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ORIGINAL ARTICLE

Markers of childhood lupus nephritis indicating disease activity

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Abstract Current treatment regimens for childhood lupus nephritis (LN) are associated with significant side-effects and toxicity in vulnerable phases of growth and development. The paucity of biomarkers particularly in childhood impedes the appropriate clinical management and the development of new therapeutics. We analyzed markers of immune system (BAFF, RANTES), complement (Bb, C1q, C3d-CIC, C5a) and endothelial cell activation (sVCAM-1) in children with LN (n=22, mean age 14.8±4.7 years), nephrotic syndrome (n=13) and age-matched healthy controls (n=20) to define parameters that correlate with LN activity. Complement fragments of the alternative (Bb, p=0.0004) classical (C3d-CIC, p<0.0001) and common pathway (C5a, p<0.0001) and the levels of BAFF (p< 0.0001), RANTES (p=0.0002) and sVCAM-1 (p=0.0004) were significantly higher in active compared to inactive LN. Activation of complement was associated with the occurrence of anti-C1q antibodies and reduced complement

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C1q. Complement-activation fragments highly correlated with the markers for immune system and endothelial cell activation. The ensemble of these parameters may be of great value in identifying early flares or remissions of childhood LN, and moreover may prove useful in the assessment of new treatments and in determining the optimization of their use.

Keywords Anti-C1q antibodies · B cell activating factor · C1q · Complement activation products · Lupus nephritis · Soluble VCAM-1 · RANTES

Introduction

Childhood lupus nephritis (LN) remains a significant diagnostic and therapeutic challenge due to its complex etiopathogenesis, heterogeneous presentation, and unpredictable course.

A complex interplay between abnormally activated B and T cells, antigen presenting cells, and the complement system results in the production of autoantibodies, circulating immune complexes (CIC), systemic complement activation, and finally, to multisystem injury including nephritis [1].

A crucial homeostatic cytokine for B cells that is up-regulated during inflammation and accounts for the perpetuation of systemic lupus erythematosus (SLE) is the B cell activation factor BAFF [2, 3]. RANTES (CCL5) is a key chemokine for T cell recruitment to inflammatory tissues and its expression is associated with renal damage [4, 5].

Complement itself can damage the kidney or attracts and activates leukocytes, which, in turn, cause inflammation and tissue destruction, and thus contributes significantly to the pathogenesis of LN [6–8]. During this inflammatory process, adhesion molecules including the vascular cell adhesion molecule-1 (VCAM-1) that regulate the migration of leukocytes are up-regulated. Soluble VCAM-1 (sVCAM-1) has been reported as a useful marker of adult SLE activity [9, 10]. On the other hand, deficiency of the early classical pathway component C1q induces tissue damage [11, 12] and the occurrence of autoantibodies to C1q in SLE has been shown to be associated with hypocomplementemia and severe active LN [13–15].

In daily clinical practice, various parameters are currently used to assess SLE disease activity, including anti-double-stranded DNA antibodies (anti-dsDNA) and complement C3 and C4. Nevertheless, the utility of these markers in reflecting disease activity remains controversial [16–18], and C3 and C4 not always accurately reflect complement activation [16, 19, 20]. C3 acts as an acute-phase protein, and thus an increased production can obscure its consumption [21, 22]. Complement degradation products however are unique to complement activation, and can be easily and quickly detected by enzyme-linked immunosorbent assay (ELISA), thus can be used in routine clinical practice. Though, various studies have shown relationships between complement consumption and SLE disease activity, most have been done in adults [22–24]. Studies in children with LN are limited and restricted to the analysis of individual parameters [25, 26].

The lack of reliable biomarkers for childhood LN that identify early flares or remissions and measure reliably a clinical response to therapy impedes the appropriate clinical management and the development of new therapeutics and evaluation in clinical trials.

Therefore, this multicenter study was designed (a) to determine whether parameters related to the multifactorial pathogenesis, namely complement-activation fragments of the alternative (Bb), classical (C1q, C3d fixing CIC) and final common pathway (C5a) and markers for immune system (BAFF, RANTES) and endothelial cell (sVCAM-1) activation correlate with the activity of childhood LN, and (b) to examine correlations between these markers and with complement C3, C4, and anti-ds DNA.

Patients and methods

Study population

Twenty-two pediatric patients with LN (mean age 14.8± 4.7 years) seen at the Department of Pediatrics in Innsbruck (A), Rostock (G), Muenchen (G), Heidelberg (G), Erlangen (G), Tuebingen (G), Memmingen (G), Wuerzburg (G), or Bremen (G) were included. All patients met the American College of Rheumatology (ACR) revised classification criteria for definite SLE with disease onset prior to 16 years of age [27, 28]. None of the children had a hereditary complement deficiency. Histology of renal biopsies was classified according to the International Society of Nephrology and the Renal Pathology Society [29].

To establish normal values for the parameters investigated, 20 healthy children undergoing examination for sport eligibility evaluation or surgery for hernia inguinalis, umbilicalis or phimosis, with similar age distribution were recruited at the Department of Surgery of the Innsbruck Medical University.

In order to assess the impact of immunosuppression on the markers analyzed, 13 children with nephrotic syndrome (NS), more specifically minimal change glomerulonephritis (MCGN) or focal segmental glomerulosclerosis (FSGS), and similar immunosuppressive therapy to children with LN were recruited at the Department of Pediatrics of the Innsbruck Medical University.

Table 1 Characteristics of the study population

	LN		NS	Control
	n=22		*n*=13	*n*=20
Age, years (mean ± SD)[a]	14.8±4.7		10.5±5.0	12.3±2.7
Phase of LN	Active	Inactive		
No. of patients	14	15		
Medications, No. (%) of patients				
No	3 (21)	0	1 (7.7)	20 (100)
Cyclophosphamide	5 (36)	0 (0)	0 (0)	0 (0)
S/p Cyclophosphamide	3 (21)	8 (53)	0 (0)	0 (0)
Cyclosporine A	1 (7)	2 (13)	6 (46.1)	0 (0)
Hydroxychloroquine	0 (0)	3 (20)	0 (0)	0 (0)
Mycophenolate mofetil	10 (71)	8 (53)	3 (23.1)	0 (0)
Prednisone <1 mg/kg/d	4 (29)	10 (67)	3 (23.1)	0 (0)
Prednisone≥1 mg/kg/d	7 (50)	0 (0)	0 (0)	0 (0)

[a] Age at the time of the study (p=0.11)

LN Lupus nephritis, *NS* nephrotic syndrome, *No*. Number, *S/p* status/post

At the time of diagnosis demonstrated by renal biopsy and the Systemic Lupus Erythematosus Disease Activity Index 2000 update SLEDAI-2K, flare or remission of LN, demonstrated by the renal score of the SLEDAI-2K or at routine follow-up appointments, plasma and serum samples were collected and stored in aliquots frozen at -80°C until further use.

Written informed consent was obtained from all patients and parents prior to inclusion. The study was approved by the local ethical committees.

Clinical evaluations

Clinical evidence of disease activity was assessed using the SLEDAI-2K that has been validated for use in children [30–32]. Active SLE was defined by a SLEDAI score≥8 [33]. The physician completing the SLEDAI-2K was blinded to the complement split products, anti-C1q antibody, BAFF, RANTES, and sVCAM results.

Routine laboratory tests

Blood and urine samples were obtained at each visit. Routine laboratory tests included a complete blood cell count, platelet count, measurement of blood urea nitrogen, serum creatinine, erythrocyte sedimentation rate, serum C3 and C4 levels, serum anti-double stranded DNA (dsDNA), urine analysis, urine total protein/creatinine ratio measurement.

Definition of LN activity

LN activity was defined according to the renal score of the SLEDAI-2K [32, 34, 35].

Plasma complement products (Bb, C1q, C5a), C3d fixing CIC, and BAFF determinations

Plasma samples were evaluated using commercially available ELISA kits according to the assay procedure for the presence

Table 2 Clinical and routine laboratory data in patients with LN, *n*=22

	Active LN *n*=14	Inactive LN *n*=15
WHO class II, No. of patients	2	2
WHO class III, No. of patients	4	2
WHO class IV, No. of patients	8	11
SLEDAI-2K, median (range)	15 (8-24)	0 (0-4)
Complement C3 median (range), mg/dl	69.25 (30.0-93.0)	104.0 (79.0-161.0)
Complement C4 median (range), mg/dl	9.6 (4.0-13.0)	18.75 (11.80-31.70)
Urine protein to creatinine ratio median (range), g/g	2.75 (0.98-5.89)	0.16 (0.0-0.5)
Serum creatinine median (range), mg/dl	0.74 (0.42-3.53)	0.60 (0.42-0.80)

Table 3 Longitudinal study in individual patients

Patient	WHO LN class	Treatment	BAFF (pg/ml) A/I	Anti-C1q (U/ml) A/I	C1q (μg/ml) A/I	Bb (μg/ml) A/I	C3d-CIC (μg Eq./ml) A/I	C5a (μg/l) A/I	sVCAM (ng/ml) A/I	Time between A and I[a]	Time between A and S[b]	Time between I and S[c]
1	II	Pred	1,562 / 595	103.7 / 56.5	137.6 / 139.2	2.18 / 0.98	21.8 / 7.5	2.40 / 0.24	653 / 638	2 months	19 days	2 months
2	III	Pred, MMF	3,246 / 773	203.5 / 88.4	85.0 / 150.9	2.30 / 1.58	30.3 / 7.7	5.21 / 0.87	769 / 739	1 month	1 day	1 month
3	III	Pred, CYC	3,091 / 802	243.8 / 71.6	91.4 / 118.5	2.59 / 1.18	45.5 / 10.5	7.54 / 0.44	851 / 625	8 months	1 day	2 months
4	IV	Pred, CYC	7,242 / 873	950.7 / 48.0	52.3 / 166.6	3.31 / 1.97	74.3 / 9.1	8.35 / 1.12	1,403 / 853	7 months	12 days	3 months
5	IV	Pred, CYC	4,587 / 949	745.7 / 71.9	70.0 / 92.3	2.70 / 1.21	96.4 / 18.8	9.39 / 0.47	1,854 / 611	16 months	0 days	2 months
6	IV	Pred, CYC	6,938 / 961	1,050 / 93.7	37.0 / 123.2	4.79 / 2.05	50.5 / 10.7	7.53 / 0.87	1,731 / 703	11 months	1 day	2 months
7	IV	Pred, CyA	3,247 / 978	902.3 / 35.5	58.5 / 201.0	2.54 / 1.22	25.3 / 8.3	5.53 / 0.37	1,388 / 579	12 months	2 days	11 months

A/I Active/inactive lupus nephritis (LN), *CyA* cyclosporine A, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil, *Pred* prednisone

[a] Period of time between active and inactive phase of LN; [b] Time between diagnosis of active LN and study; [c] Time between diagnosis of inactive LN and study

of Bb (Bb Plus ELISA, Quidel Corporation, San Diego, CA, USA), C1q (C1q ELISA, Hölzel Diagnostika GmbH, Cologne, Germany), C3d CIC (CIC C3d ELISA, DRG International Inc., USA), C5a (C5a ELISA, DRG International Inc., USA), BAFF (Human BAFF Immunoassay, R&D, Systems, Inc., Minneapolis, MN, USA). All assays were performed in duplicates.

Serum antiC1q antibody, sVCAM, and RANTES measurements

AntiC1q antibodies, sVCAM, and RANTES were analyzed in serum samples by commercially available ELISA kits (Anti-C1q ELISA, Orgentec Diagnostika GmbH, Mainz, Germany; human sVCAM-1 Immunoassay and human CCL5/RANTES Immunoassay, both from R&D Systems, Inc., Minneapolis, MN, USA), according to the assay procedure. All assays were performed in duplicates.

Statistical analysis

Statistics and linear regression were calculated using GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA). Mann–Whitney *U* test, Kruskal-Wallis test or Wilcoxon signed-rank test were used for comparisons and the Spearman's rank correlation test for correlations. The results are given as mean±SEM, if not otherwise indicated. Differences were considered significant if p values were less than 0.05. Receiver operator characteristic (ROC) curves of sensitivity vs. 1 - specificity were used to determine whether sVCAM-1 could discriminate children with active from those with inactive LN. ROC curves were described quantitatively using the area under the curve (AUC). The ROC curve analysis was carried out with Analyze-It software (Ver. 1.44; Analyze-It Software).

Results

Patients' characteristics

Twenty-two patients with LN (mean age 14.8±4.7 years, females (f)/males (m): 15/7), 13 patients with NS (f/m: 4/9) and 20 healthy controls (HC; f/m: 5/15) were enrolled in the study, and details are given in Table 1. In all patients (LN, nephrotic syndrome) the disease onset was prior to 16 years of age. Medications at the time of blood withdrawal are shown in Table 1. Three patients with LN had no treatment because of first diagnosis at study entry, and one patient with MCGN because of complete remission. Patients with NS were in remission at the time of the study, median duration of disease was 72 (range: 4-168) months.

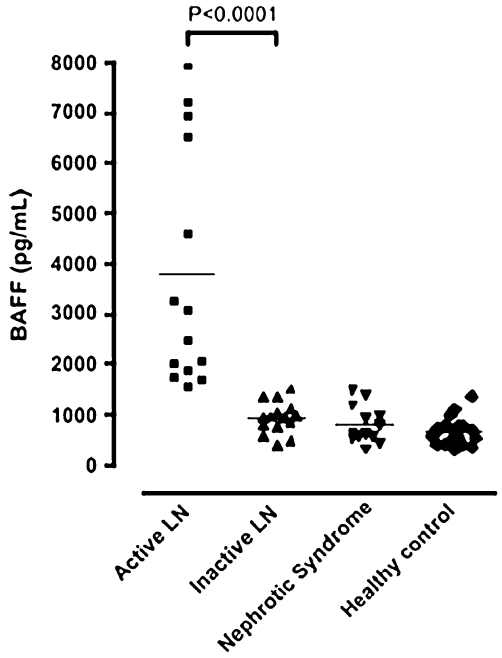

Fig. 1 Increased BAFF concentrations during flares of childhood lupus nephritis (*LN*). Plasma concentrations of BAFF were measured by ELISA. Levels in children with active LN were compared to those in children with inactive LN or nephrotic syndrome or healthy age-matched controls. Each *symbol* indicates an individual subject. The mean for each group is shown as a *horizontal line*

Patients with LN

The median duration between the diagnosis of LN and the time of the study was 593 (range: 0-3156) days. LN was the first manifestation of SLE in 20 out of 22 patients, and in two patients, LN was diagnosed 1 year 8 months and 2 years after the first manifestation of SLE (arthritis). The median time of active LN when the samples where obtained was 2 (range: 0-91) days. The median time of remission of LN when the samples were obtained was 2.5 (range: 1-33) months.

Clinical and routine laboratory findings in patients with LN are shown in Table 2. At the time of blood withdrawal, only five of the patients had additional manifestations of lupus other than nephritis (arthritis, n=2; new rash, n=5; mucosal ulcers, n=2; leukopenia, n=2). Descriptors of the SLEDAI present at the time of blood withdrawal in the study population included arthritis, urinary casts, hematuria, proteinuria, pyuria, new rash, mucosal ulcers, low complement, increased DNA binding, and leukopenia.

From seven patients, blood was obtained twice for analysis during the active and inactive phase of LN (Table 3).

Increased BAFF and RANTES levels in children with active LN

The mean BAFF concentration was significantly higher in patients with active (3,791±632 pg/ml) than in those with inactive LN (956±84 pg/ml, p<0.0001), NS (824±102 pg/ml, p<0.0001) or healthy controls (HC; 667±61 pg/ml, p< 0.0001, Fig. 1). In patients with inactive LN, BAFF levels were comparable to those of patients with NS (p=0.3334), although there was a slight difference between mean BAFF levels in inactive LN and HC (p=0.011, Fig. 1).

Children with LN showed increased levels of RANTES compared to HC (437.9±79.15 pg/ml in active LN and 105.9± 21.22 pg/ml in inactive LN versus 41.63±3.819 pg/ml in HC, p<0.0001).

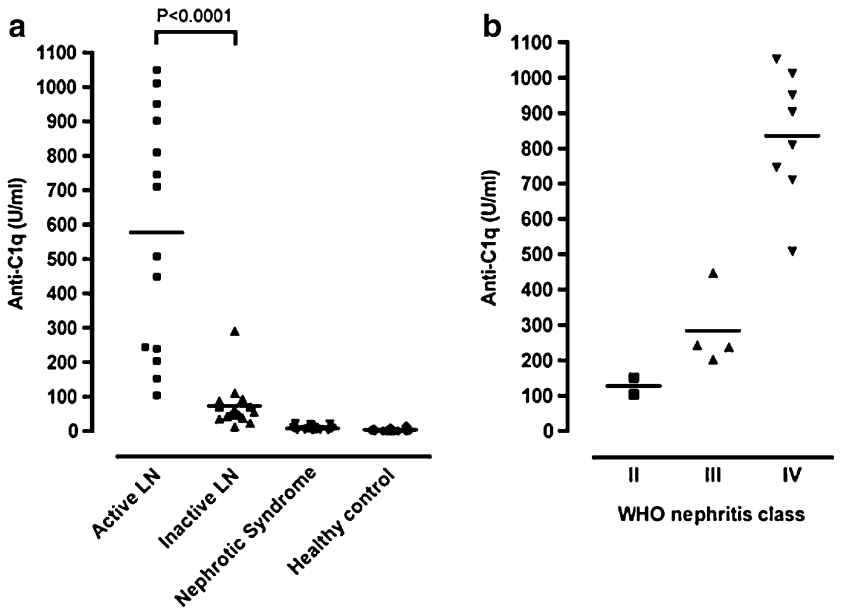

Fig. 2 Children with active LN show high levels of anti-C1q antibodies. **a** Concentrations of anti-C1q were detected by ELISA in serum samples from children with active or inactive LN, NS, or age-matched healthy controls. **b** In children with active LN, anti-C1q levels were further grouped according to the WHO classes of LN. *Symbols* represent individual data points, and the *horizontal lines* represent means

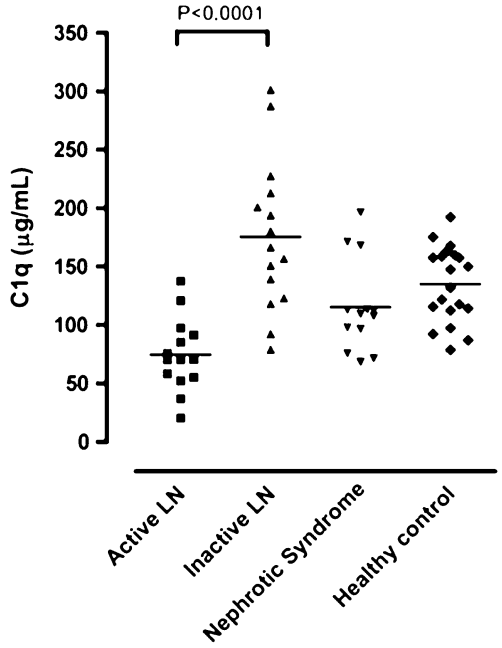

Fig. 3 Decrease of C1q during flares of LN in children. Plasma C1q concentrations in children with active or inactive LN, NS, or age-matched healthy controls were analyzed by ELISA. Each *symbol* indicates an individual subject. The mean for each group is shown as a *horizontal line*

Antibodies against C1q in children with severe active lupus nephritis

Children with active LN showed significantly higher concentrations of anti-C1q antibodies (576.9±92.3 U/ml) than those with inactive LN (73.1±17.0 U/ml, $p<0.0001$), NS (7.0±1.9 U/ml, $p<0.0001$) or HC (3.3±0.9 U/ml, $p<0.0001$, Fig. 2a). Also, in children with inactive LN, mean anti-C1q antibody levels were higher than in children with NS ($p<0.0001$) or HC ($p<0.0001$, Fig. 2a). Among children with active LN, those with WHO class IV nephritis showed the highest levels of anti-C1q (Fig. 2b)

Low levels of the first component of the classical pathway C1q in active lupus nephritis

Mean concentrations of C1q in children with active LN (74.4±8.3 µg/ml) were significantly lower than those in children with inactive LN (175.4±16.6 µg/ml, $p<0.0001$), NS (115.3±11.1 µg/ml, $p=0.0082$) or HC (135.0±7.224 µg/ml, $p<0.0001$), Fig. 3. Children with inactive LN, however, had increased C1q compared to children with NS ($p=0.0087$) as well as HC ($p=0.0474$), Fig. 3. In children with LN, a significant inverse correlation was detected between the levels of anti-C1q antibodies and circulating C1q ($r=-0.7213$, $p<0.0001$).

High concentrations of Bb, C3d fixing CIC, and C5a in active LN

In children with active LN, the mean concentrations of Bb (2.59±0.22 µg/ml) were significantly higher than those in children with inactive LN (1.68±0.13 µg/ml, $p=0.0004$), and the latter again were higher than in children with NS (0.94±0.07 µg/ml, $p=0.0003$) or HC (0.89±0.05 µg/ml, $p=0.0003$, Fig. 4a).

As shown in Fig. 4b and c, children with active LN showed high levels of C3d fixing CIC (42.3±7.2 µg Eq./ml) and of C5a (5.83±0.72 µg/l), which were significantly different from those in children with inactive LN (10.8±1.5 µg Eq./ml, $p<0.0001$; 0.51±0.11 µg/l, $p<0.0001$, respectively), NS (8.3±0.84 µg Eq./ml, $p<0.0001$; 0.22±0.05 µg/l, $p<0.0001$, respectively) or HC (6.2±0.41 µg Eq./ml, $p<0.0001$; 0.19±0.04 µg/l, $p<0.0001$, respectively).

Changes of soluble VCAM-1 concentrations according to LN activity

As shown in Fig. 5, sVCAM-1 was increased in children with active LN (1,255±116 ng/ml) compared to inactive LN (674±31 ng/ml, $p=0.0004$), NS (607±35 ng/ml, $p<0.0001$) or HC (596±29 ng/ml, $p<0.0001$). However, there were no significant differences in sVCAM-1 concentrations among the other groups (inactive LN, NS, HC). ROC curves of sensitivity vs. 1 - specificity were used to determine whether sVCAM-1 could discriminate children with active from those with inactive LN. A sVCAM-1 value of 769 ng/ml had a sensitivity of 86% and specificity of 80% for discriminating active from inactive LN. The AUC for sVCAM-1 was 0.93, thus sVCAM-1 can be used to identify active LN in children.

Longitudinal studies in individual patients with LN

From seven patients, blood samples were obtained twice for analysis in the active and inactive phase of LN. Changes of the immune system, complement and endothelial cell activation markers in these patients, the treatment regimens given at the time of diagnosis of active LN, the period of time between active and inactive phase of LN, between diagnosis of active or inactive LN and study are shown in Table 3.

Correlations of C3, C4, and anti-dsDNA with markers for immune system or endothelial cell activation and complement-activation fragments in LN

While in children with LN the complement-activation fragments correlated highly ($p<0.001$) with the markers for immune system and endothelial cell activation and C1q, complement C3 correlated moderately ($p<0.01$),

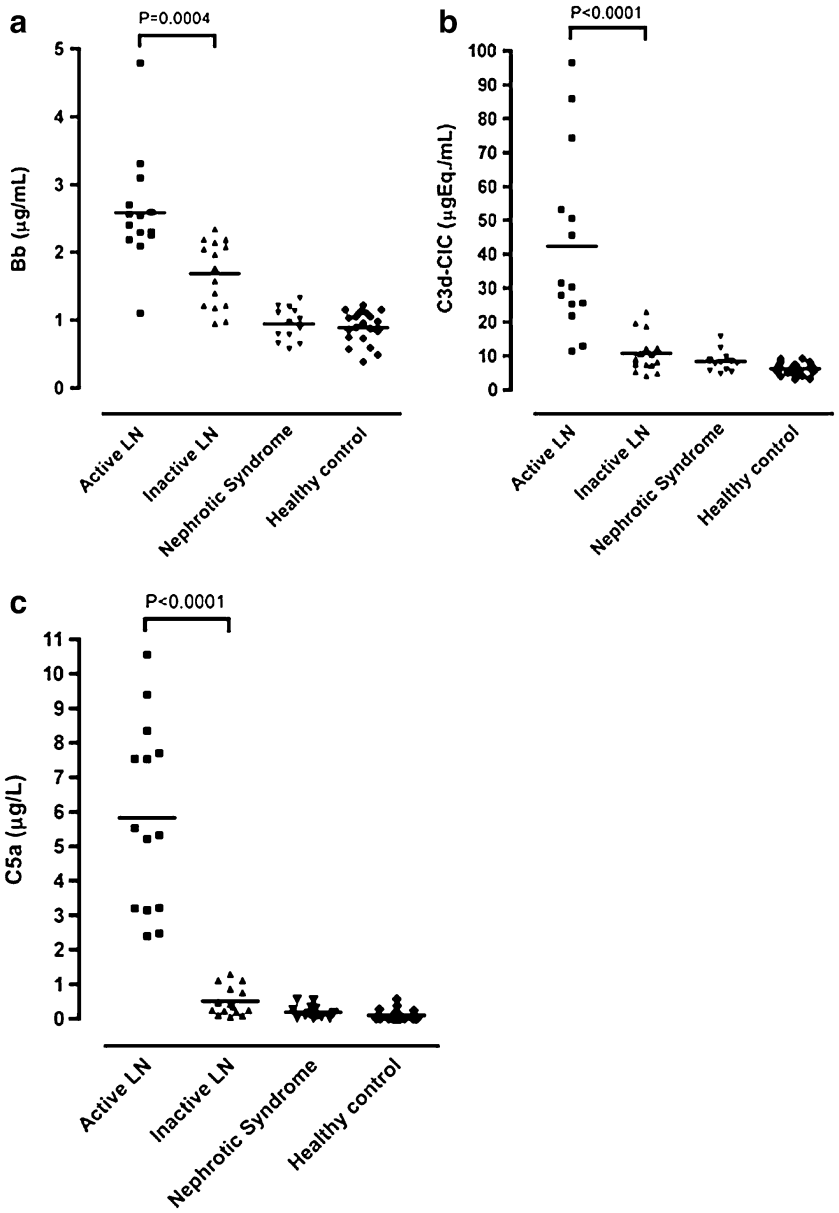

Fig. 4 High levels of complement split products in active LN. In children with active or inactive LN, NS, or age-matched healthy controls plasma concentrations of **a** Bb, **b** C3d-CIC, and **c** C5a was measured using ELISA. *Symbols* represent individual data points, and the *horizontal lines* represent means

complement C4 slightly ($p<0.05$), and anti-dsDNA correlated slightly only with BAFF, C1q, and antiC1q (Table 4).

Discussion

In this study, we demonstrated that in children with LN complement split products of the alternative (Bb), classical (C3d-CIC), and common complement pathway (C5a) were significantly increased while C1q was reduced during active disease. This study is the first to compare levels of BAFF, RANTES, anti-C1q antibodies, complement activation products, and sVCAM-1 in the same pediatric patients with LN, thus taking into consideration the multifactorial pathogenesis. We also found significant correlations between these parameters and with LN activity.

Considering the cascade of immunological and complement-related events leading to renal inflammation, we propose that in children with LN the measurement of Bb, C1q, anti-C1q antibodies, C3d-CIC, C5a, BAFF, RANTES, and sVCAM-1 allows early and reliable detection of renal flares and of response to therapy, prior to changes in complement C3 and C4, renal function, proteinuria, and urinary sediment.

However, our observations are limited to a small study population. Validation in large longitudinal studies that also include recently described urinary biomarkers for disease activity in childhood LN [4, 36–38], correlations with the activity and chronicity scores in renal biopsy, and lupus

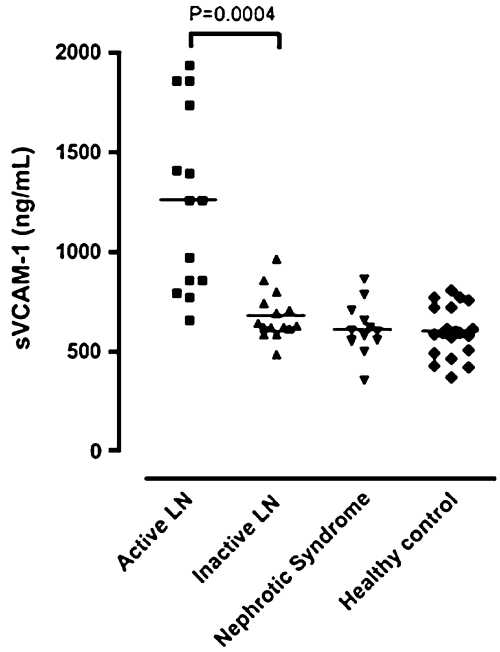

Fig. 5 Rise of sVCAM-1 in children with active LN. sVCAM levels were detected in serum samples from children with active or inactive LN, NS, or healthy age-matched controls. Each *symbol* indicates an individual subject. The mean for each group is shown as a *horizontal line*

patients without nephritis is required to enhance the clinical utility, to assess the precise predictive value of these parameters, and to define levels that indicate the necessity for therapeutic intervention or discontinuation.

Auto-antibodies against C1q are directed against a highly functional molecule that plays important roles in preventing autoimmunity [39–41]. Here, we show a strong correlation between the occurrence of anti-C1q antibodies, up-regulation of BAFF, consumption of C1q, and elevated levels of sVCAM-1 in children with active LN. Macrophages and dendritic cells that are significantly altered in patients with SLE are the major source of C1q [42–44]. IFN-α that is highly expressed during active LN [45, 46] inhibits the synthesis of C1q, and thus could contribute (in addition to anti-C1q antibodies) to the low levels of C1q in active LN, while the distinct cytokine milieu in inactive LN might favor the synthesis of C1q, which could explain the elevated levels observed in our study.

There is increasing evidence from animal models that the alternative pathway is importantly involved in LN [47, 48]. Here, we show that in children with LN, the alternative complement pathway is indeed activated during renal flares, indicating its pathogenic significance and diagnostic value. The major cause of complement activation in SLE although is thought to be the formation of immune complexes that in turn activate complement via the classical pathway [49]. In children with active LN we found high levels of circulating C3d fixing CIC. This is in line with previous studies in adults, showing that serum levels of C3d-fixing immune complexes correlate significantly with renal mesangial C3d deposits and disease activity [50].

The anaphylatoxins, especially C5a, are the key mediators of the complement system that induce renal injury [51, 52]. Experimental models showed a critical role of C5a in the pathogenesis of LN; and inhibition of C5 was found to be protective [53, 54]. As demonstrated, children suffering from LN have high C5a levels during active disease, indicating its pathogenic significance in childhood LN.

Complement activation enhances leukocyte infiltration and the production of pro-inflammatory cytokines in the kidney [55]. VCAM-1 induced in endothelial cells following activation by cytokines [56] is expressed in lupus nephritis [57, 58]. Children with systemic complement activation and active LN had high levels of sVCAM-1, suggesting that sVCAM-1 can serve as a marker of the ongoing inflammatory processes in LN.

Although current treatment regimens as demonstrated are able to suppress disease activity and complement activation in children with LN, medication associated toxicity is high. Moreover, despite treatment, some patients

Table 4 Correlations of C3, C4, and anti-dsDNA with markers for immune system or endothelial cell activation and complement-activation fragments

	Bb r / p	C1q r / p	antiC1q ab r / p	C3d-CIC r / p	C5a r / p	BAFF r / p	RANTES r / p	sVCAM r / p
C3	-0.48 / 0.0144	0.50 / 0.0106	-0.57 / 0.0032	-0.61 / 0.0012	-0.50 / 0.0111	-0.48 / 0.0141	-0.57 / 0.0032	-0.66 / 0.0079
C4	-0.43 / 0.0313	0.45 / 0.0231	-0.45 / 0.0251	-0.50 / 0.0116	-0.47 / 0.0158	-0.47 / 0.017	-0.42 / 0.0347	-0.42 / 0.0350
dsDNA	0.27 / 0.2032	-0.42 / 0.0379	0.41 / 0.0399	0.42 / 0.0455	0.32 / 0.1142	0.43 / 0.0400	0.33 / 0.1053	0.33 / 0.1118
BAFF	0.58 / 0.0009	-0.73 / 0.0013	0.64 / 0.0002	0.65 / 0.0001	0.66 / 0.0004			
RANTES	0.59 / 0.0007	-0.83 / 0.0001	0.88 / 0.0001	0.61 / 0.0005	0.59 / 0.0009			
sVCAM	0.59 / 0.0007	-0.60 / 0.0006	0.59 / 0.0009	0.58 / 0.0009	0.61 / 0.0005			

dsDNA anti-double-stranded DNA antibodies; *antiC1q ab* anti-C1q antibodies, *C3d-CIC* C3d fixing circulating immune complexes

develop progressive renal injury resulting in end-stage renal disease, and those patients who respond to treatment remain at risk of disease relapse [59].

The use of inhibitors of the terminal complement pathway that leave the beneficial effects of the classical pathway unaffected may represent an important therapeutic strategy for human LN.

We conclude that the magnitude of complement activation occurring during flares of LN in childhood can be quantified by measuring its split products. In view of the multi-factorial pathogenesis, we propose that the ensemble of the markers, namely BAFF, RANTES, complement components of the classical, alternative, and common pathway (C1q, Bb, C3d-CIC, C5a), antibodies to C1q and sVCAM-1, may be of great value in the early identification of flares or remissions of childhood LN to reliably measure a clinical response and thus to guide therapy in daily clinical practice. Moreover, their use may provide an important advance in the assessment of new treatments and in determining the optimization of their use.

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