Title Page

Urinary CD4+ T cells identify SLE patients with proliferative Lupus nephritis and can be used to monitor treatment response

Enghard P[1], Rieder C[2], Kopetschke K[2], Klocke JR[2], Undeutsch R[2,3], Biesen R[2], Dragun D[1], Gollasch M[1], Schneider U[2], Aupperle K[2], Humrich JY[2], Hiepe F[2], Backhaus M[2], Radbruch A[3], Burmester GR[2], Riemekasten G[2]

1. Charité Universitätsmedizin Berlin
Department for Nephrology and Intensive Care Medicine
Augustenburger Platz 1
13353 Berlin

2. Charité Universitätsmedizin Berlin
Department for Rheumatology and Clinical Immunology
Charitéplatz 1
10117 Berlin

3. German Arthritis Research Center (DRFZ)
a Leibnitz Institute
Charitéplatz 1
10117 Berlin

Corresponding author:
Philipp Enghard
Email: Philipp.Enghard@Charite.de

Key Words: Systemic lupus erythematosus, Lupus nephritis, T cells, Biomarker, Urine

Urinary CD4+ T cells identify SLE patients with proliferative Lupus nephritis and can be used to monitor treatment response

Enghard P, Rieder C, Kopetschke K, Klocke JR, Undeutsch R, Biesen R, Dragun D, Gollasch M, Schneider U, Aupperle K, Humrich JY, Hiepe F, Backhaus M, Radbruch A, Burmester GR, Riemekasten G

Abstract

Objectives: Proliferative lupus nephritis (LN) is one of the major concerns in the treatment of systemic lupus erythematosus (SLE). Here we evaluate urinary CD4+ T cells as a biomarker of active LN and indicator of treatment response.

Methods: Urinary CD3+CD4+ T cells were quantified using flow cytometry in 186 urine samples from 147 SLE patients. 14 patients were monitored as follow up. 31 patients with other nephropathies and 20 healthy volunteers were included as controls.

Results: In SLE urinary CD4+ T cell counts ≥800/100ml were observed exclusively in patients with active LN. ROC analysis documented clear separation of SLE patients with active and non-active LN (area under the curve 0.9969). All patients with contemporary kidney biopsy showing proliferative LN presented high urinary CD4+ numbers. In patients monitored under therapy a normalisation of urinary CD4+ T cell counts indicated lower disease activity and better renal function. In contrast, patients with persistence or increase of urinary T cells displayed worse outcomes.

Conclusions: Urinary CD4+ T cells are a highly sensitive and specific marker to detect proliferative LN in SLE patients. Furthermore, monitoring urinary CD4+ T cells may help identify treatment responders and treatment failure and enable patient tailored therapy in the future.

Introduction

Lupus nephritis (LN) is one of the most common manifestations of systemic lupus erythematosus (SLE) and implies a significant risk for the patient.[1] Despite advances in the treatment of LN, the occurrence of nephritis is still associated with a relevant burden of morbidity and mortality.[2] The patient is threatened from two sides: undertreatment, implicating uncontrolled autoimmunity, and overtreatment, resulting in toxicity and danger of serious infections.[3]

The diagnosis of LN is usually suspected in patients with systemic signs of SLE activity and urinary abnormalities, and is confirmed by kidney biopsy. The sooner the diagnosis of LN is established the better is the prognosis.[4-6] However, urinary findings may be misleading or unspecifically elevated, while kidney biopsy is an invasive procedure not free of risk.[7]

An ideal biomarker would non-invasively identify patients with acute LN and quantitatively reflect the inflammation in the kidneys to allow monitoring of the disease and patient tailored treatment. We hypothesize that a SLE marker specific for LN would most likely be found in urine. Additionally, we expect the sensitivity of such a marker to be a function of its involvement in the pathogenesis of LN, thus a marker that reflects an indispensable key event in the local pathogenesis is likely to be sensitive for the underlying disease.

Using flow cytometry based analysis of cells that are considered crucial in the pathogenesis of SLE has yielded valuable biomarkers. Quantification of plasmablasts in the peripheral blood as well as assessing the expression of interferon induced markers on monocytes both correlate closely with disease activity in lupus patients.[8, 9] In our previous work we have reported that CD4+ T cells can be found in abundance in the urine of patients with active LN. The CD4+ T cells in the urine and in kidney biopsies were enriched for CXCR3 expressing cells compared to the blood, suggesting CXCR3 mediated recruitment of CD4+ T cells into the kidney.[10] Importantly, the amount of urinary CD4+ T cells in SLE patients correlated with active renal involvement, suggesting urinary T cells as a potential

biomarker for LN.[10] Meanwhile these results have been confirmed by another group,[11] however a larger trial on T cells as biomarker for LN is still missing.

Infiltration of inflammatory cells into the kidneys is a well established element of LN, particularly the proliferative forms.[12-14] This infiltration predominantly consists of CD4+ T cells,[15, 16] and the extent of the infiltrate is one of the best predictors of renal outcome.[12-14] The phenotype of the CD4+ T cells in the urine is qualitatively reminiscent of the renal infiltrating cells rather than the T cells circulating in the peripheral blood.[10] Hence, the assumption that the CD4+ T cells found in the urine originate from the inflamed kidney interstitium is reasonable, and strengthened by the observed close correlation with LN activity.

In the present study we analyzed a large cross sectional cohort of SLE patients, with and without renal involvement, for the presence of urinary CD4+ T cells. Patients with other nephropathies and healthy volunteers were analyzed as a control group. Furthermore, a cohort of patients with LN was monitored under treatment in the follow up.

Patients and Methods

Patients

We collected and analyzed 186 samples from 147 SLE patients. 36 SLE patients were measured more than once; 19 of these 30 patients were measured twice, 3 Patients were analysed three times, the respective measurements were all seperated by at least 12 month; these data sets were included in all analyses (Figs 1-5). 14 SLE patients were monitored at shorter intervals and these follow up measurements were only included in the data set presented in Figure 6. 4 further patients with SLE were analyzed in whom the kidney biopsy revealed a non-SLE related nephropathy. These 4 patients were only included in the correlation with histology and the ROC analysis (Fig 3 and 4). For all Patients the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was calculated, a score system containing 24 items reflecting the disease activity. Patients with a SLEDAI ≥10 were considered to have severely active SLE disease; patients with a SLEDAI <10 were considered inactive to moderately active. 104 of these samples were taken from SLE patients with past or present involvement of the kidneys, 64 samples were obtained from SLE patients without any signs or history of renal affection.

The patient group with active SLE and past or present renal involvement contains patients with active nephritis, but not exclusively (n = 104). Active nephritis was defined by current renal biopsy demonstrating LN; in absence of a current biopsy, active LN was defined as SLEDAI ≥10 and at least two renal elements of the SLEDAI (n = 29). Proliferative/inflammatory nephritis was defined as patients with active nephritis, except cases of biopsy proven pure class I or V nephritis (n = 26).

Of 29 Patients defined as active LN, 25 had an active biopsy showing LN, 4 were defined over the SLEDAI and rSLEDAI. Two of these 4 patients had prior kidney biopsy and now presented with worsening kidney function and increasing proteinuria. One further patient had active SLE disease with newly onset proteinuria, erythrocyteuria and urinary casts, but was not biopsied. One patient had increasing creatinine values, newly onset proteinuria and erythrocyteuria combined with active SLE, however without undergoing kidney biopsy.

For all patients with active disease, the renal SLEDAI (rSLEDAI) was determined by adding up the renal features of the regular SLEDAI (proteinuria, erythrocyteuria, leucocyteuria, casts). In cases without documented sediment the feature casts was omitted. Kidney biopsy was judged as being current if it had been undertaken ≤4 weeks apart from urine analysis.

31 patients with other nephropathies and 20 healthy volunteers were included as a control group. The 31 patients with other nephropathies consisted of 13 patients with diabetic nephropathy, 10 patients with ANCA associated vasculitis and 8 patients with different proteinuric syndromes (1x cast nephropathy, 1x renal amyloidosis, 1x minimal change, 2x focal segmental glomerulosclerosis, 1x global glomerulosclerosis, 2x membranous glomerulonephritis). All patients provided personal consent to participate in our study for research purposes. Samples were collected between 2006-2012 from patients of the Departments of Rheumatology and Clinical Immunology, Nephrology and Intensive Care Medicine, Charité University Hospital, Berlin, Germany. Patients from our initially reported cohort were reanalyzed for clinical data not previously provided and included in the present study.

The study was approved by the ethics committee of the Charité University Hospital (Charité EA1/034/10) and was conducted according to the ethical guidelines at our institution and the Helsinki Declaration.

Sample preparation and flow cytometry

Urine samples were collected, immediately centrifuged and washed with PBS/BSA. The median sample size was 100ml urine. The usual standing time for the urine was 4-6 h; urine samples from the previous day were discarded. Cells were stained with anti-hCD3-PE (clone UCHT-1 DRFZ, Berlin, Germany or clone HIT3a Biolegend, San Diego, USA), anti-hCD4-FITC (clone TT1 DRFZ, Berlin, Germany or OKT4 Biolegend, San Diego, USA). To block unspecific binding, cells were stained in PBS/BSA containing 10% human IgG (Flebogamma, Grifols, Langen, Germany); to exclude dead cells, propidium iodide (Sigma-

Aldrich, Germany) was immediately added prior to flow cytometry. To calculate cell numbers, the entire samples were acquired and the amount of cells was normalized as cells per 100ml initial urine sample. The cells were analyzed using a Calibur flow cytometer (BD Pharmingen, Heidelberg, Germany). Data were analyzed using Flowjo Software (Tree Star, Ashland, USA).

Routine laboratory values

Values for creatinine, urinary sediment, proteinuria, erythrocyte-/haemoglobinuria and leucoyteuria were all retrieved from the medical records. Creatinine was measured by the Jaffe reaction, 24 h urinary protein excretion using a turbimetric assay. Spot proteinuria, erythrocyte-/haemoglobinuria and leucoyteuria were determined using a dipstick assay (Bayer Diagnostics, Germany). Urinary sediments were analyzed by trained nephrologists after centrifugation of fresh urine.

Statistics

Medians, Mann-Whitney tests, Spearman correlations and the ROC curve were calculated using Graphpad Prism 3.0 (GraphPad Software, San Diego, USA).

Results

Elevated amounts of urinary CD4+ T cells are observed in patients with active LN

Urine samples from 143 patients with SLE were monitored for the presence of CD4+ T cells, 25 repeated measurement separated by at least 12 month were included (n = 168, patient characteristic are given in Table 1). In patients with past or present renal involvement the number of urinary CD4+ T cells clearly correlated with the disease activity ($p < 0.0001$, Spearman $r = 0.5987$, $n = 104$), while in patients with no renal involvement no significant correlation was observed ($p = 0.2114$, Spearman $r = 0.2174$, $n = 64$) (Figure 1B).

In patients with known renal involvement and severely active disease (SLEDAI ≥10) a median of 2437 CD4+ T cells/100ml urine were detectable (n = 32). This number of CD4+ T cells in the urine was significantly higher in SLE patients with active disease and renal involvement compared to the other SLE patients or healthy controls ($p < 0.0001$). In patients with known previous renal involvement but only low to moderate disease activity (SLEDAI <10), a median of 29 CD4+ T cells/100ml were found, which was not significantly more than the cell counts in SLE patients without renal disease ($p = 0.1155$). In SLE patients with no past or present renal involvement, regardless of the disease activity (SLEDAI 0-15), we found only low amounts of CD4+ T cells (median 19 CD4+ T cells/100ml, n = 64). In healthy controls the urine was almost devoid of CD4+ T cells (median 3/100ml, n = 20), which was significantly fewer cells than the amounts observed in SLE patients, regardless of disease activity or renal involvement ($p = 0.0003$ and $p = 0.0074$ comparing healthy controls to patients with inactive LN and SLE patients without renal involvement, respectively) (Figure 1C).

CD4+ T cells numbers of 800/100ml or higher were exclusively found in SLE patients with active disease and renal involvement, however not all of these patients had elevated urinary T cell counts. To assess which patients with active disease and past or present renal affection presented high urinary T cells numbers, and which did not, the number of urinary cells in the active patients was correlated with the renal SLEDAI (rSLEDAI). The rSLEDAI significantly correlated with the amount of urinary CD4+ T cells ($p = 0.0171$, Spearman r =

0.4185, n = 32). Patients with active disease and low urinary CD4+ T cell counts (<800/100ml) all had a relatively low rSLEDAI (0-8), while the patients with elevated T cell counts had higher rSLEDAIs (4-16) (Figure 1D).

	SLE Renal Involvement active disease	SLE Renal Involvement no active disease	SLE No renal involvemet	Other Nephropathies	Healthy controls
n	32	72	64	31	20
age	32 (19-59)	42 (21-74)	44 (22-72)	57 (36-92)	26 (22-58)
female/male	29/3	59/13	59/5	10/21	12/8
Creatinine (mg/dl)	0.96 (0.51-2.76)	0.81 (0.47-6.89)	0.76 (0.56-2.1)	2.5 (0.49-12.48)	np
Proteinuria (mg/24h)	1190 (60-20400)	152 (40-4530)	60 (40-320)	2110 (40-21000)	np
SLEDAI	16 (10-25)	4 (0-8)	4 (0-15)	na	na
Immunosuppressive	32xPred, 19xCyc	59xPred, 12xCyc	58xPred, 2x Cyc	10xPred, 2xCyc	-
Treatment	6xMMF, 3xAza,	18xMMF, 15xAza	13xMMF, 32xAza	5xAza, 1xBorte	
	1x Borte, 15x HCQ	2xCyA, 1xMTX, 33xHCQ	6xMTX, 35xHCQ	4xRtx, 1xDox, 1xMTX	

Table 1. Characteristics and medication of patients. Shown are the data form 143 SLE patients, the characteristic of the 22 patients measured on several occasions seperated by at least 12 month are included (n = 168). Furthermore the characteristics of the 31 patients with other nephropathies and 20 healthy controls are displayed. Abbreviations: Pred - Prednisolone, Cyc - pulse cyclophosphamide, MMF - mycophenolate mofetil, Aza - azathioprine, MTX - methotrexate, HCQ - hydrochloroquine, CyA – Ciclosporine A, Rtx - Rituximab, Dox – Doxorubicin, Borte – Bortezomib, np – not performed, na - not applicable.

Urinary CD4+ T cell counts correlate with plasma creatinine, proteinuria and erythrocyte-/haemoglobinuria but not with leucocyteuria

Urinary CD4+ T cell numbers correlated with kidney function, assessed by plasma creatinine ($p = 0.0007$, Spearman $r = 0.2685$, $n = 157$). The amount number of urinary CD4+ T cells also correlated with proteinuria ($p < 0.0001$, Spearman $r = 0.3883$, $n = 141$) and erythrocyte-/haemoglobinuria ($p < 0.0001$, Spearman $r = 0.5397$, $n = 153$). Notably, high urinary CD4+ T cell counts were also observed in patients without the presence of erythrocytes or haemoglobin in the urine, excluding bleeding as the main source of the T cells in urine. No correlation was found between the presence of T cells and leucocyteuria ($p = 0.4468$, Spearman $r = 0.0629$, $n = 153$) (Figure 2).

A urine sediment was documented in 14 patients with active SLE and renal involvement. All of these patients had urinary CD4+ T cell counts >800/100ml. 5 of these patients had an

active sediment, 5 had inconclusive sediments with dysmorphic erythrocytes in insignificant numbers and no casts, and 4 had non-nephritic sediment. 12 of these patients (5 with active sediment, 3 inconclusive, 4 non-nephritic) had received kidney biopsy in parallel, demonstrating proliferative LN.

Elevated numbers of urinary T cells identify patients with proliferative LN

29 SLE patients undergoing kidney biopsy were analyzed in parallel. All patients with type IV LN or a combination of IV and V presented high urinary T cell numbers (n = 20). Similarly, two patients with atypical inflammatory LN (one extracapillary, proliferative necrotizing glomerulonephritis, one Pauci immune with interstitial lymphocytic nephritis) exhibited elevated CD4+ numbers in the urine. In contrast, patients with class I or pure class V nephritis showed only low numbers of urinary T cells (n = 3) (Figure 3).

In four SLE patients biopsied for suspected LN, the histology revealed a non SLE related kidney pathology. Two patients had a hypertensive nephropathy with CD4+ T cell counts of 20 and 724/100ml. One patient had focal segmental glomerulosclerosis with urinary CD4+ T cell counts >1000/100ml. The fourth patient had minimal change nephropathy, which was considered by the treating clinicians as potentially SLE associated, with very few urinary T cells (Figure 3).

Urinary CD4+ T cells are highly sensitive and specific in diagnosing inflammatory LN in SLE patients

A receiver operator characteristic (ROC) curve was used to calculate the sensitivity and specificity to identify proliferative/inflammatory LN in SLE patients. This group contained all 20 patients with biopsy proven proliferative LN, the two patients with biopsy showing atypical LN and four patients without current biopsy (n = 26). The non-inflammatory group contained all other SLE patients inclusive the patients with biopsy proven class I or V nephritis and the four SLE patients in whom biopsy indicated a non SLE related pathology (n=146). The area under the ROC curve (AUC) was 0.9969. A cut-off of 800 CD4+ T cells yielded a sensitivity of 100% and specificity of 98.0% to identify inflammatory LN in SLE patients (Figure 4).

In our cohort proteinuria and erythrocyte-/haemoglobinuria showed an inferior diagnostic performance than urinary T cells. The AUC for proteinuria to detect proliferative/inflammatory LN was 0.9123 and 0.8942 for erythrocyte-/haemoglobinuria. The respective AUC to indicate active LN was 0.9209 for proteinuria and 0,8788 for erythrocyte-/haemoglobinuria. Applying a cut-off of 500mg/d proteinuria yields a sensitivity of 88.9% and specificity of 83.5% to detect active LN. The diagnostic performance of leucocytes in the urine and creatinine was even worse (AUC of 0.5890 and 0.6281 detect active LN).

Elevation of urinary CD4+ T cells is not a unique feature of LN

Given the high diagnostic precision to identify inflammatory LN in SLE patients in our cohort, we determined whether elevation of urinary T cells is specific for LN compared with other kidney pathologies. A fraction of patients with diabetic nephropathy and ANCA associated vasculitis presented elevated numbers of urinary CD4+ T cells. In contrast, patients with proteinuric syndromes due to different aetiologies (minimal change glomerulonephritis, cast nephropathy and renal amyloidosis) showed no elevation of urinary T cells (Figure 5).

Monitoring urinary CD4+ T cells indicates treatment response

12 patients with acute LN (nine with biopsy proven class IV LN, one with biopsy proven extracapillary, proliferative necrotizing glomerulonephritis, two diagnosed without biopsy) were monitored under treatment. Of these 12 patients, 9 had normalized the amount of their urinary CD4+ T cells below the cut-off value of 800 cells/100ml urine within six months. These patients were considered “responders”. Three patients showed persistence of elevated amounts of urinary T cells >800/100ml despite six months of treatment. Two further patients who initially presented with normal urinary T cells counts were monitored and showed an increase of their urinary CD4+ T cells >800/100ml six months later. The patients that had not normalized or increased their urinary CD4+ T cells during the observation period were considered “non-responders”.

Six months after the initial analysis the responders showed a significant reduction in their disease activity (initial median SLEDAI 16, six months later SLEDAI 2, p = 0.0006). The non-responders showed no reduction in disease activity (initial median SLEDAI 12, six months later SLEDAI 12). Additionally, the responding group presented lower disease activity on month six than the non-responders (median SLEDAI 3 versus 12, p = 0.0141) (Figure 6A). The plasma creatinine levels were compared by calculating the creatinine at the six month visit as a percentage of the initial creatinine. The responders had lowered their creatinine levels (median 73.55% of initial, 9/9 values available), which was significantly lower than the non-responding group (median 103.1% of initial, p = 0.0120, 5/5 values available) (Figure 6B). Similarly, the responders had a lower median proteinuria (percentage of the initial proteinuria, median 64.48%, 6/9 values available) than the non-responders (median 104.4%, 5/5 values available); however this did not reach statistical significance (p = 0.1255) (Figure 6C).

Discussion

In the present paper we report that urinary CD4+ T cells i) are elevated in SLE patients with active proliferative/inflammatory nephritis, ii) have an excellent sensitivity and specificity to identify proliferative nephritis in patients with SLE, and iii) can be used to monitor the treatment response of LN. In our study urinary CD4+ T cells >800/100ml identified SLE patients with active inflammatory nephritis. The high sensitivity and specificity to detect proliferative LN in patients with SLE is close to providing clinicians with a “black or white” performance of an optimal biomarker.

In current clinical routine, SLE patients are mainly screened for renal involvement using creatinine, proteinuria and urinary sediment.[17] Although useful, these parameters have certain limitations. Creatinine and proteinuria can be persistently elevated and thereby unable to differentiate present kidney damage from acute nephritis. The sediment is potentially able to close this diagnostic gap; however it is only semi-quantitative and observer dependent. The mainstay for diagnosing LN is renal biopsy, which is not free of risk and is not suitable for monitoring the follow up. Furthermore, the interobserver agreement between renal pathologists classifying LN biopsies has recently been reported to be surprisingly low, including treatment relevant features such as grading a sample to be proliferative or not.[18]

Several candidate urinary biomarkers have been published in recent years, most detected by ELISA or by RT-PCR (reviewed in [19-21]). Among the most promising novel biomarkers are molecules involved in the recruitment of cells into the kidney (chemokines and adhesion molecules) and molecules reflecting the renal inflammation (cytokines). Prominent examples are urinary MCP-1, VCAM-1, CXCL16 and TWEAK, which have all been reported in several independent publications.[22-26] However, the levels of these soluble urinary biomarkers show considerable overlap between SLE patients with active LN and those without active LN. Therefore, these molecules will be unable to clearly separate SLE patients with and without nephritis, limiting their clinical application.

Not surprisingly, elevated CD4+ T cells in the urine were not a unique finding in LN. Patients with other nephropathies that are known to be associated with an inflammatory infiltration also resulted in high urinary CD4+ T cell counts. This limitation is presumably shared by all urinary biomarkers reflecting the interstitial inflammation. Consequently, quantification of urinary T cells will not be helpful discriminating between lupus nephritis, ANCA vasculitis or another inflammatory nephropathy, but may be suitable to decide whether a SLE patient has active proliferative/inflammatory renal involvement or not.

A cohort of patients was monitored for urinary CD4+ T cells in the follow up. Under treatment some patients normalized their urinary CD4+ T cells, while others showed persistence or even an increase. Similar observations of responders and non-responders were reported for urinary levels of MCP-1 and urinary mRNA levels of CXCL10, CXCR3, TGFbeta and VEGF.[23, 27] Here we were able to demonstrate that patients that had normalized their urinary CD4+ T cells within six months after diagnosis of nephritis had a better outcome than patients with persistence or increase of these cells. Interestingly three of the eight responders had normalized their urinary CD4+ T cell counts as early as 2-3 months after initiation of treatment, suggesting that monitoring urinary T cells may be predictive of the outcome. Moreover, it is reasonable to hypothesize that patients with persistence of urinary CD4+ T cells would benefit from more immunosuppression, while the treatment in patients with normalized cells may be reduced more rapidly.

Flow cytometry of blood cells is routinely performed in most hospitals, so the requirements for quantification of urinary CD4+ T are widely available. Although CD4+ T cells have a limited survival in urine (82% die in 24h, data not shown), the survival over a time span of 4-6h was sufficient to detect the reported differences between patients with active and inactive LN. Furthermore in our data set neither urinary tract infections nor menstruation resulted in urinary CD4+ T cells numbers ≥ 800/100ml (data not shown). Hence the quantification of urinary CD4+ T cells is an accessible technique with a robust readout that awaits to be exploited by the rheumatology and nephrology community.

Taken together, urinary CD4+ T cells represent a promising biomarker in the assessment of proliferative LN. Urinary CD4+ T cells may be used to monitor treatment response and enable a patient tailored treatment of LN in near future. Current limitations include the sparsity of data about non-proliferative forms of LN and lack of extensive data on follow up measurements. We hope that the current paper lays the basis for a large, multi-center study on urinary CD4+ T cells as biomarker fur LN.

Competing Interests

The authors have no competing interests.

Author Contributions

Dr. Enghard had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design: Enghard, Rieder, Kopetschke, Klocke, Undeutsch, Humrich, Riemekasten

Acquisition of data: Enghard, Rieder, Kopetschke, Klocke, Undeutsch

Analysis and interpretation of data: Enghard, Rieder, Kopetschke, Klocke, Undeutsch, Biesen, Dragun, Gollasch, Schneider, Aupperle, Humrich, Hiepe, Backhaus, Radbruch, Burmester, Riemekasten

Manuscript preparation: Enghard, Rieder, Kopetschke, Klocke, Undeutsch, Biesen, Dragun, Gollasch, Schneider, Aupperle, Humrich, Hiepe, Backhaus, Radbruch, Burmester, Riemekasten

Statistical analysis:

Enghard, Rieder, Kopetschke, Klocke, Humrich.

Acknowledgements

We thank all the patients and healthy volunteers for sample donations.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 650) and grants from the University Hospital Charité Berlin

1. Mills JA. Systemic lupus erythematosus. *N Engl J Med* 1994;**330**:1871-9.
2. Ward MM. Changes in the incidence of endstage renal disease due to lupus nephritis in the United States, 1996-2004. *J Rheumatol* 2009;**36**:63-7.
3. Moore RA, Derry S. Systematic review and meta-analysis of randomised trials and cohort studies of mycophenolate mofetil in lupus nephritis. *Arthritis Res Ther* 2006;**8**:R182.
4. Faurschou M, Starklint H, Halberg P, et al. Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure. *J Rheumatol* 2006;**33**:1563-9.
5. Esdaile JM, Joseph L, MacKenzie T, et al. The benefit of early treatment with immunosuppressive agents in lupus nephritis. *J Rheumatol* 1994;**21**:2046-51.
6. Fiehn C, Hajjar Y, Mueller K, et al. Improved clinical outcome of lupus nephritis during the past decade: importance of early diagnosis and treatment. *Ann Rheum Dis* 2003;**62**:435-9.
7. Preda A, Van Dijk LC, Van Oostaijen JA, et al. Complication rate and diagnostic yield of 515 consecutive ultrasound-guided biopsies of renal allografts and native kidneys using a 14-gauge Biopty gun. *Eur Radiol* 2003;**13**:527-30.
8. Jacobi AM, Odendahl M, Reiter K, et al. Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 2003;**48**:1332-42.
9. Biesen R, Demir C, Barkhudarova F, et al. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. *Arthritis Rheum* 2008;**58**:1136-45.
10. Enghard P, Humrich JY, Rudolph B, et al. CXCR3+CD4+ T cells are enriched in inflamed kidneys and urine and provide a new biomarker for acute nephritis flares in systemic lupus erythematosus patients. *Arthritis Rheum* 2009;**60**:199-206.
11. Dolff S, Abdulahad WH, van Dijk MC, et al. Urinary T cells in active lupus nephritis show an effector memory phenotype. *Ann Rheum Dis* 2010;**69**:2034-41.
12. Yu F, Wu LH, Tan Y, et al. Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. *Kidney Int* 2010;**77**:820-9.
13. Park MH, D'Agati V, Appel GB, et al. Tubulointerstitial disease in lupus nephritis: relationship to immune deposits, interstitial inflammation, glomerular changes, renal function, and prognosis. *Nephron* 1986;**44**:309-19.
14. Esdaile JM, Levinton C, Federgreen W, et al. The clinical and renal biopsy predictors of long-term outcome in lupus nephritis: a study of 87 patients and review of the literature. *The Quarterly journal of medicine* 1989;**72**:779-833.
15. Boucher A, Droz D, Adafer E, et al. Characterization of mononuclear cell subsets in renal cellular interstitial infiltrates. *Kidney Int* 1986;**29**:1043-9.

16. Caligaris-Cappio F, Bergui L, Tesio L, et al. HLA-Dr+ T cells of the Leu 3 (helper) type infiltrate the kidneys of patients with systemic lupus erythematosus. *Clinical and experimental immunology* 1985;**59**:185-9.
17. Hahn BH, McMahon MA, Wilkinson A, et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis care & research* 2012;**64**:797-808.
18. Grootscholten C, Bajema IM, Florquin S, et al. Interobserver agreement of scoring of histopathological characteristics and classification of lupus nephritis. *Nephrol Dial Transplant* 2008;**23**:223-30.
19. Mok CC. Biomarkers for lupus nephritis: a critical appraisal. *Journal of biomedicine & biotechnology* 2010;**2010**:638413.
20. Enghard P, Riemekasten G. Immunology and the diagnosis of lupus nephritis. *Lupus* 2009;**18**:287-90.
21. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. *Clinical reviews in allergy & immunology* 2011;**40**:138-50.
22. Singh S, Wu T, Xie C, et al. Urine VCAM-1 as a marker of renal pathology activity index in lupus nephritis. *Arthritis Res Ther* 2012;**14**:R164.
23. Rovin BH, Song H, Birmingham DJ, et al. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. *J Am Soc Nephrol* 2005;**16**:467-73.
24. Wu T, Xie C, Wang HW, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. *J Immunol* 2007;**179**:7166-75.
25. Schwartz N, Rubinstein T, Burkly LC, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. *Arthritis Res Ther* 2009;**11**:R143.
26. Schwartz N, Su L, Burkly LC, et al. Urinary TWEAK and the activity of lupus nephritis. *J Autoimmun* 2006;**27**:242-50.
27. Avihingsanon Y, Phumesin P, Benjachat T, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. *Kidney Int* 2006;**69**:747-53.

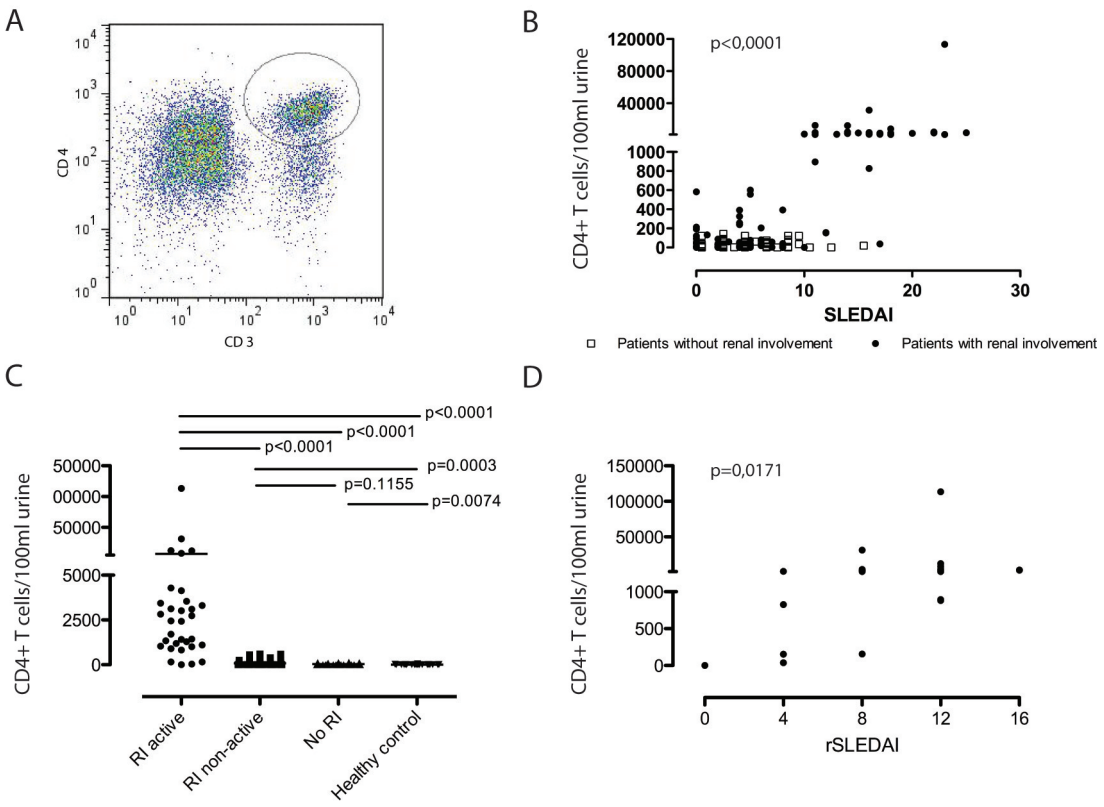

Figure 1. CD4+ T cells are found abundantly in the urine of patients with LN. **A.** Representative Dot-Plot showing CD3+CD4+ T cells in the urine of a patient with acute LN (gated on living lymphocytes). **B.** The amount of urinary CD4+ T cells correlates with the SLEDAI in SLE patients with renal involvement (filled circles, $p < 0.0001$, Spearman $r = 0.5987$). In SLE patients without renal involvement very few urinary CD4+ T cells are detectable regardless of disease activity (open boxes, $p = 0.2114$, Spearman $r = 0.2174$). **C.** CD4+ T cell counts ≥800/100ml urine are exclusively found in SLE patients with active disease and renal involvement. **D.** The number of urinary CD4+ T cells in patients with active disease closely correlates with the rSLEDAI.

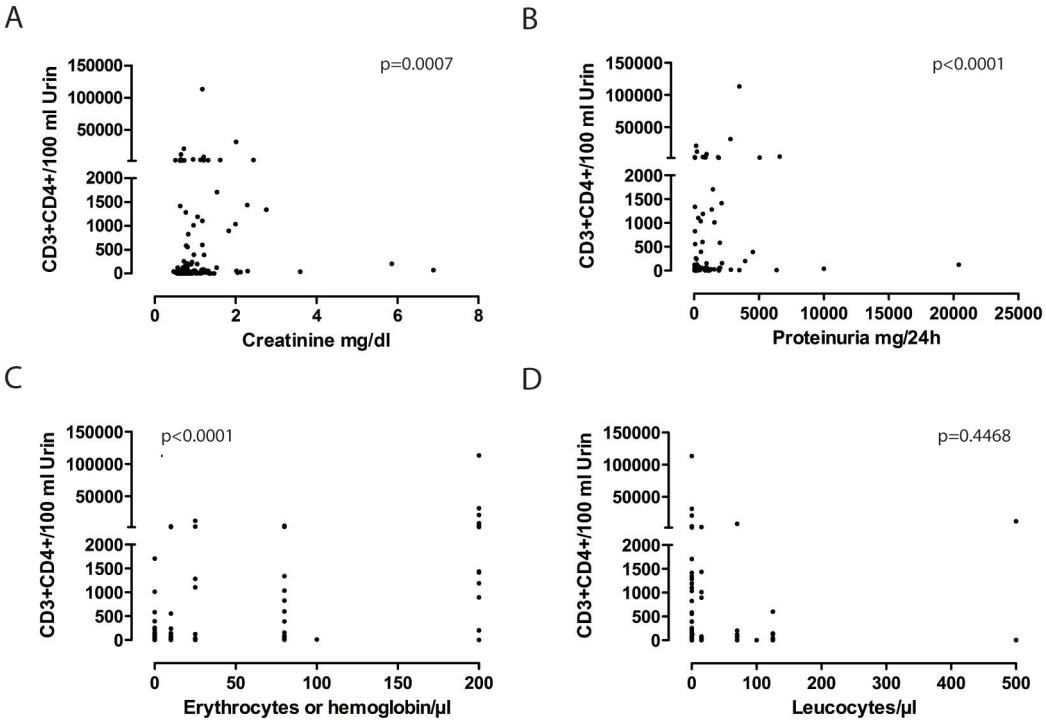

Figure 2. Correlation of the number of urinary CD4+ T cells with different markers for renal pathology. **A.** Urinary CD4+ T cells correlate with the plasma creatinine (p = 0.0007, Spearman r = 0.2685). **B.** Significant correlation between the amount of CD4+ T cells and proteins in the urine (p < 0.0001, Spearman r = 0.3883). **C.** Urinary CD4+ T cells and erythrocyte-/haemoglobinuria correlate (p < 0.0001, Spearman r = 0.5397). **D.** No correlation of urinary CD4+ T cells with leucocyteuria (p = 0.4468, Spearman r = 0.0629).

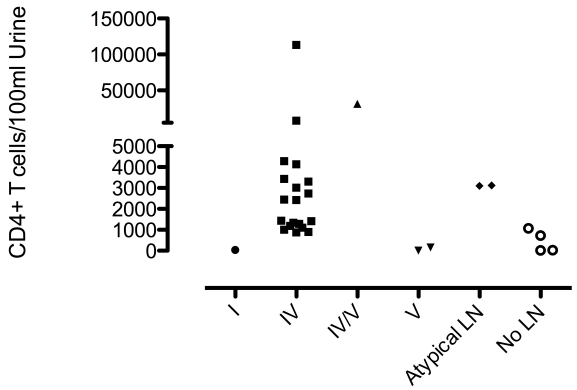

Figure 3. Results of concurrent kidney biopsies and number of urinary CD4+ T cells in 29 patients. All patients with LN IV and atypical inflammatory LN have highly elevated urinary CD4+ T cell counts. No elevated urinary CD4+ counts in pure class I or V LN.

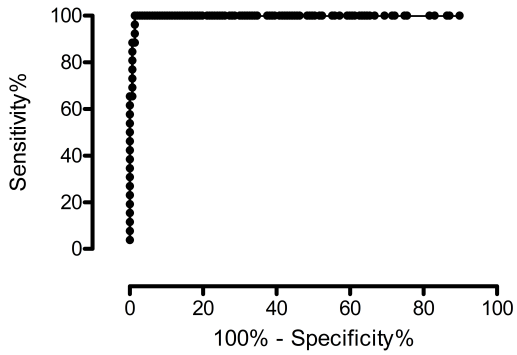

Figure 4. Receiver operator characteristic (ROC) showing the diagnostic performance of urinary CD4+ T cells to detect proliferative/inflammatory LN in SLE patients. Area under the curve 0.9969. Employing a cut-off of 800 CD4+ T cells yielded a sensitivity of 100% and specificity of 98.0%.

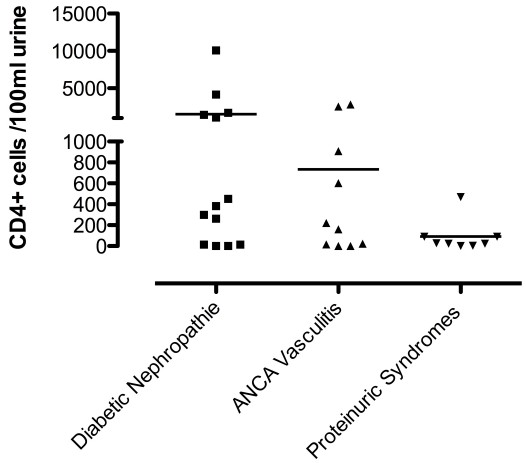

Figure 5. Elevated number of urinary CD4+ T cells is not a unique finding in LN. Patients with diabetic nephropathy and ANCA associated nephritis also present with elevated numbers of urinary CD4+ T cells.

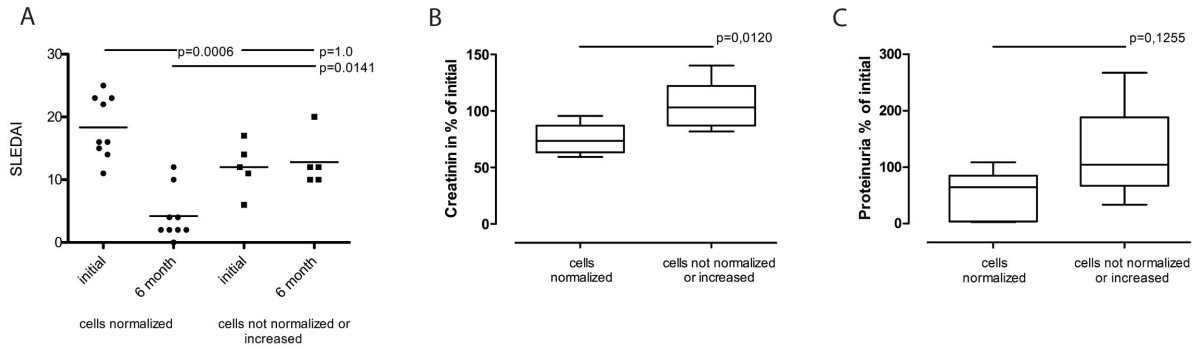

Figure 6. Follow up of patients under therapy. **A.** Patients who normalized their urinary CD4+ T cell counts <800/100ml within six months show a significant reduction of their SLEDAI. In contrast, patients with persistent or increased urinary CD4+ T cell numbers show no treatment response in their SLEDAI. **B.** Patients with normalized urinary CD4+ cells have significantly decreased creatinine levels then patients with increase or persistence of these cells (six month creatinine as % of initial creatinine). **C.** Higher proteinuria levels in patients with increase or persistence of their urinary CD4+ T cells, although not statistically significant (six month proteinuria as % of initial proteinuria).