2* values in class V patients were higher than those in class IV and class III. The MCA showed that higher R2* values were associated with pathological features in class V patients. **Conclusions:** The extent of renal hypoxia in patients with LN was more serious compared with the healthy volunteers. Differentiated mechanisms of renal oxygenation are possibly involved in proliferative and non-proliferative LN patients. R2* values may be linked with multiple clinical and pathological indexes.

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Introduction

Lupus nephritis (LN) is one of the most common secondary glomerulonephritides in China. The clinical and pathological manifestations of LN are diverse and associated with different therapeutic responses and prognoses in patients with this disease. A precise description of renal histopathological lesions and an appropriate classification of LN are essential for nephrologists both as a treatment guide and for predicting prognosis in patients [1]. Although renal biopsies can provide pathological information, they are invasive and can be risky as systemic lupus erythematosus (SLE) patients tend to have a greater chance of bleeding especially those with coagulation abnormalities or renal atrophy. For these reasons, noninvasive, ideal methods for assessing renal structure and function have been pursued by nephrologists.

Functional magnetic resonance imaging (fMRI) techniques, such as blood oxygen level-dependent (BOLD) imaging, have shown considerable promise for evaluating renal function in both healthy patients and those with renal diseases [2, 3]. The BOLD MRI technique has proven useful for evaluating renal hypoxia in diverse renal diseases, including renal artery stenosis [4], ureteral obstruction [5], diabetes mellitus [6], and renal allograft rejection [7]. However, few studies of BOLD MRI and the pathology of lupus nephritis have focused on the relationship between pathological patterns of the disease and iconographical indexes.

Homeostasis of renal oxygenation is mediated by complex systems, which regulate regional blood perfusion and oxygen consumption. Multiple regulators such as nitric oxide (NO) and prostaglandins have been recognized as main regulators of renal regional perfusion and tubular transport activity [8]. Serum levels of these regulators will be changed in lupus nephritis patients, which were proven by previous investigations [9, 10]. Based on these researches, we hypothesized that renal parenchymal oxygenation would be altered in lupus nephritis patients.

To our best knowledge, only two renal BOLD-MRI studies in lupus nephritis have been published as yet. One study was accomplished by Rapacchi and his colleagues in order to identify potential quantitative MRI biomarker of the renal impairment in lupus nephritis patients [11]. In this study, Rapacchi et al investigated 10 lupus nephritis patients and 10 matched controls participants at 1.5 Tesla platform. Multi-parametric renal quantitative MRI protocol including blood oxygen level dependent (BOLD), diffusion weighted imaging (DWI), arterial spin labelling (ASL) and T1 rho MRI were tested. Unfortunately, BOLD MRI protocol parameter T2* values did not demonstrated significant differences between the LN and control groups. However, BOLD T2* values showed excellent reproducibility (intra-class correlation coefficient ranging from 0.82~0.91, within-subject coefficient of variation ranging from 2.2%~5.3%). Another study was achieved by Li and his colleagues three years ago [12]. The aim of the study was to assess the utility of BOLD and DWI MRI in discrimination renal involvement and pathological changes in lupus nephritis patients. A total of 65 LN patients and 16 healthy volunteers underwent BOLD and DWI MRI inspection. R2* and apparent diffusion coefficient (ADC) values were measured as quantitative biomarker. This investigation showed that the mean R2* values of the renal cortex and medulla in LN patients were all significantly lower than that in volunteers. Moreover, correlation analysis indicated that negative correlation between R2* values of the renal medulla and proteinuria ($r=-0.244$, $p<0.05$) as well as the degree of tubulointerstitial lesion ($r=-0.242$, $p<0.05$). These two previous studies implied that BOLD MRI might be used as a non-invasive technique in probing renal pathophysiological changes.

The purpose of this study was to explore the feasibility of using BOLD MRI for the assessment of renal hypoxia in patients with LN. The correlation between iconographical features and renal pathological features was also investigated.

Patients and Methods

Participants

This single-centre observational study was approved by the *Ethics Committee of Tianjin Medical University General Hospital* and informed consent was obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki. Twenty-three patients and eighteen healthy volunteers were studied from January 2015 to June 2016. All of healthy volunteers who were included in our study were recruited from different division of our hospital. Detail information of healthy volunteers were listed below. Ten volunteers came from physical examination centre. None of them were confirmed with explicit diseases. Residual participants came from division of general surgery (4 participants), division of endocrinology (2 participants), division of ophthalmology (1 participant) and division of dermatology (1 participant), respectively. No kidney disease or renal injury evidence were found among these participants. The male/female ratio were 0.125 (2/16) and 0.095 (2/21) in healthy volunteers group and lupus nephritis patients group, respectively. Gender ratio between two groups were comparative.

Patients who fulfilled the 2012 International Collaborating Clinics classification criteria for systemic lupus erythematosus were included [13]. Disease activity was assessed using the SLEDAI (SLE Disease Activity Index) [14]. The serum creatinine value was used to calculate the current estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [15].

Renal histopathology

Renal biopsy specimens were fixed in 4.5% buffered formaldehyde for light microscopy. Consecutive serial 2-um thick sections were used for histological staining. Stains used included hematoxylin and eosin, periodic acid-Schiff, silver mehtenamine, and Masson's trichrome. Renal histopathological data from 18 patients with lupus nephritis, as proven by renal biopsy, were diagnosed according to the ISN/RPS 2003 classification [16] by two experienced pathologists. The pathologists classified and scored the biopsies separately, and were masked to patients' data and scores from other observers. Patients with renal biopsy specimens that contained less than 10 glomeruli were excluded. For this study, cases of III+V were classified as class III and cases of IV+V were classified as class IV. Pathological parameters, such as activity indices and chronicity indices were assessed by renal pathologists using a modified system involving semi-quantitative scoring of specific biopsy features, as previously reported [17].

Magnetic Resonance Imaging Techniques

Magnetic resonance imaging was performed using a 3.0-T Imager (GE Discovery 750 3.0T, General Electric, USA). The scanner had a maximum gradient strength of 50mT/m and a slew rate of 200mT/m/sec. A Torsopa eight-channel body coil was used. Images were acquired for morphologic evaluation using a T_1 weighted fat-suppressed sequence. Images were acquired with a T_1 INPHASE+FAT sequence. The field of view (FOV) was 380×380mm, section thickness 7.0mm, section width 1.0mm, repetition time (TR)/echo time (TE) 180/2.1. The BOLD-MRI was performed using a T2* spoiled gradient recalled echo (SPGR) sequence. The field of view was 380×380 mm, matrix 192×160, TR100ms, and TE 2.4ms, 6.2ms, 10.0ms, 13.8ms, 17.6ms, 21.4ms, 25.2ms, and 29.0ms. Echo number was 8.00, flip angle 35°, bandwidth 19.23kHz, section thickness 7.0mm, section width 1.0, section number 8 and scan time 25sec.

Image Analysis

R2* maps were constructed on an ADW 4.5 Workstation using the FUNCTOOL program. Three consecutive renal coronal anatomical planes were selected in each kidney. The region of interest (ROI) was set as a 1.0cm (width) by 3.0cm (height) rectangle. Three ROIs were placed on each R2* renal coronal plane. The ROIs were placed in a direction from the renal cortex to the renal medulla (see Figure 1). Three consecutive renal planes were selected from each side of the kidney so that each participant provided eighteen ROIs. R2* values of each voxel from selected ROIs were obtained using MATLAB 7.10 (MathWorks Inc, Natick, MA, USA). R2* values from each ROI were calculated indexes and included the arithmetic mean, geometric mean, harmonic mean, range, variance, quartile, skewness, and kurtosis.

Fig. 1. The shape of the ROI was set as a rectangle with a 1.0 cm width and 3.0 cm height. Three isolated ROIs were placed on one renal coronal R2* colorful plane along the direction from the cortex to medulla.

Statistical Analyses

Multivariable analysis. Multivariate analysis of variance (MANOVA) models were used to compare the differences in indexes of R2* values between groups. The MANOVA provides an omnibus test of statistical significance. If this omnibus test was statistically significant for the key independent variable, then post-hoc comparisons were run to identify which group was statistically different. Four MANOVA output statistics (Wilk's Lambda, Lawley-Hotelling trace, Pillai's trace and Roy's largest root) were used to judge whether differences between groups existed. *Bonferroni* Method was used in post-hoc test.

Multiple Correspondence Analysis. A multiple correspondence analysis (MCA) was used to analyse the relationship between R2* values and clinical or pathological parameters. MCA is a descriptive/exploratory technique designed to analyse simple two-way and multi-way tables containing some measures of correspondence between the rows and columns. The object of MCA is to visualize the relationship of categorical parameter in a graphical manner. Details of categorized information of clinical and pathological parameters are illustrated in Table 1.

All analyses were carried out using the IBM®SPSS®Statistics software (version 22.0.0.0 IBM Corporation; Armonk, NY, USA). Statistical significance was determined as $P < 0.05$.

Results

Demographic information of participants

Twenty-three patients were included in the lupus nephritis group and underwent a

Table 1. Value assignment of clinical and pathological indexes in lupus nephritis

Indexes description	Scale category	Value assignment
Age	less than 20 years	1
	20 to 40 years	2
	40 to 60 years	3
	greater than 60 years	4
Proteinuria	less than 1.0g/24h	1
	1.0 to 3.5g/24h	2
	greater than 3.5g/24h	3
eGFR	greater than 90ml/min	1
	90 to 60ml/min	2
	less than 60ml/min	3
Glomerular cell proliferation	none	1
	less than 25%	2
	25% to 50%	3
	greater than 50%	4
Leucocyte exudation	none	1
	mild	2
	moderate	3
	extensive	4
Karyorrhexis and fibrinoid necrosis	none	1
	less than 25%	2
	25% to 50%	3
	greater than 50%	4
Cellular crescents	none	1
	less than 25%	2
	25% to 50%	3
	greater than 50%	4
Hyaline deposits	none	1
	mild	2
	moderate	3
	extensive	4
Interstitial inflammation	none	1
	mild	2
	moderate	3
	extensive	4
Glomerular sclerosis	none	1
	less than 25%	2
	25% to 50%	3
	greater than 50%	4
Fibrous crescents	none	1
	less than 25%	2
	25% to 50%	3
	greater than 50%	4
Tubular atrophy	none	1
	mild	2
	moderate	3
	extensive	4
Interstitial fibrosis	none	1
	mild	2
	moderate	3
	extensive	4
Thicken basement membranes	no	1
	yes	2
R2* value	less than Q1	1
	Q1~Q2	2
	Q2~Q3	3
	greater than Q3	4

BOLD MRI examination. The average age of patients was 35.52 ± 13.34 years (ranging from 15 to 62 years). The mean urine protein was 3.71 ± 2.81 g/24h (ranging from 0.34 to 9.47 g/24h). Mean eGFR was 102.74 ± 24.79 ml/min/1.73m² (ranging from 39 to 138 ml/min/1.73m²). A total of eighteen patients underwent renal biopsy. The renal pathological patterns were listed, according to the 2003 ISN/RPS classification system for lupus nephritis, as follows: III-(A/C) pattern (2 cases), III-(A/C)+V pattern (3 cases), IV-G(A/C) pattern (3 cases), IV-S(A) pattern (1 case), IV-G(A/C)+V pattern (4 cases) and V pattern (5 cases). Eighteen participants were recruited and underwent BOLD MRI examinations in the healthy volunteer group. The average age of healthy volunteers

was 36.78 ± 13.66 years (ranging from 16 to 68 years). Mean eGFR of healthy volunteers was 112.28 ± 18.84 ml/min/1.73m² (ranging from 84 to 146 ml/min/1.73m²). There was no statistically significant difference in age or eGFR between the LN patients and healthy volunteers ($P=0.769$ and $P=0.184$, respectively).

Comparison of renal R2 value between lupus nephritis patients and healthy volunteers*

Mean renal R2* values, including arithmetic mean, geometric mean, harmonic mean and median, were higher in LN patients than in healthy volunteers ($P<0.001$ for all parameters). The renal R2* values of the two discreteness parameters, range and quartile, were also higher in LN patients than in healthy volunteers ($P=0.047$ and $P=0.007$, respectively). Details are provided in Table 2 and Figure 2.

Table 2. Comparison of R2* values functions between groups with LN and healthy volunteers

R2* values functions	Lupus nephritis (N=23)	Healthy volunteers (N=18)	P values
Arithmetic mean	21.368±3.690	20.054±4.039	<0.001
Geometric mean	21.291±3.676	19.985±4.028	<0.001
Harmonic mean	21.215±3.665	19.918±4.016	<0.001
Median	21.344±3.716	19.994±4.070	<0.001
Range	9.296±4.356	8.554±5.773	0.047
Quartile	2.421±1.206	2.182±1.160	0.007
Variance	3.462±4.782	3.066±4.094	0.235
Skewness	-0.007±0.670	0.063±0.949	0.238
Kurtosis	3.187±1.874	3.650±4.321	0.051

Fig. 2. Comparison of R2* value data characteristics between LN and healthy volunteers.

Table 3. Comparison of R2* value between different lupus nephritis group and healthy volunteers group

R2* values functions	III type (N=5)	IV type (N=8)	V type (N=5)	Control(N=18)	P value
Arithmetic mean	19.576±2.854	20.284±3.857	24.291±2.482	20.054±4.086	<0.001
Geometric mean	19.488±2.819	20.228±3.855	24.201±2.481	19.985±4.028	<0.001
Harmonic mean	19.403±2.797	20.172±3.853	24.109±2.479	19.918±4.016	<0.001
Median	19.529±2.823	20.298±3.880	24.278±2.679	19.994±4.070	<0.001
Range	10.126±6.143	8.407±3.520	9.069±2.709	8.554±5.773	0.046
Quartile	2.301±0.942	1.985±0.866	3.037±1.388	2.182±1.160	<0.001
Variance	3.979±8.662	2.304±1.701	4.332±2.704	3.066±4.094	0.003
Skewness	0.051±0.785	-0.020±0.619	-0.229±0.695	0.063±0.949	0.052
Kurtosis	3.505±2.276	3.250±1.747	2.823±1.537	3.650±4.321	0.230

Fig. 3. Comparison of R2* value data characteristics among different renal pathological patterns in LN patients.

Comparison of renal R2* values in patients with different pathological patterns of lupus nephritis

We compared the 9 parameters of R2* values among the three different pathological types of lupus nephritis patients and healthy volunteers. Except for two parameters (skewness and kurtosis), the majority of parameters (arithmetic mean, geometric mean, harmonic mean, median, range, quartile and variance) showed statistics significance between four groups. Details are provided in Table 3. To analyze the difference of renal oxygenation between three pathological types and healthy control, we compared the means of R2* values by using post-hoc analysis. We found that arithmetic means of R2* values in V type patients (24.291±2.482) were higher than III type (19.576±2.854, $p < 0.001$), IV type (20.284±3.857, $p < 0.001$) and healthy control (20.054±4.086, $p < 0.001$). No statistic difference of the R2* values were found among the III type, IV type and control groups. Similar results were found in other means of R2* values. Details are provided in Figure 3. When focusing on the comparison arithmetic means of R2* values in class III and class IV LN, with and without class V, further analysis showed that patients with class V had higher R2* values (20.797±3.824) than those without class V (19.457±3.210, $p = 0.003$). Similar results were found in other means of R2* values. Details are provided in Table 4.

Multiple correspondence analysis

The MCA model of R2* values and pathological characteristics explained 71.48% variability between the two dimensions (44.82% for the first and 24.66% for the second). The complex interrelationships among the pathological indexes and R2* values were primarily explored. Using MCA, the correlation between the multiple indexes and renal R2* values could be visualized. As shown in Figure 4, higher R2* values and several graded pathological characteristics are located in the lower right quadrant. In

Table 4. Comparison of R2* values between type III type and type IV LN with and without type V pathological patterns

R2* values functions	Without V type (N=6)	With V type (N=7)	P value
Arithmetic mean	19.457±3.210	20.797±3.824	0.003
Geometric mean	19.396±3.205	20.720±3.811	0.003
Harmonic mean	19.336±3.200	20.646±3.805	0.003
Median	19.477±2.262	20.752±3.801	0.005
Range	8.366±2.745	9.895±6.342	0.010
Quartile	2.081±0.882	2.121±0.938	0.730
Variance	2.402±1.798	3.569±7.948	0.089
Skewness	-0.042±0.571	0.068±0.805	0.207
Kurtosis	3.068±1.421	3.704±2.450	0.010

Fig. 4. Multiple correspondence analysis of 18 LN patients referring to R2* values and renal pathological parameters.

this region of the plot, the graded characteristics include thickened basement membranes, moderate hyaline deposits, no interstitial inflammation, no interstitial fibrosis and no tubular atrophy. On the contrary, lower R2* values and several other graded pathological charac-

teristics are grouped in the upper left quadrant. These classified characteristics are composed of extensive glomerular cell proliferation, moderate cellular crescents, moderate karyorrhexis and fibrinoid necrosis, moderate leucocyte exudation and mild glomerular sclerosis and interstitial inflammation. By using MCA, the different $R2^*$ values may be linked with some special pathological conditions. Details are provided in Figure 4. Furthermore, the relationship between

$R2^*$ values and clinical indexes such as patient age, proteinuria level and eGFR were analysed using MCA. Lower $R2^*$ values and younger ages are located in the upper left quadrant. In contrast, higher $R2^*$ values and older ages are distributed in the lower right quadrant. Details are provided in Figure 5.

Discussion

In the current study, higher renal $R2^*$ values were detected in LN patients. This phenomenon implies that there is a lower level of tissue oxygenation in the renal parenchyma of LN patients. In cellular level, several activated-mechanism are believed involving in renal hypoxia in lupus nephritis. For example, Deng et al. found that hypoxia-inducible factors (HIF)-1 α was highly expressed in lupus nephritis patients and lupus mice and could induce the proliferation of MCs [18]. HIF is consisting of α and β heterodimers which is key regulators of gene expression in response to declining pO₂ [19]. In normal physiological conditions, HIF is decomposed by oxygen-sensitive HIF-prolyl-hydroxylases. When HIF-prolyl-hydroxylases were inhibited under pathologic conditions, HIF rapidly accumulated and HIF-mediated genes are subsequently unregulated in affected renal regions, which act as ameliorate hypoxia, counteract oxidative stress and improve cell survival [19]. Although the HIF-mechanism is a renal protective mechanism, increased HIF may result in renal cellular proliferation and increment of oxygenation consumption [20]. If renal hypoxia cannot be resolved, HIF-mediated mechanism will be activated continuously and lead to vicious cycle. Moreover, excess formation of reactive oxygen species (ROS) is another mechanism of renal hypoxia. ROS may not only enhance tubular transport activity and mitochondrial respiration,

Fig. 5. Multiple correspondence analysis of 18 LN patients referring to $R2^*$ values and clinical parameters.

but also reinforce these effect by feed-forward loop [21, 22]. Enhanced reparative processes, such as poly ADP-ribose polymerase-mediated DNA repair or autophagy, may intensify oxygen consumption [8].

Although the renal $R2^*$ values could be detected and visualized using BOLD MRI, this technique failed to differentiate changes in oxygen delivery by renal blood flow, oxygen consumption, cellular and tissue oxygen utilization and arteriovenous diffusion of oxygen [23]. However, higher renal $R2^*$ values may reflect lower oxygenation in the kidney. According to characterization of the pathological changes of lupus nephritis, we possibly speculated mechanism of renal hypoxia. First, proliferation of mesangial cells and matrix can be seen in a majority of lupus nephritis cases and moderate to extensive proliferation can compress glomerular capillary vessels. In addition, proliferation of endothelial cells may cause stenosis of capillary vessels resulting in possible restriction of renal blood flow, further compromising tissue oxygenation [24]. Secondly, almost half of LN patients have extensive proteinuria (exceeded 3.5g/d). Renal tubular triggered reabsorption compensatory mechanism because of excess protein filtration from glomerular. Enhanced protein reabsorption then leads to increased oxygen consumption [25]. This study also found that the range and quartile of $R2^*$ values in LN patients were higher than those in healthy volunteers indicating amplification in renal $R2^*$ value dispersion. Although the distribution feature of $R2^*$ values were not evaluated in the present study, it is possible that irregular renal tissue oxygenation distribution may be attribute to renal medulla, which is prone to be impacted by hypoxia. Several possible mechanisms may be involved in this pathophysiological role. The first is the discrepancy of blood flow distribution between the cortex and medulla. Most blood is directed towards the renal cortex in order to optimize glomerular filtration and reabsorption. However, about 40% and 10% of blood influx into the cortex flows into the outer and inner medulla, respectively. Relatively low medullary blood flow favors the maintenance of the cortical-medullary solute gradient and facilitates concentration of urine [26]. Physiological differences result in a tendency for the medulla to be more anoxic. The second is the sophisticated relationship between the tissue partial pressure of oxygen (PaO_2) and the oxyhemoglobin saturation dissociation curve. When the PaO_2 is above 60 mmHg, the oxyhemoglobin saturation dissociation curve is relatively flat. Whereas, there is a steep gradient in the oxyhemoglobin dissociation curve when the PaO_2 is less than 26.6 mmHg. Previous research has shown that PaO_2 in the majority of cortexes is generally higher (above 60 mmHg), whereas medullary PaO_2 rarely exceeds 26.6 mmHg [27]. Thus, smaller changes in medullary PaO_2 may result in greater changes in deoxyhemoglobin concentration, as indicated by $R2^*$ values measured using BOLD MRI.

Interestingly, there was contradictory results of renal $R2^*$ values in LN group between our present study and Li et al investigation. We speculated that two possible reason resulted in this difference. The first possible reason was that different degree of tubulointerstitial lesion might result in different level of $R2^*$ values. In Li et al. investigation, negative correlation between $R2^*$ values of renal medulla and the degree of tubulointersitital lesions was found. In other words, the more serious tubulointerstitial lesions lupus nephritis patients have, the lower $R2^*$ values will be detected. The similar result were also found in our present study. Multiple correspondence analysis figure indicated relative lower $R2^*$ values and moderate tubular atrophy and interstitial inflammation distributed in same area. Based on these findings, we surmised that both higher and lower $R2^*$ values would be possibly detected by BOLD MRI in lupus nephritis patients. The level of $R2^*$ values dependent on degree of tubulointerstitial lesions. The second possible reason was different ROI technique. In Li et al study, conventional small ROIs technique was used to acquire measurement samples. However, higher fluctuations of measured $R2^*$ values were usually found because of renal depth heterogeneity, particularly in the medulla [28]. By contrast, we applied a new ROIs technique called "rectangle ROIs". The selected ROIs area was composed of different depth of renal cortex and medulla. Final measured $R2^*$ values were the means of all ROIs pixels $R2^*$ values. We also surmised that different selective ROIs technique might result in different

measured $R2^*$ values. For example, in more recent study, Piskunowicz compared with two ROI measurement technique. One was the conventional ROI technique, the other was a novel ROI technique called concentric objects technique or onion peel technique. They found that $R2^*$ values (both cortical $R2^*$ and medullary $R2^*$) in non-CKD patients were higher than those in CKD patients by using conventional ROI technique. However, the opposite result were found by using novel ROI technique [29].

The present study also examined discrepancies in $R2^*$ values among three pathological patterns of lupus nephritis. Relatively higher $R2^*$ values were found in class V lupus nephritis as illustrated by MANOVA test analyses. Similar results were found in sub-group analyses of class III and class IV. Patients with pattern V may have higher $R2^*$ values compared with the sub-group of patients without pattern V pathological features. Previous experimental and clinical research has shown different immune pathogeneses among proliferative (class III or class IV) and nonproliferative (class V) lupus nephritis. The mechanism of renal injury in membranous LN is generally believed to be related to epithelial cell and glomerular basement membrane immune complex deposition [30]. Although the cause of more extensive hypoxia in class V LN remains elusive, it is possible that thickened capillary basement membrane compromises oxygen diffusion. Li et al. evaluated renal pathophysiology using diffusion weighed imaging (DWI) and BOLD. They compared the $R2^*$ and ADC values of kidneys in two groups with LN. Patients in the group with class V had higher $R2^*$ and ADC values. The latter parameter is a quantitative index, which stands for capacity of water molecular diffusion [12].

In order to explore the correlation between $R2^*$ values and multiple clinical and pathological factors, a multivariate technique, MCA, was used to visualize the relationship within a set of categorical variables. In the current study, the higher $R2^*$ was located in lower right quadrant of the MCA plot. Thickened basement membranes, moderate hyaline deposits, lack of tubular atrophy and interstitial fibrosis were also distributed in the same area. These pathological features are categorized as class V LN. In other words, class V LN may be associated with higher $R2^*$ values. This result was similar to findings from the MANOVA analysis. Based on these results, it appears that the mechanism of renal hypoxia in class V LN can be distinguished from that in pure proliferative LN, such as class III and class IV. MCA plot of the relationship between $R2^*$ values and pathological features provided other puzzling findings. For example, the proportion of $R2^*$ values less than $Q1$ were located in left upper quadrant and a proportion of mildly increased $R2^*$ values ($R2^*$ values between $Q1$ to $Q2$ and $Q2$ to $Q3$) were clustered in left lower quadrant. Correspondingly, glomerular active lesions, such as extensive glomerular cell proliferation, karyorrhexis, fibrinoid necrosis, leucocyte exudation and cellular crescents and tubulointerstitial active lesions, such as moderate tubular atrophy and interstitial inflammation, were scattered in the same area. According to the principle of MCA plot exploration, parameters located in same areas appear to be linked with each other. Two possible mechanisms may explain this phenomenon. First, a majority of glomeruli were distributed in the renal cortex, in which plenty of blood flow was inflexed. Although extensive glomerular lesions could be seen, adequate oxygen supplementation would reduce or prevent tissue hypoxia. Secondly, there was vast ionic transport pump scatter in both the proximal and distal convoluted tubules. Under normal physiological conditions, these ionic pumps consume a massive amount of oxygen. Tubule injury could damage these pumps resulting in a reduction in oxygen consumption. These possible mechanisms may have caused the decrease in $R2^*$ value.

The current study also investigated the correlation between $R2^*$ values and clinical parameters using MCA, including patient age, magnitude of proteinuria, and eGFR. In the MCA plot, higher $R2^*$ values were linked with relative old age. This is similar to results of a previous study in healthy populations [31]. Renal vasodilators may be involved in renal oxygenation in the aging kidney. Long et al. found that nitric oxide levels decreased in the serum and renal cortex of older animals. There is a decline in renal blood flow and renal vascular resistance is reinforced when nitric oxide decreases [32, 33]. Animal models

proved that prostaglandin E_2 (PGE₂) could reduce renal oxygen consumption by inhibiting activity of Na and K-ATPase [34]. The PGE₂ level was approximately twice as high in younger subjects compared to older subjects [35]. An interesting result provided by MCA plot was that lower R2* values tended to correlate with higher proteinuria levels. A similar finding was also reported in the Li et al. study. In their study, the R2* values of the renal medulla were negatively correlated with 24 hour proteinuria [12]. A possible explanation was that extensive proteinuria damaged the tubular ionic pump resulting in a decline in oxygen consumption. Finally, there was no obvious correlation between R2* values and eGFR, as indicated by the MCA plot. Similar findings were reported in a large sample clinical study. Michaely et al. investigated the relationship between R2* values and eGFR in 342 patients with chronic kidney disease. Neither medullary nor cortical R2* values showed significant alterations in patients with various stages of kidney disease or renal function. Although the precise mechanism has not been clarified, some renal pathophysiologists believe that the preglomerular arteriovenous shunting mechanism may maintain intrarenal oxygenation homeostasis when renal blood flow changes [4].

The conventional renal BOLD MRI measurement method was used to quantify R2* values in the renal cortex and medulla. However, it is difficult to distinguish renal cortex and medulla in MR imaging, especially for serious renal disorders. Another issue was the location of the ROIs in the renal MR imaging. It is well known that R2* levels vary gradually from the cortex to the medulla and reach a very hypoxic zone in the deepest sections of the medullary pyramids. No explicit renal cortical-medullary boundary has been found in previous studies. For example, Piskunowicz et al. developed a novel ROI technique to analyse BOLD MRI images. This technique was called the concentric object or 'onion peel' technique. In this technique, the kidney parenchyma was divided into six layers of equal thickness, from the outermost cortex to the deepest medulla. Using this ROI technique, R2* values of six layers were surveyed in patients with chronic kidney disease. The results showed that the R2* values of the six layers were 24.9, 23.2, 27.8, 31.3, 33.6, and 33.4 respectively [29]. In order to prevent R2* value variation due to discrepancy in ROI location, a 'rectangle' ROI technique was used for the present study.

There were several limitations in the current study. First, there were too few participants to gain a robust result. Only 23 LN patients and 18 healthy volunteers underwent BOLD MRI. The majority of participants ranged in age from 20 to 40 years, with only a few aged 16 years or below or above 65. Moreover, other pathological classes and subclasses, such as class II, III-C, III-A, IV-G(C) or IV-S(A) were not included in this study. We were not sure of the precise deviation in sampling errors in our current results. Secondly, intra-observer and inter-observer variations in R2* values were not tested using our 'rectangle' ROI technique. Also, R2* stabilization between our 'rectangle' ROI technique and other ROI techniques, such as conventional small or large ROIs, were not compared. Thirdly, the MCA technique is a statistical exploration technique and is not a statistical test, which gives robust results. The correlation between R2* values and clinical or pathological parameters, resulting from MCA plots, needs to be proven with more pathophysiological evidence.

Conclusion

In conclusion, the extent of renal hypoxia in patients with LN was more serious than in the healthy population. Differentiated mechanisms of renal oxygenation were possibly involved in proliferative and non-proliferative LN patients. R2* values may be linked with multiple clinical and pathological indexes, however, the relationship is still perplexing and sophisticated.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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