Evaluation of non-contrast MRI biomarkers in lupus nephritis

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Abstract

Objective

To investigate the association of novel non-contrast MRI biomarkers with standard measurements of renal function and renal disease activity in lupus.

Methods

A pilot study of lupus nephritis (LN) and lupus non-nephritis (LNN) patients, and healthy volunteers (HV), was undertaken. Multi-modal renal MRI was performed including sequences for arterial spin labelling (ASL) measuring blood flow, diffusion tensor imaging (DTI), measuring microstructural disruption, and effective transverse relaxation time (T_2) which is a biomarker of micro-haemorrhage. MRI measurements were compared with urinary protein creatinine ratio (uPCR) and estimated glomerular filtration rate (eGFR) measurements in the whole study population, then differences in imaging measurements between the groups were explored.*

Results

21 patients (6 LN, 8 LNN and 7 HV) completed the study, although ASL data were not available in 4 subjects. In the whole cohort, eGFR correlated significantly with the apparent diffusion coefficient measurement from DTI in the medulla ($r=0.47$, $p=0.03$). uPCR correlated strongly with the fractional anisotropy (FA) DTI measurement in the cortex and moderately with T_2 measurements (rho=-0.71, $p<0.001$ and rho=-0.53, $p=0.013$, respectively). Delayed blood flow to the medulla was found in LN subjects and there was a trend towards lower FA values in the cortex, suggesting micro-structural disruption ($p=0.04$ and $p=0.07$, respectively).*

Conclusion

This preliminary study demonstrates that non-contrast renal MRI biomarkers are associated with standard measures of disease activity in lupus. The potential utility of these non-invasive biomarkers warrants further investigation, as there is an unmet need for reliable biomarkers of disease activity in lupus nephritis.

Key words

systemic lupus erythematosus, lupus nephritis, biomarkers, magnetic resonance imaging

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Background

Lupus nephritis (LN) remains a significant cause of mortality (1). Current standard assessments of disease activity include urinary protein quantification and estimated glomerular filtration rate (eGFR) using plasma creatinine (2). However these parameters are influenced by other factors, such as renal damage, and there is a need for non-invasive biomarkers that can identify active disease (3).

Advances in magnetic resonance imaging (MRI) provide better tools for the evaluation of renal tissue structure and function. The most commonly investigated MRI approaches use gadolinium-based contrast agents (GBCAs), but they are now contra-indicated in severe renal disease. Therefore, the need for alternative non-GBCA techniques has been recognised.

Arterial spin labelling (ASL) is an MRI technique that can be used to measure regional renal blood flow and perfusion. A radio-frequency magnetic labelling pulse is applied across a portion of blood at the origin of the renal arteries. With the altered spin polarisation, the blood acts as an endogenous contrast agent. Flow is measured by subtracting images with altered *versus* normal spin polarisation. ASL measurements correlate with eGFR and discriminate between healthy and diseased kidneys (4, 5). We aimed to evaluate three ASL biomarkers in SLE, namely ASL-derived blood flow, labelling bolus arrival time (BAT) and labelling bolus end time (BET).

Additionally, we aimed to evaluate three further MRI biomarkers, each a proxy of microstructural change. Diffusion tensor imaging (DTI) measures the magnitude and orientation of water diffusion in a tissue, showing whether tissues have relatively ordered or chaotic structure. The apparent diffusion coefficient (ADC) is a measure of the diffusion path-length of tissue water molecules, and is increased when diffusion is relatively unimpeded as in oedema or necrosis. Anisotropy describes the dominate direction of diffusion. Highly structured tissues, such as renal tubules, exhibit high fractional anisotropy (FA). Damaged or inflamed tissue becomes disorganised leading to lower FA. DTI has been used to detect early changes in diabetic nephropathy independent of eGFR (6, 7).

T_2*-weighted MRI exploits the paramagnetic properties of haem. T_2* is an MRI relaxation time – a tissue property within a magnetic field that influences MRI signal intensity. It is inversely dependant on paramagnetic iron content (*e.g.* deoxyhaemoglobin) and therefore can act as a proxy for microhaemorrhage or hypoxia. T_2* has been employed in studies to investigate the effects of anti-hypertensives on renal medullary tissue oxygenation (8, 9).

The aims of the current study were to evaluate these quantitative MRI measurements in SLE, to assess the association of imaging measurements with standard disease activity measures and to explore differences between SLE patients with and without nephritis.

Methods

Study setting and patient population

This was a pilot study of SLE patients and age- and sex-matched controls. Ethical approval was granted from the regional ethics committee. Written informed consent was obtained from participants.

Patients aged 30 to 70 with ≥4 ACR revised criteria for SLE (10) were recruited from rheumatology departments in the North of England. LN patients had a biopsy showing International Society of Nephrology/Renal Pathology Society (ISN/RPS) class III-V disease, within the prior 36 months. Healthy volunteers (HV) were recruited via local advertisement. Exclusion criteria included eGFR<30mL/min/1.73m^3, other renal diseases, hypertension, anti-phospholipid syndrome, recent change in anti-proteinuric agent, contraindication to MRI.

Data collection

Following a screening visit bloods and urine were acquired and eGFR (11) and urinary protein: creatinine ratio (uPCR) were measured. Clinical assessment was undertaken including disease activity (SLEDAI-2000)(12) and damage (ACR/SLICC Damage Index) (13), then renal MRI was performed.

Table I. Cohort characteristics (median (IQR) or frequency (%) where *).

Characteristics	Lupus nephritis (n=6)	Lupus non-nephritis (n=8)	Healthy controls (n=7)	*p*-value
Age, years	32.5 (29,37)	34.5 (23,44)	34 (30,36)	0.92
Female*	4 (66.67)	8 (100)	6 (85.71)	0.14
eGFR(MDRD) [mL/min/1.73m^2]	97 (87,115)	85 (75,105)	82 (74,89)	0.27
Urinary PCR (mg/mmol)	21 (6,76)	11 (8,16)	6 (5,12)	0.21
Proteinuria (urinary PCR>20)*	**3 (50%)**	**1 (12.5%)**	**0**	**0.005**
SLEDAI-2000	**7 (4,10)**	**0 (0,1.5)**	**-**	**0.02**
Ds-DNA anti-body titre	25.5 (6.95, 166)	1 (0.9,23)	-	0.29
Positive ds-DNA*	4 (66.67)	2 (25.0)		0.11
C3	0.73 (0.53, 0.98)	0.90 (0.81, 1.01)	-	0.186
C4	0.09 (0.06, 0.14)	0.19 (0.18, 0.20)	-	0.198
Low complement*	**1 (12.50)**	**5 (83.3)**		**0.018**
SDI	0 (0, 1)	0 (0, 0.5)	-	0.75
Disease duration (years)	5 (2, 11)	7.5 (2.5, 16.5)	-	0.65
Imaging parameters				
ASL				
Flow (cortex)[ml 100 ml^{-1} min^{-1}]	250.96 (106.40, 275.64)	248.50 (214.43, 266.34)	237.29 (148.82, 303.57)	0.96
Flow (medulla)[ml 100 ml^{-1} min^{-1}]	212 (97.40, 396.24)	171.69 (155.88, 240.12)	188.77 (171.86, 212.98)	0.94
BAT (cortex)[s]	0.359 (0.342, 0.524)	0.316 (0.252, 0.365)	0.265 (0.227, 0.354)	0.16
BAT (medulla)[s]	**0.430 (0.298,0.634)**	**0.234 (0.226, 0.261)**	**0.247 (0.178, 0.269)**	**0.04**
BET (cortex)[s]	1.54 (1.511, 2.239)	2.47 (1.256, 2.611)	2.106 (1.590, 2.136)	0.35
BET (medulla) [s]	0.900 (0.882, 2.046)	1.197 (0.911, 1.250)	0.999 (0.679, 1.357)	0.72
DTI				
FA (cortex)[range 0-1]	0.176 (0.147, 0.184)	0.185 (0.178, 0.196)	0.192 (0.189, 0.206)	0.07
FA (medulla)[range 0-1]	0.363 (0.61, 0.391)	0.363 (0.341, 0.373)	0.368 (0.353, 0.371)	0.81
ADC (cortex)[10^{-3} mm^2 s^{-1}]	2.598 (2.343, 2.615)	2.465 (2.347, 2.6435)	2.656 (2.558, 2.695)	0.45
ADC (medulla)[10^{-3} mm^2 s^{-1}]	2.518 (2.350, 2.688)	2.376 (2.258, 2.629)	2.537 (2.362, 2.596)	0.89
T^{2*} (whole kidney)[s]	0.061 (0.059, 0.064)	0.0621 (0.059, 0.068)	0.0629 (0.059, 0.066)	0.58

Imaging methods

Scans were performed on a 1.5 T Philips Achieva scanner (Philips Healthcare, The Netherlands). Detailed imaging protocols are described in the supplemental data. Briefly, T_1-weighted, single-slice ASL, 3-slice DTI and single-slice T_2* sequences were acquired including both kidneys, avoiding renal pelvis and major vessels. Images were analysed using in-house software. Kidney segmentation was performed manually for all sequences and segmentation of cortex and medulla for the ASL and DTI sequences (not possible for T_2*). For each kidney, median values were calculated for each parameter in cortex and medulla (where applicable), to reduce bias from individual voxel outliers. For each subject, an average of left and right kidney median values was taken to give a single value for each imaging biomarker.

Statistical analysis

Correlations between imaging measurements and eGFR and uPCR were tested using Spearman's rank test. Differences between the groups were tested using Kruskal Wallis test.

Results

Twenty-six participants were recruited to the study between 2011 and 2012. Five SLE patients failed screening (1=ineligible, 2=ill health, 2=consent withdrawn). 6 LN patients, 8 LNN patients and 7 HV completed the study.

Cohort characteristics

Baseline characteristics are described in Table I. Although there was no significant difference in uPCR values between groups, the number of patients with uPCR>20 was significantly higher in the LN group [3/6 (50%) *vs*. 1/8 (12.5%) *vs*. 0/7 (0%), p=0.005]. The median time from biopsy to MRI was 17.5 (7,100) weeks. On biopsy, four patients had class IV and two had class V nephritis. All had active glomerular disease, three had active tubulo-interstitial disease. One case had chronic glomerular changes, three had chronic tubular changes. None had thrombotic disease or vasculitis (detailed findings in supplemental data). LN patients had a higher SLEDAI-2000 score (median [IQR] 7 [4, 10] *vs*. 0 [0, 1.5] in LN and LNN, respectively, p=0.02) and were more likely to have low complement levels (83.33% *vs*. 12.5%in LN and LNN, respectively, p=0.018). There was no significant difference in anti-double stranded DNA levels between the lupus groups.

Imaging results

A technical failure meant that ASL data were available for 3/6 LN, 7/8 LNN and 7/7 HV participants. Otherwise, acceptable imaging quality was achieved in all participants. Figure 1 shows an image from an LN patient for each MRI biomarker.

Correlations of imaging measurements with eGFR and uPCR can be seen in Table II. There was a trend towards correlation between medullary flow and eGFR (rho[r] = 0.46, p=0.064). Medullary ADC correlated with eGFR (r=0.47, p=0.03) and there was a strong inverse correlation between cortical FA and uPCR (r=-0.723, p<0.01). T_2*

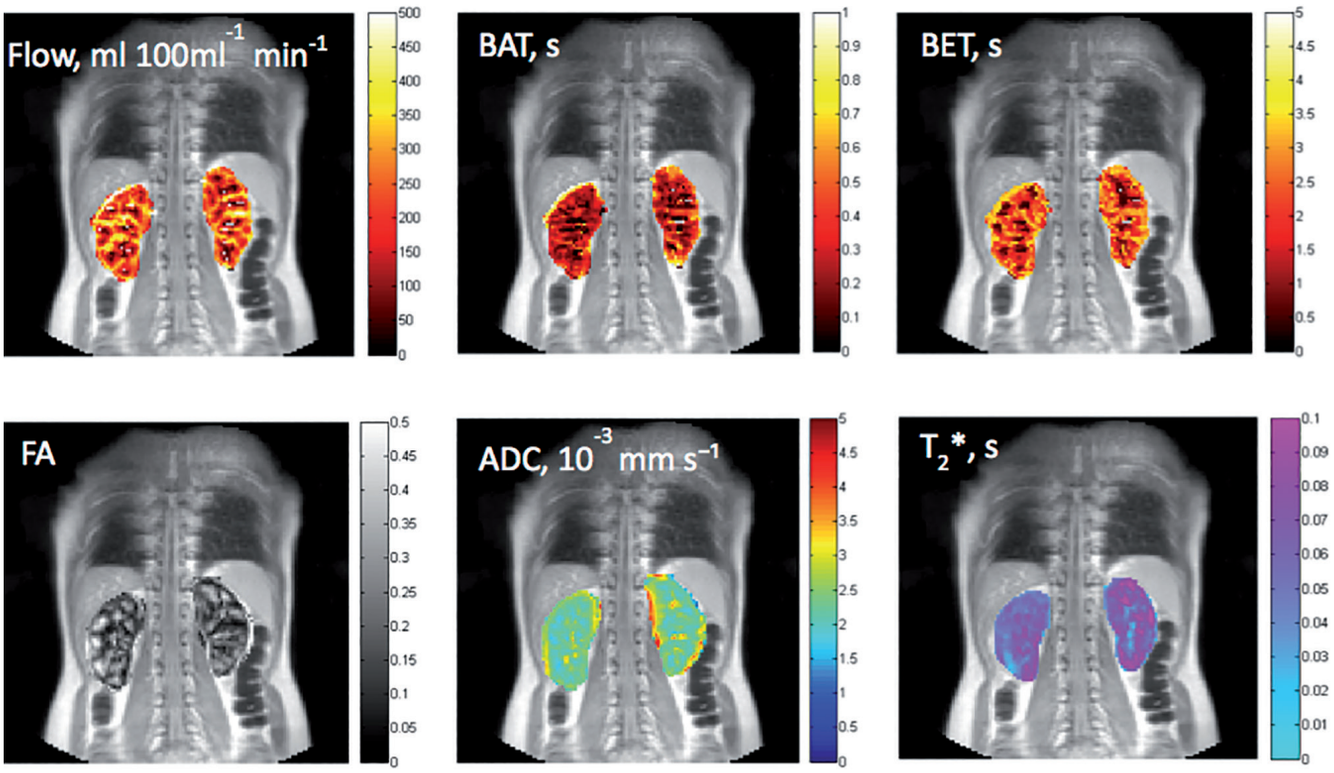

Fig. 1. Example images of ASL parameters (flow, BAT and BET), DTI parameter (FA and ADC) and T_2* in a LN patient. Some registration artefact is evident around the edge of the kidneys.

values correlated inversely with uPCR (r=-0.53, *p*=0.013).

Comparison between groups

Group biomarker estimates are found in Table I (box-plots in supplemental data). BAT was increased in the LN group compared with other groups (0.43 (0.298,0.634)s *vs*. 0.23 (0.226,0.261) s *vs*. 0.247 (0.178,0.269)s, *p*=0.04). There was a trend towards lower cortical FA values in the LN group (0.176 (0.147,0.184) *vs*. 0.185 (0.178,0.196) *vs*. 0.192 (0.189,0.206), *p*=0.07).

Table II. Correlation of MRI parameters with GFR and PCR in the whole cohort (relationships with *p*-values less than 0.05 are highlighted in bold font).

MRI parameter	Correlation co-efficient with eGFR	*p*-value	Correlation with uP:CR	*p*-value
Flow (cortex)	0.378	0.135	-0.12	0.632
Flow (medulla)	0.458	0.0643	-0.114	0.664
BET (cortex)	-0.37	0.144	0.251	0.331
BET (medulla)	-0.135	0.606	0.296	0.249
BAT (cortex)	0.0735	0.779	0.415	0.098
BAT (medulla)	0.333	0.19	0.182	0.485
FA (cortex)	-0.92	0.691	**-0.7232**	**0.0002**
FA (medulla)	-0.325	0.151	0.124	0.590
ADC (cortex)	0.414	0.062	-0.012	0.957
ADC (medulla)	**0.474**	**0.0299**	0.010	0.964
T_2* (whole kidney)	-0.169	0.464	**-0.532**	**0.0131**

Discussion

The need for more sensitive biomarkers for LN is well recognised and we sought to evaluate a combination of non-contrast biomarkers in lupus for the first time. We found no significant association between eGFR and ASL measurements, although studies in other patient populations have found significant correlations (5). We did, however, note a trend towards association between medullary flow and eGFR across the groups. Interestingly we found a delay in bolus arrival time (BAT) in the LN group, despite equivalent eGFR. This could suggest subtle changes in bulk blood flow to the kidney in LN patients, not detected using eGFR. One other study has employed renal ASL in LN. Rapacchi *et al*. conducted a repeatability study in 10 LN patients and 10 HVs (15). Increased perfusion was noted in LN patients. However, different imaging protocols were employed than in the current study and correlation with eGFR or uPCR was not performed. In particular, previous studies (5, 15) did not perform multiple inversion times that allow BAT to be evaluated, which appears in our work to be a biomarker of interest. Omitting a significant change in BAT from the ASL analysis may lead to variation in perfusion measurement due to an under-parameterised model, rather than a genuine physiological change. The literature supports a potential role for ASL-MRI to measure renal perfusion (5, 15).

This was the first study to evaluate DTI-MRI in LN. Previous studies have

demonstrated association between diffusion parameters and eGFR in diabetic and renal transplant populations but have not examined the association with proteinuria (6, 7). Lu *et al*. found differences in DTI measurements between diabetic patients with normal eGFR and healthy controls suggesting that DTI-MRI may be sensitive in early renal disease. In the current study a strong negative correlation between uPCR and fractional anisotropy (FA) was observed and could represent the relationship between micro-architectural disruption and proteinuria. There was also trend towards lower FA values in LN patients compared with other groups. This would be consistent with disruption of normal tissue architecture in LN. It is unclear if differences in DTI values can be attributed to active inflammation, fibrosis or a combination of pathologies. Further study with MRI performed at time of biopsy is required to investigate further.

While the inverse association between T_2* measurements and uPCR is an interesting finding, T_2* can be influenced by other factors such as vessel geometry. Therefore the signal cannot be definitively attributed to renal microhaemorrhage. Further work including histological sampling is required to establish the degree of specificity. Medullary T_2* most accurately reflects tissue oxygenation however lack of segmentation meant we were unable to evaluate this in our study.

There were a number of study limitations. The small sample size means that findings should be interpreted with caution. We could not determine if associations between the imaging and non-imaging measures were due to disease activity or damage. There was a narrow range in eGFR which limited our power to evaluate associations. Also we did not evaluate other vascular parameters such as arterial stiffness, which may have influenced renal blood flow (17). Additionally, multiple associations were explored without correction for multiple testing. Thus findings should be viewed as preliminary data on which to base further studies rather than conclusive. Although slice positioning in ASL aimed to minimise signal contribution from macro-vascular flow, contribution from smaller branching arteries could not be excluded. Thus measurements were not strictly of perfusion, but included some regional blood flow. Recent advances in imaging analysis may further improve the accuracy if these MRI measurements in future studies.

Despite these limitations, this study suggests that non-contrast MRI is well tolerated and measurements are associated with standard measures of LN activity. The literature, in other disease populations, also supports its use in evaluation of renal function. The strengths of these MRI biomarkers are their non-invasive nature and their sensitivity to change, making them most useful in the setting of longitudinal studies and for disease monitoring in the individual patient. Further validations studies at the time of biopsy and in a longitudinal setting are required. However, these techniques could provide a much needed non-invasive biomarker to assess LN.

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