



## A Markov Multi-State model of lupus nephritis urine biomarker panel dynamics in children: Predicting changes in disease activity



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### ABSTRACT

**Background:** A urine 'biomarker panel' comprising alpha-1-acid-glycoprotein, ceruloplasmin, transferrin and lipocalin-like-prostaglandin-D synthase performs to an 'excellent' level for lupus nephritis identification in children cross-sectionally. The aim of this study was to assess if this biomarker panel predicts lupus nephritis flare/remission longitudinally.

**Methods:** The novel urinary biomarker panel was quantified by enzyme linked immunoabsorbant assay in participants of the United Kingdom Juvenile Systemic Lupus Erythematosus (UK JSLE) Cohort Study, the Einstein Lupus Cohort, and the South African Paediatric Lupus Cohort. Monocyte chemoattractant protein-1 and vascular cell adhesion molecule-1 were also quantified in view of evidence from other longitudinal studies. Serial urine samples were collected during routine care with detailed clinical and demographic data. A Markov Multi-State model of state transitions was fitted, with predictive clinical/biomarker factors assessed by a corrected Akaike Information Criterion (AICc) score (the better the model, the lower the AICc score).

**Results:** The study included 184 longitudinal observations from 80 patients. The homogeneous multi-state Markov model of lupus nephritis activity AICc score was 147.85. Alpha-1-acid-glycoprotein and ceruloplasmin were identified to be the best predictive factors, reducing the AICc score to 139.81 and 141.40 respectively. Ceruloplasmin was associated with the active-to-inactive transition (hazard ratio 0.60 (95% confidence interval [0.39, 0.93])), and alpha-1-acid-glycoprotein with the inactive-to-active transition (hazard ratio 1.49 (95%

**Abbreviations:** AGP, alpha-1-acid-glycoprotein; ACR, American College of Rheumatology; act sed, active urinary sediment; AICc, Akaike Information Criterion score; anti-dsDNA antibodies, anti-double stranded DNA antibodies; AUC, area under the curve; BILAG, British Isles Lupus Assessment Grade; BP, Blood pressure; C3, complement factor 3; C4, complement factor 4; CI, confidence intervals; CP, ceruloplasmin; Cr, creatinine; eGFR, estimated GFR; ELISA, enzyme-linked immunosorbant assay; HR, hazard ratio; IQR, interquartile range; JSLE, Juvenile Systemic Lupus Erythematosus; LN, lupus nephritis; LPGDS, lipocalin-like-prostaglandin-D synthase; MCP-1, monocyte chemoattractant protein-1; mg, milligrams; ng, nanograms; NHS, national health service; pc, corrected p value; pg, picograms; S creat, serum creatinine; SA, South Africa; Sev Hyper, severe hypertension; TF, transferrin; U dip, urine dipstick protein levels; UACR, urine albumin creatinine ratio; UK, United Kingdom; US, United States; VCAM-1, vascular cell adhesion molecule-1

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confidence interval [1.10, 2.02])). Inputting individual alpha-1-acid-glycoprotein/ceruloplasmin values provides 3, 6 and 12 months probabilities of state transition.

**Conclusions:** Alpha-1-acid-glycoprotein was predictive of active lupus nephritis flare, whereas ceruloplasmin was predictive of remission. The Markov state-space model warrants testing in a prospective clinical trial of lupus nephritis biomarker led monitoring.

## 1. Background

Juvenile-onset systemic lupus erythematosus (JSLE) is a severe, multi-system autoimmune disease that displays a more aggressive course than adult-onset SLE [1–3]. Lupus nephritis (LN) develops in up to 80% of JSLE patients within the first 5 years from diagnosis [1,4–9]. LN is characterised by a relapsing and remitting course, requiring prompt treatment to prevent permanent renal damage. The clinical presentation of LN is however heterogeneous, and there are significant limitations in LN diagnosis and monitoring. Following an LN flare, proteinuria can take a significant period of time to normalize [10] making it difficult to differentiate whether there is continued LN activity, irreversible renal damage, or if this is part of the normal LN recovery phase [11]. Changes in serum creatinine tend to lag behind changes in biopsy appearance, and are influenced by the child's age, gender and height [12]. There have also been reports of 'clinically silent LN' in patients with biopsy-defined LN but no proteinuria, normal urinalysis and normal renal function [13].

Renal biopsy is currently the gold standard for diagnosing and monitoring LN in children, however, it is associated with significant risks of bleeding, infection and requires a general anesthetic or sedative [14–16]. At diagnosis, renal biopsy provides prognostic information and guides treatment, so the benefits outweigh these risks; however, it has a limited role in ongoing monitoring, with no clear consensus as to the indications for repeat biopsy [17]. A number of studies have demonstrated poor inter-observer agreement in LN class classification between pathologists [18–21], questioning the value of renal biopsies as a gold standard in LN monitoring.

Novel non-invasive LN urine biomarkers are increasingly being investigated. Initial studies focused on individual urinary biomarkers, which were cross-sectionally shown to outperform both traditional and novel serum biomarkers for active LN identification [22–28]. Improvement in both diagnostic and prognostic ability has been demonstrated by using combinations of urinary biomarkers within 'biomarker panels' [29–31]. In a study including 91 JSLE patients from the United Kingdom (UK) and United States (US) of America (61 from the UK; and 30 from the US), we have previously demonstrated on a cross-sectional basis, that a combination of urinary biomarkers including alpha-1-acid-glycoprotein (AGP), ceruloplasmin (CP), lipocalin-like-prostaglandin-D synthase (LPGDS) and transferrin (TF) displayed an excellent ability for active LN identification (UK AUC = 0.920, US AUC = 0.991) [30].

Further prospective investigation of the above biomarkers is warranted to improve understanding of the relationship of these biomarkers to fluctuations in disease activity over time. Although urinary monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) did not emerge as part of the biomarker panel in our previous cross-sectional study [30], further evaluation within a longitudinal study is of interest as low urinary MCP-1 levels have been shown to be a good predictor of future renal disease improvement in JSLE [28]. Urinary VCAM-1 has also been shown to longitudinally correlate with renal SLICC score, urine protein-creatinine-ratio and physician's global assessment in adult SLE [32]. The aim of this study was therefore to longitudinally determine if a urinary biomarker panel (AGP, CP, LPGDS, TF, VCAM-1 and MCP-1), or its constituent members, are able to predict LN flare and remission, using samples from three ethnically distinct JSLE cohorts from the UK, US and South Africa (SA).

## 2. Methods

### 2.1. Patients

UK JSLE Cohort Study [1] patients were recruited from Alder Hey Children's NHS Foundation Trust, Liverpool, and Great Ormond Street NHS Hospital for Children, London, UK. The US Cohort included patients followed at the Children's Hospital at Montefiore, Bronx, New York, US [33] and SA Cohort patients attend Red Cross Memorial and Groote Schuur Hospital Hospitals, Cape Town, South Africa [34]. Urine samples were collected during routine clinical care with detailed demographic data, self-reported ethnicity data, clinical laboratory results and medication information. Within the SA cohort, the term 'Coloured' is used as standard ethnic label for people of mixed ethnic origin who possess ancestry from Europe, Asia, and various Khoisan and Bantu ethnic groups of Southern Africa. The time between urine samples and the number of longitudinal samples varied between patients. Inclusion criteria were as follows a) patients diagnosed with JSLE before 19 years of age and b) achieving  $\geq 4$  of the revised American College of Rheumatology (ACR) SLE classification criteria [35]. Patients were excluded if they a) were  $\geq 19$  years of age at JSLE diagnosis, b) had  $\leq 4$  ACR SLE classification criteria, c) had a urinary tract infection or d) if they had other diseases which could explain their ACR SLE classification criteria.

### 2.2. Renal disease activity classification

Patients were categorized at each clinic visit according to the renal domain of British Isles Lupus Assessment Grade 2004 (BILAG2004) disease activity score, defined as follows; BILAG2004 grade A/B: severe, moderate disease respectively, grade C patients: mild/improving renal disease, grade D: inactive disease but previous system involvement, grade E: system has never been involved [36]. A binary outcome of 'any LN activity' vs inactive LN was utilized for the longitudinal analysis. The longitudinal 'any LN activity group' included patients with a renal domain BILAG score of A, B or C & previous histological confirmation of LN, or inactive LN (renal domain BILAG score D or E). Grouping of renal BILAG D and E episodes together in this way is consistent with previous urine biomarker studies [28,37,38], with identical clinical criteria required to score a BILAG score of D or E, regardless of whether the patient has or has not had previous renal involvement (urine dipstick negative for protein, urine protein or albumin-creatinine ratio of  $< 25$  mg/mmol and no rising blood pressure). During the longitudinal study patients therefore either remained in the same LN disease activity state, or transitioned (inactive→active LN, or vice-versa) on the basis of their renal BILAG score. Inclusion of patients with a history of LN, who had attained inactive LN is important to appreciate whether the biomarkers fluctuate during periods of LN inactivity.

### 2.3. Urine sample processing

The urine sample processing protocol was the same as within the previous cross-sectional urine biomarker panel study [30]. In brief, random mid-stream urine samples were processed as soon as possible or kept refrigerated or on ice for up to 2 h until processing. Urine samples were centrifuged at 2000 rpm for 10 min. Urine supernatant was divided in to 1 ml aliquots and stored without any additives at  $-80$  °C. Samples were transferred on dry ice to the University of Liverpool, UK.

## 2.4. Laboratory techniques

The techniques used for urinary biomarker quantification were also the same as those of the previous cross-sectional urinary biomarker panel study [30]. Pre-coated enzyme-linked immunosorbent assay (ELISA) kits were used for quantification of urinary CP (St Charles, Assay Pro, USA), TF (GenWay, San Diego, USA), LPGDS (BioVendor, Brno, Czech Republic), AGP and MCP-1 (R&D Systems Ltd., Minneapolis, USA). An R&D systems duo-kit (R&D Systems Ltd., Minneapolis, USA) was used to quantify urinary VCAM-1. All biomarker results were standardised for urinary creatinine (Cr) concentration and presented in units (nanograms or picograms) per milligram creatinine (mgCr).

## 2.5. Statistical analysis

Serial biomarker and clinical-demographic data (age, sex, disease duration, ethnicity, C3/C4, and anti-dsDNA antibodies) were considered within the analysis. Chi-squared or Kruskal-Wallis tests were used to compare demographic factors across the patient cohorts. Some predictors contained missing (completely at random) data which would reduce the sample size available for the model, therefore multiple imputation based on the Bayesian Alternating Conditional Expectation algorithm [39] was used to avoid this. Heterogeneity was found in the distributions of some of the prognostic factors (statistical significance assessed via Kolmogorov-Smirnov and Fisher tests, for continuous and binary data, respectively). To address this, Huber's robust "sandwich" estimate [39] of the covariance matrix of model parameters was applied, taking into account the intra-cluster correlation of the data in each cohort. This correction to the covariance matrix increased the standard errors of the parameter estimates, reducing the chances of false rejections of statistical tests, and increasing the width of the confidence intervals. The status data (follow-up time and LN disease state) is panel-observed [40], i.e. we never observe the exact time of state transitions; as such, some of them might be completely unobserved. We only observe states at some sampling times (corresponding to the follow-up visits), which are not in general uniform. The nature of the data therefore required specialized statistical analysis techniques, such as Markov multi-state modelling. It should be noted that although it would seem appropriate to use a simpler logistic regression model due to the binary nature of the LN disease state, doing so would be wrong, since technically the likelihood of the logistic model cannot represent the unobserved association between a state transition and the time when it happens [40].

A homogeneous (independent of any prognostic factor) Markov multi-state model of disease state transitions was fitted. The model assumed that individuals independently transition among the two states of active and inactive LN, or could remain in a state of active or inactive LN (see Fig. 1). At a given time-point, the model provides the instantaneous probability of a patient staying in the same state or transitioning (inactive→active LN, or vice versa). The model was then extended so that the state transitions could depend upon prognostic factors. Within the present data set there were 13 potential predictors (novel biomarkers and clinical/demographic factors), which would require a model with 28 parameters.

No formal definition of an *effective sample size* for Markov Multi-State models exists in the literature to date [39]. The somewhat restrictive definition of the *smallest number of observed state transitions* (smallest  $n = 10$ , inactive→active disease state transition) was therefore noted, and using the empirical assessment of model complexity in regression analysis [39], it was deemed that no more than two predictors could be used in the model to avoid overfitting. The expected relative Kullback-Leibler information [41] estimated by the Akaike Information Criterion (AICc) score was used to select the two strongest candidate predictors. The AICc score is essentially a measure of information loss induced by approximating the data generating process with a model; the best model is therefore the one with the lowest AICc

score. All data analysis was undertaken using R version 3.1.1 with the *msm* [40] and *rms* libraries [42]. Additional software was written by Dr. A. Eleuteri.

## 3. Results

### 3.1. Patients

A total of 80 patients were included in the study; 57 from the UK, 13 from the US and 10 from SA. Across all cohorts there were approximately three times more females than males, with no significant difference in the sex distribution ( $p = 0.899$ ). The median age at diagnosis (in years) was 11.9 in the UK Cohort, 12.3 in the US and 9.7 in the SA Cohort. The median disease duration at the time of first biomarker measurement was 2.2 years within the UK Cohort, 0.8 years in the US and 2.1 years within the SA Cohort ( $p = 0.727$ ). The majority of patients had 2 or 3 follow up visits over a median period of 12 months for the UK Cohort, 20 months in the US Cohort and 8 months in the SA Cohort (see Table 1). 14/80 (17.5%) patients were receiving LN induction treatment, and 66/80 (82.5%) patients were receiving LN maintenance treatment at the time of the first biomarker measurement.

UK Cohort patients were mainly Caucasian (46%) and Asian (30%), including those of Indian, Pakistani, Bangladeshi, Chinese and other Asian origin) with the remaining patients of Black origin (23%, including those of African, Caribbean or mixed Black/Caucasian origin). US Cohort patients were largely African American (56%) or Hispanic (41%) with the other 3% of patients being of Asian origin. The majority of SA patients were either of Coloured (56%, see section 2.1) or Black origin (32%), with the remaining patients being Asian (8%) or Caucasian (4%). 33/80 patients had at least one episode of active LN during the longitudinal follow up period. Of these, 22/33 had proliferative LN (isolated class III, IV or combined with class V LN), 3/33 (9%) had membranous LN (class V), 4/33 (12%) had class II LN. In 4/33 (12%) no biopsy report was available.

### 3.2. The longitudinal data

This study included 184 observations, 124 from the 57 UK patients, 27 from 13 US patients, and 33 from 10 SA patients. Across the data set there were 10 transitions from the inactive→active LN disease state, 18 from active→inactive LN, with 93 and 43 remaining inactive or persistently active between the time points. The urine biomarker raw data values for all samples included in the longitudinal analysis are shown in Fig. 2.

### 3.3. Development of a Markov Multi-State model of LN urine biomarker dynamics

The AICc values for individual models based upon different predictors are shown in Table 2. At this stage, we assumed that all prognostic factors impacted upon all state transitions. The homogeneous model had a AICc of 147.85, therefore models with a lower AICc had better evidence than the homogeneous model. AICc scores are expressed on a logarithmic scale, so even a small difference between the scores can translate into a large difference in evidence and predictive power. The two models with the highest evidence included AGP (AICc 139.81) and CP (AICc 141.40). The AICc values for VCAM-1 and anti-

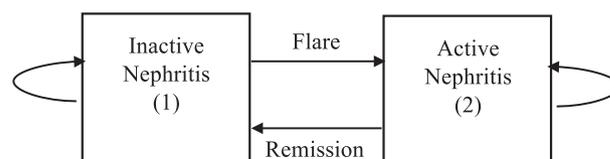


Fig. 1. State transitions assumed by the Markov Multi-State model.

**Table 1**  
Patient demographics.

	UK Cohort (n = 57)	US Cohort (n = 13)	SA Cohort (n = 10)	p-Value
Gender	F = 42, M = 15	F = 10, M = 3	F = 8, M = 2	0.899
Age at diagnosis (years)	11.9 [8.5–13.3]	12.3 [9.9–17.7]	9.7 [8.8–12.8]	0.096
Duration of disease at 1st biomarker measurement (yrs)	2.2 [0.6–4.1]	0.8 [0.2–5.2]	2.1 [0.1–2.9]	0.727
Follow-up period (months)	12 [6–23]	20 [5–41]	8 [5–18]	0.447
Number of follow-up visits	3.0 [2.0–3.0]	2.0 [2.0–2.0]	2.5 [2.0–5.0]	0.212

Values include counts for gender and median values and interquartile ranges (in square brackets) for all other data. F = female, M = male. Yrs = years. Chi-squared or Kruskal-Wallis tests were used to compare demographic factors across the patient cohorts.

dsDNA antibodies were also less than the homogeneous model's AICc (142.59 and 147.35 respectively), but they were not included in the multi-predictor models to prevent over-fitting.

Including both AGP and CP in a model, where both factors impacted on all state transitions in combination, the AICc was 157.19, so this more complex model did not improve the model's evidence. The hazard ratios for this model (with 95% CIs) are shown in Table 3. The two hazard ratios for AGP were > 1, and for CP were < 1. However, inspection of the associated confidence intervals revealed that they were large and included the value 1 in the case of CP for inactive→active transitions, and in the case of AGP for active→inactive transitions. This suggested that the data was not informative enough to reliably assess the impact of CP/AGP on all state transitions, and that individual biomarkers specifically impacted the different state transitions.

A simplified model was fitted with each biomarker only impacting on one state transition, i.e. AGP for the inactive→active transition and CP for the active→inactive transition. The new simplified model's AICc was 127.53, which was the lowest among both the single factor models, and the previous full model. Table 3 shows the hazard ratios with 95% CIs for the simplified model.

The equations for the final model are written as follows:

$$\log \lambda_{12} = -0.54 + 0.40 \log AGP$$

$$\log \lambda_{21} = 0.75 - 0.50 \log CP$$

where  $\lambda_{ij}$  denotes the transition hazard from state  $i$  to state  $j$ , and the logarithm is natural.

### 3.4. Assessing the performance of the optimal Markov Multi-State model

Table 4 shows the optimal model's performance in predicting state prevalence up to one year after biomarker quantification, in three-months intervals. The observed state is that which is observed from the renal BILAG scores data used to fit the model. The expected state is that which is predicted by the optimal Markov Multi-State model, where AGP impacts on the inactive to active state transition (predicting flare) and CP on the active to inactive transition (predicting remission). It highlights that the 'expected' state prevalence predicted by the model and that 'observed' in the longitudinal renal BILAG data are well matched.

### 3.5. Using the Multi-State Markov model of LN urine biomarker dynamics - a worked example

Using a test subjects AGP and CP values (patient had active LN at the time of biomarker quantification), predictions of LN disease state over 3, 6 and 12 months can be demonstrated. For example, where:

- CP is 2090 ng/mgCr (log CP 7.64)
- AGP is 14,043 ng/mgCr (log AGP 9.55)

The Markov Multi-State model predicts that they will remain in an active LN state for a mean time of 0.41 years [0.16, 1.03]. Over the course of 12 months, the probabilities of state transition are shown in

Table 5. Therefore, the probability that the patient will continue to have active LN at 3 months is moderate (probability 60%), however by 6 and 12 months the probability of remaining active falls (probability 45% at 6 months and 36% at 12 months). Table 6 shows the actual LN disease course of this patient during the subsequent follow-up visits, showing that at 3 months the patient had a renal BILAG B score, becoming inactive (renal BILAG D) by 6 months.

## 4. Discussion

A urine biomarker panel including AGP, CP, LPGDS and TF has previously been shown to be excellent for identifying LN on a cross-sectional basis [30]. The main aim of this study was to use longitudinal data from three JSLE cohorts from the UK, US and SA to see if a combination, or constituents of the biomarker panel are able to predict LN flare and remission, using Markov Multi-State modelling. The two biomarkers with the highest evidence for longitudinally predicting changes in LN activity were found to be AGP and CP. The optimal model suggested that AGP was best at predicting flare, and CP was best for predicting remission. This highlights that distinct urinary biomarkers can be of key relevance at distinct points in the LN disease course. The model was derived using longitudinal data from patients at different stages of the disease course (e.g. initial induction, maintenance treatment phase, re-flare following initial LN remission), and is therefore appropriate for use at different stages of the disease process. Using this model, it is possible to develop individual patient predictions of LN disease state over 12 months.

The current study evaluated both novel urine biomarkers and clinical/demographic factors within the Markov Multi-State models, demonstrating novel urine biomarkers to have the strongest impact on LN disease course prediction. Due to the low number of flares observed in the study (n = 10), using the empirical assessment of model complexity in regression analysis [39], no more than two predictors could be used in the model to avoid overfitting. AGP and CP produced the lowest AICc values and were carried forward into the optimal model, however, if more LN disease state transitions data were available; VCAM-1 (AICc = 142.59), and anti-dsDNA antibodies (AICc = 147.35) would also have been considered within the combined models.

In a recent paper assessing a range of classical blood markers for active LN identification (including C3, C4, anti-dsDNA antibodies, ESR, CRP, haemoglobin, white cells, neutrophils, lymphocytes, platelets and immunoglobulins), it was demonstrated that the optimal combination of classical blood markers was only able to demonstrate 'fair' ability for LN identification (AUC 0.724) [43]. In a study of 53 adult SLE patients assessing anti-dsDNA antibody and C3/C4 levels 3–9 months preceding a flare, sensitivity and specificity for predicting renal and non-renal flares was in the region of 50% and 75% respectively, for all three test, with positive and negative likelihood ratios being close to 1.0 [44]. In clinical practice, normal complement and anti-dsDNA antibodies may provide reassurance that active LN is improbable, however, overall studies looking at the accuracy of these tests for differentiating between patients with active and inactive LN are conflicting [44–46].

Classical renal biomarkers (e.g. proteinuria, serum creatinine, eGFR) present limitations during the follow up of LN patients.

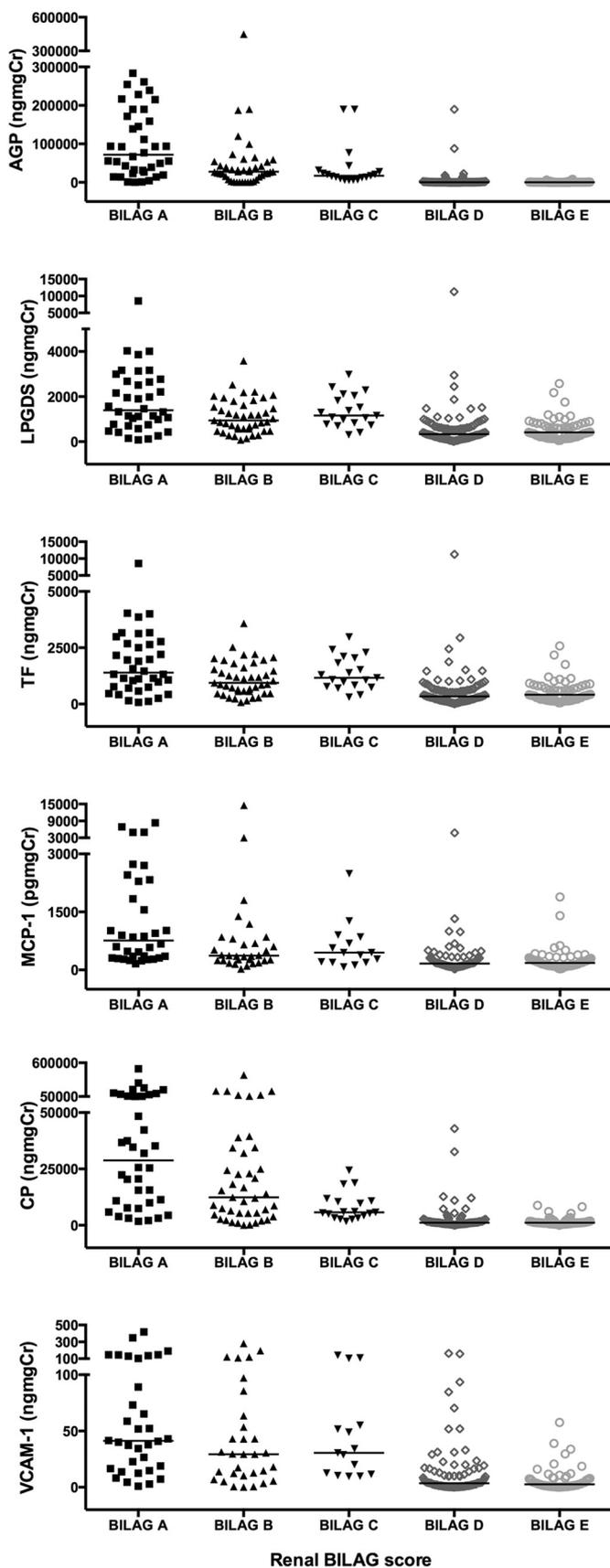


Fig. 2. Urine biomarker values for UK, US, SA Cohort patients included in the longitudinal analysis according to renal BILAG score. Legend – Horizontal line represents the median biomarker concentration.

Following an LN flare, proteinuria has been shown to take a significant period of time to normalize in adult [11], and paediatric [10] SLE patients, making it difficult to differentiate whether ongoing proteinuria reflects the recovery phase after a flare, is due to irreversible renal damage, or continued LN activity, with recent studies identifying ongoing evidence of LN activity on renal biopsy in patients who have attained clinical renal remission based upon classical renal biomarker results [47,48]. Changes in serum creatinine tend to lag behind changes in GFR, and are also influenced by the child's age, gender and height, influencing the use of serum creatinine use as a reliable biomarker in children [12]. Spot protein to creatinine ratio measurements are frequently used as an alternative to 24-hour urinary protein quantification, however the correlation between spot protein/creatinine ratio and 24-hour urine protein is poor in those with a high or medium LN activity index scores [49]. Along with the results of the current study, these data highlight that such classical markers may be of limited value for LN identification, as compared to novel urine biomarkers. Urine is increasingly becoming recognised as the most useful and desirable medium for biomarker discovery in LN given its close proximity to native renal cells, with plasma and serum being more complex sample types that are in contact with multiple organs, and therefore less likely to yield kidney specific biomarkers [50].

The results of the current study are in-keeping with those of a previous paper which showed AGP to be elevated at least 3 months before an LN flare becomes diagnosed clinically [29]. The same study also showed AGP levels to remain elevated in improving LN. This is at odds with the current study which shows AGP levels to be predictive of flare and CP levels to be predictive of remission. A further study has demonstrated that lower CP levels at baseline have been associated with good response to treatment, and after three months of treatment a significant decrease in CP is seen in treatment responders [51], supporting the results of the current study. We were unable to sub-divide the patients according to their treatment in view of the number and complexity of the longitudinal treatment strategies (number of DMARDs, dosage, sequence, combined treatment, and differences in steroid treatment), precluding any meaningful analyses. Inclusion of such data would greatly increase the complexity of the model and require a much larger sample size.

Within the cross-sectional study [30], renal BILAG C samples were excluded, to identify biomarkers which clearly differentiated between active and inactive LN. In contrast when designing the current study, renal BILAG C patient episodes were included within the active LN group, as exclusion of these visits would have led to long gaps between urine sampling episodes, and inclusion of a third outcome category would increase the complexity of the modelling (and the required sample size). Inspection of the longitudinal renal BILAG C urine biomarker values showed them to cluster with renal BILAG A/B episodes (see Fig. 2) rather than D/E episodes. This approach is also in-keeping with a study [52] by Yee et al., which showed fluctuation between adjacent renal BILAG categories of A → B or B → C to occur without LN treatment changes. Clinically it was also considered more appropriate to group patients with 'any LN activity' together in this way. Renal BILAG D and E episodes have been grouped together in many previous urine biomarker studies [28,30,37,38], and the raw data in Fig. 2 support this. Within a future larger study, more patient groups could be considered within a more complex model.

Certain limitations warrant recognition and should be addressed in future work. The definition of active LN was based upon the composite renal BILAG score as this can be serially monitored easily (as opposed to biopsies). Use of this pragmatic composite score does however preclude the direct comparison of the constituent classical renal markers with novel urine biomarkers (as the BILAG score is calculated from proteinuria, blood pressure, serum creatinine, GFR, active urine sediment, and recent biopsy findings). In other words, the renal BILAG score is the 'dependent variable' within the Markov Multi-State model, therefore the constituent classical renal markers cannot be added to the model as

**Table 2**  
AICc values obtained by fitting Markov Multi-State models to different predictors.

No factor	LPGDS	MCP-1	CP	AGP	VCAM-1	TF
147.85	152.14	154.74	141.40	139.81	142.59	151.64

Ethnicity <sup>a</sup>	Sex	Age	Disease duration	Anti-dsDNA antibody	C3	C4
151.41	154.35	154.57	153.89	147.35	155.88	157.12

<sup>a</sup> Three categories were considered for ethnicity: Caucasian, Asian, and any black origin. LPGDS = lipocalin-like-prostaglandin-D synthase, MCP-1 = monocyte chemoattractant protein 1, Ceruloplasmin = CP, AGP = alpha-1-acid-glycoprotein, VCAM-1 = vascular cell adhesion molecule-1, TF = transferrin, anti-dsDNA antibody = anti-double stranded DNA antibody, C3 = complement factor 3, C4 = complement factor 4.

**Table 3**  
Hazard ratios for state transition in the full and simplified models.

Full model – both AGP and CP impact on all state transitions		
State transitions	AGP	CP
Inactive → active LN	1.80 (1.08, 3.01)	0.46 (0.17, 1.28)
Active → inactive LN	1.22 (0.78, 1.91)	0.33 (0.18, 0.62)
Simplified model – AGP impacts on the inactive → active transition (predicting flare), and CP on the active → inactive transition (predicting remission)		
Inactive → active LN	1.49 (1.10, 2.02)	–
Active → inactive LN	–	0.60 (0.39, 0.93)

Hazard ratios (evaluated at mean factor values) and 95% confidence intervals shown. AGP = alpha-1-acid-glycoprotein, Ceruloplasmin = CP, LN = lupus nephritis.

**Table 4**  
Observed and expected state prevalence's over a 1-year period using the optimal Markov Multi-State model.

Time	Inactive LN		Active LN	
	Observed	Expected	Observed	Expected
3 months	50	51	23	22
6 months	41	42	18	17
9 months	33	35	16	14
1 year	21	22	11	10

In the optimal model, AGP impacts on the inactive to active state transition (predicting flare) and CP on the active to inactive transition (predicting remission). The observed state is that which is observed from the renal BILAG scores. The expected state is that which is predicted by the optimal Markov Multi-State model. LN = lupus nephritis.

**Table 5**  
A test subjects' probabilities of state transition at 3, 6 and 12 months following biomarker quantification.

Time <sup>a</sup>	From	To	
		Inactive LN	Active LN
3 months	Inactive LN	0.79	0.21
	Active LN	0.40	0.60
6 months	Inactive LN	0.71	0.29
	Active LN	0.55	0.45
12 months	Inactive LN	0.66	0.34
	Active LN	0.64	0.36

<sup>a</sup> Time = time post biomarker measurement. Patient had active LN at the time of initial biomarker quantification. Probabilities of transition shown. LN = lupus nephritis.

'independent variables'. The majority of patients had proliferative LN and a single renal biopsy, making it difficult to compare urine biomarker levels between different LN sub-classes.

The strengths of the current study are the international

**Table 6**  
Clinical LN disease course of a patient during follow up visits post urine biomarker quantification.

Time	Renal BILAG	BP	UACR	Serum creat	eGFR	Active sediment
0	A	98/60	628	39	152	NA
3 months	B	102/50	9.6	49	120	Y
6 months	D	98/59	3.2	49	118	N
12 months	D	100/60	0.9	51	116	N

Time = time following biomarker quantification, BILAG = British Isles Lupus Assessment Grade score, BP = blood pressure in millimeters of mercury, UACR = urine albumin creatinine ratio in milligram/milimol, Serum creat = serum creatinine in micromoles per litre, eGFR = estimated GFR in millitres/min/m squared, Act Sed = active urinary sediment, Y/N = yes/no, NA = not assessed. The patient had class III LN demonstrated on biopsy (ISN/RPS classification) prior to the first urine sample.

representation within the study population, and the rigorous statistical methods applied whereby use of the AICs complexity control criterion protects against overfitting of the model, and acknowledges the low number of LN flares observed in the study (n = 10). The Markov Multi-State model warrants further testing in a larger, prospectively conducted study of biomarker-led LN monitoring or a clinical trial, featuring more transitions in LN disease state. Such a study could be adequately powered to facilitate assessment of other markers which may improve the model further when assessed in combination (e.g. VCAM-1 and anti-dsDNA antibodies) without risking overfitting of the model. JSLE and adult-onset SLE differ in many ways, with more renal involvement [1,2,53], greater risk of renal failure and irreversible renal damage being seen in JSLE [54,55]. Children also tend to have fewer co-morbidities (e.g. diabetes, cardiovascular disease), making it incorrect to apply this model directly to adult SLE patients. An adult study could allow the Markov Multi-State model to be assessed in a much larger patient group given the higher incidence of SLE in adults than children.

**5. Conclusions**

Within this study a Markov Multi-State model of LN urine biomarker disease dynamics has been developed, demonstrating that AGP is predictive of active LN flare, and CP is predictive of remission in three independent cohorts from across three continents. This model can be used to develop individual patient predictions of LN disease state over 12 months, representing a significant step in the advancement urinary biomarker panel led monitoring of LN. To move this towards clinical translation, the model warrants further testing in a larger, prospective, rigorously conducted clinical trial of urine biomarker led LN monitoring.

**Disclosure statement**

The research was carried out in accordance with the declaration of

Helsinki. The authors do not have any financial interests that could create a potential conflict of interest or the appearance of a conflict of interest with regard to this work.

### Ethics approval and consent to participate

Patient assent/consent and parental consent was obtained to participate in all three cohort studies, and full ethical approvals were in place from the National Research Ethics Service North West, Liverpool East, UK (reference 06/Q1502/77) and the Institutional Review Board at Einstein-Montefiore (IRB 2000-154), and the University of Cape Town ethics committee (HREC No 424-2013). Within the SA cohort, consent was carried out in the language preferred by the patient and family (Afrikaans, Xhosa, or English). Where the patients or family were unable to read the participant information sheet, this was read to them and explained in their preferred language by the physician or research nurse.

### Consent for publication

Not applicable.

### Availability of data and material

The raw biomarker data from this study are shown in Fig. 2. Further access to the data can be requested by interested investigators by contacting the corresponding author – Dr. Eve Smith ([esmith8@liverpool.ac.uk](mailto:esmith8@liverpool.ac.uk)) or chief investigator Prof Michael Beresford ([m.w.beresford@liverpool.ac.uk](mailto:m.w.beresford@liverpool.ac.uk)) on reasonable request.

### Authors contributions

EMDS, AE, ACF and MWB all participated in design of the study and the interpretation of the data. EMDS, BG, LL, AP, DW, TR, CAJ, PN, SDM, CP, KT, CP and CS participated in the conception of the study and the acquisition of clinical data and samples. EMDS and RC carried out the laboratory work. AE performed the statistical analysis. All authors were involved in drafting the manuscript and revising it critically for important intellectual content. They have also all read and given final approval of the version to be published.

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