Journal of Translational Autoimmunity 3 (2020) 100027

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.journals.elsevier.com/journal-of-translational-autoimmunity/

Expression of BAFF, APRIL, and cognate receptor genes in lupus nephritis and potential use as urinary biomarkers

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ARTICLE INFO

Keywords:
BAFF
APRIL
Lupus nephritis
Systemic lupus erythematosus
Gene expression
Urinary biomarkers

ABSTRACT

Background: B-cell activating factor (BAFF), a proliferation-inducing ligand (APRIL), and their receptors BAFF-R, BCMA, and TACI are crucial factors for the survival of B lymphocytes. Recent evidence has also demonstrated the importance of BAFF/APRIL signaling in lupus nephritis (LN). This study evaluated the relationships between LN clinical characteristics and the urinary expression levels of BAFF, APRIL, and cognate receptors to assess their potential value as disease biomarkers.

Methods: Expression levels of these genes were assessed in urine samples collected from systemic lupus erythematosus (SLE) patients before renal biopsy using reverse transcription real-time PCR.

Results: Thirty-five patients with LN were included. Most of the patients were female (82.86%) with median Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) of 15. BAFF mRNA was detectable in 28.57%, APRIL mRNA in 42.85%, BR3 mRNA in 48.57%, and TACI mRNA in 42.85% of urine samples. On the other hand, urinary (u)BCMA mRNA was not found in any sample. Urinary expression of most biomarkers was detected with greater frequency in class III and IV LN compared to class V LN. The expression level of uBR3 mRNA was correlated with SLEDAI-2K and histological activity index.

Conclusion: Urinary expression of BAFF/APRIL signaling factors, especially TACI, APRIL, and BR3 mRNAs, may be useful biomarkers for LN.

Authors' contribution

David Aguirre-Valencia, MD: Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration. Lady J. Ríos-Serna, BSc: Methodology, Validation, Writing - Original Draft, Visualization. Iván Posso-Osorio, MD: Investigation, Resources. Juan Naranjo-Escobar, MD: Investigation, Resources. Daniel López, MD: Investigation, Resources. Vanessa Bedoya-Joaqui, MD: Investigation, Writing - Original Draft, Visualization. Ivana Nieto-Aristizábal, MD: Investigation, Resources, Writing - Review & Editing, Visualization. Andrés M. Castro: Formal analysis. Lorena Díaz-Ordoñez, BSc, MSc: Methodology. Erika P. Navarro, MD: Investigation, Resources. María-Claudia Barrera, BSc, MSc: Methodology, Resources. Alex Echeverri, MD: Conceptualization, Resources. Carlos A. Cañas, MD: Conceptualization, Resources, Writing - Review & Editing. Gabriel J. Tobón, MD, PhD: Conceptualization, Methodology, Validation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision.

1. Introduction

Systemic lupus erythematosus (SLE) is a potentially fatal chronic autoimmune disease that can target multiple organ systems [1]. Clinically significant renal involvement (lupus nephritis, LN) is frequent in SLE, and this pathology can affect any structural component of the kidney

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https://doi.org/10.1016/j.jtauto.2019.100027
Received 26 August 2019; Received in revised form 10 December 2019; Accepted 13 December 2019

with varying degrees of severity [2]. About 50%–60% of SLE patients exhibit LN [2,3], and severe LN can significantly raise disease-related morbidity and mortality [4].

The pathogenesis of SLE is complex, involving the defective clearance of apoptotic bodies, immune-complex deposition, and activation of type I interferons (IFNs) among other aberrant processes. Elevated IFNα increases production of B-cell activating factor (BAFF), a cytokine of the tumor necrosis factor [TNF] family [5–7] crucial for peripheral B-cell maturation and regulation of B-cell survival [8–11]. The cytokines BAFF and a proliferation-inducing ligand (APRIL) exert critical regulatory effects on B lineage cells through interactions with three distinct receptors: TNF receptor superfamily member 13C (also known as the BAFF receptor [BAFF-R] or BLyS receptor 3 [BR3]), TNF receptor superfamily member 17 (also known as B-cell maturation antigen [BCMA]), and TNF receptor superfamily member 13B (also known as transmembrane activator and cyclophilin ligand interactor [TACI]) [8,12]. Both BAFF and APRIL can bind to BCMA and TACI, whereas BAFF also binds to BR3 [14,15].

Both BAFF and APRIL have been implicated in autoimmune diseases like SLE and Sjögren's syndrome [13,16–19]. In mouse models of LN, elevated serum BAFF correlates with double-stranded (ds) DNA autoantibody level, disease severity, and immune-complex glomerulonephritis [12,20–22]. Furthermore, the selective blockade of BAFF prevents the development of LN in mice [23]. B-cell infiltration has also been demonstrated in the kidneys of proliferative LN patients [24], resulting in elevated renal BAFF and APRIL that can be detected in urinary sediment [25], suggesting potential utility of these factors as urinary disease biomarkers.

Several animal model studies have demonstrated pathogenic roles for BAFF, APRIL, and their receptors in SLE and LN, and human clinical studies have demonstrated immunohistochemical expression of BAFF in glomeruli and tubular epithelial cells [24–29]. However, there are many obvious advantages of urinary detection. Nonetheless, there have been only two published studies of urinary BAFF and APRIL [25,30], while no study has examined the presence of their receptors (BR3, TACI, and BCMA) in urinary sediment. In this study, we assessed the mRNA expression levels of BAFF, APRIL, and cognate receptors in the urinary sediment of LN patients to examine their potential as disease biomarkers.

2. Material and methods

2.1. Research design

This was a cross-sectional study conducted from April 2016 through April 2019 at the Rheumatology Unit, Fundación Valle del Lili, a regional referral hospital in Cali, Colombia. Patients were enrolled in the course of time in a non-probability sampling mode of convenience.

2.2. Study population

Thirty-five patients with SLE according to the 2012 Classification Criteria for Systemic Lupus Erythematosus (SLICC) [31] and active LN (as confirmed by subsequent kidney biopsy) were enrolled in the study. The criteria for active LN were urine proteinuria >0.5 g/day and urine red blood cells (RBC)/white blood cells (WBC) > 5/high power field or raised serum creatinine > 0.3 mg/dL [32]. The Department of Pathology provided LN classification (comporting with the International Society of Nephrology/Renal Pathology Society (ISN/RNP) 2004 classification) [33], as well as activity and chronicity indexes. Urine samples were obtained on the day of kidney biopsy prior to the procedure (in order to avoid interference by eventual haematuria); and also prior to any immunosuppressant or induction scheme for LN. The use of urine samples was approved by the Ethics Committee for Human Research of the Fundación Valle del Lili (IRB No. 977) in adherence to the Declaration of Helsinki. All subjects were fully informed about the purpose and methods of the present study and gave informed consent.

2.3. Lupus activity parameters

Lupus severity was evaluated by standard clinical serologic activity measures (anti-dsDNA, C3, C4, renal function) and urine measures (proteinuria/creatinuria ratio and active urinary sediment).

The following standard values of serologic activity markers were determined: anti-dsDNA by enzyme-linked immunosorbent assay if 30–200 IU/mL or indirect immunofluorescence (IIF) if titer ≥1:10, low C3 (<90 mg/dL) and low C4 (<10 mg/dL) by enzyme immunoassay, creatinine (mg/dl) and blood urea nitrogen (mg/dL) by enzymatic methods, proteinuria/creatinuria ratio by immunoturbidimetric assay/enzymatic methods, and active urinary sediment by microscopy.

2.4. Isolation of RNA

Urine specimens were collected in 50-mL sterile disposable centrifuge tubes and centrifuged at 3,000 g for 30 min at 4 °C. The supernatant and pellet were separated and stored at −80 °C until processing. Total RNA was isolated from the pellet using the High Pure RNA Isolation Kit (Roche Diagnostics, Indianapolis, IN, USA) according to a standardized protocol. Briefly, the urinary cell pellet was resuspended in 200 μL PBS and lysed by adding 400 μL Lysis/Binding Buffer and vortexing for 15 s. The lysate was purified by centrifugation for 30 s at 12000 rpm in a High Pure Filter Tube (Roche). Isolated RNAs were then incubated for 15 min at environmental temperature with 100 μL DNAsa mix, washed twice with 500 μL Wash buffer I and II by centrifugation for 30 s at 12000 rpm, and washed again with 200 μL Wash buffer II by centrifugation for 2 min at maximum speed. Finally, 60 μL of elution buffer was added, the tubes incubated for 5 min at room temperature, and RNAs retrieved from the tube filters by centrifugation for 1 min at 12000 rpm. The quantity and purity of the isolated RNA were verified by absorbance at 260 nm and 260 nm:280 nm absorbance ratio, respectively, using the NanoDrop® 2000 Spectrophotometer (Thermo Fisher Scientific Inc.). First-strand cDNA was synthesized using the Transcriptor First-Strand cDNA Synthesis Kit (Roche Diagnostics) according to the manufacturer's instructions.

2.5. Evaluation of gene expression

Urinary mRNA levels of BAFF APRIL, TACI, BR3, and BCMA were evaluated by real-time quantitative polymerase chain reaction (qPCR) using predesigned primers (Sigma-Aldrich, Inc. St Louis, USA). Expression of the GAPDH gene was used as the endogenous reference standard (Table 1).

Each PCR reaction was run in duplicate using the FastStart Essential DNA Green Master Kit (Roche Diagnostics) in a 20-μL reaction volume composed of 10 μL 2x Master Mix, 1 μL reverse primer (10 mM), 1 μL forward primer (10 mM), 6 μL RNAse-free water, and 2 μL of cDNA template. The amplification reaction was performed using an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and the following thermocycle settings: 50 °C for 2 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for 45 s. The comparative ΔCt method was used to estimate gene expression. Dissociation curve analysis was conducted to confirm the specificity of the amplicons.

2.6. Statistical analysis

All statistical analyses were performed using Stata® version 14 (StataCorp, College Station, TX, USA). Continuous variables are expressed as mean ± standard error or median (interquartile range: IQR) as appropriate and categorical variables as frequencies and percentages. Group means/medians were compared by Mann-Whitney test and frequencies by Chi-square or Fisher's exact test. Spearman's rank correlation analysis was used to test for potential associations of lupus activity and renal parameters with urinary gene expression levels. A $p < 0.05$

Table 1
Primers used to amplify the genes tested.

Gene	Direction of primer	Primer Sequence (5'→3')	Length of PCR product (bp)
BAFF	Sense	AAGTGCCCTAGAAGAAAAAG	162
	Anti-Sense	AAAGTCACCAGACTCAATTC	
APRIL	Sense	CTTTAACTGTGAGGTTACCAAC	177
	Anti-Sense	TCTTCCTCATCTTCTCCTTC	
TACI	Sense	AGTTCTATGACCATCTCCTG	79
	Anti-Sense	AAGTATGCACATTGCTTAGG	
BR3	Sense	AGACCTCACCATCTTTGAC	92
	Anti-Sense	CGGTCTTTTACTCATAGTGC	
BCMA	Sense	TTGCATACCTTGTCAACTTC	91
	Anti-Sense	TTTCACTGAATTGGTCACAC	
GAPDH	Sense	TGCACCACCAACTGCTTAGC	87
	Anti-Sense	GGCATGGACTGTGGTCATGA	

(two-tailed) was considered statistically significant for all tests.

3. Results

3.1. Patient characteristics

Thirty-five patients with LN were studied for urinary expression of the target genes (mean age 31.9 ± 12.35 years and 82.86% female). The median duration of the disease before kidney biopsy was 48 months (IQR: 12–96 months). The medication before the procedure consisted on corticosteroid pulses. None of the patients received Rituximab, Belimumab, nor induction scheme for LN prior to renal biopsy. Median Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) at the time of LN diagnosis was 15 (IQR: 8–21). Levels of serum complement C3 and C4 were low in 74.29% and 60% of the LN patients, respectively, whereas most patients presented high anti-dsDNA titers (91.46%). Most patients had severe proteinuria (median proteinuria/creatinuria ratio of 2.3, IQR: 1.3–5.5) but normal serum creatinine (median 0.95 mg/dL IQR: 0.66–2.07). Median activity and chronicity indexes were 4 (2–11) and 3 (0–6), respectively (Table 2).

3.2. Detection frequencies of BAFF, APRIL, and cognate receptors in urine samples from LN patients

Urinary (u)BAFF mRNA was detectable in 28.57% of all samples, uAPRIL mRNA in 42.85%, uBR3 mRNA in 48.57%, and uTACI in 42.85%, while uBCMA mRNA was not found in any sample. In two patients with class II mesangial nephritis, only uAPRIL mRNA was found. Expression of uBAFF and uAPRIL mRNA was detected in both class III and IV LN patients. Two patients with class III LN exhibited expression of uBR3 and uTACI mRNA. Expression of uBR3 mRNA was present in 6/10 patients with class IV LN, while uTACI mRNA was present in 4/10. Little expression of either cytokines or receptors was detected in isolated class V LN (Table 3).

3.3. Correlations of urinary mRNA expression with lupus activity and renal parameters

Expression of uBAFF mRNA was not associated with any measured parameter or variable. On the other hand, uAPRIL mRNA expression was significantly correlated with epithelial cell casts ($r = 0.51, p \leq 0.001$), but not with other lupus activity or renal parameters. Both uBR3 and uTACI mRNA levels were positively correlated with granular casts ($r = 0.50$, $p < 0.001$ and $r = 0.45$, $p \leq 0.01$, respectively) and anti-dsDNA antibody titer ($r = 0.48$, $p < 0.01$ and $r = 0.40$, $p \leq 0.05$, respectively). In addition, we found a significant correlation of uTACI mRNA with low C4 ($r = 0.49$, $p < 0.05$) and of uBR3 mRNA with SLEDAI-2K ($r = 0.33$, $p < 0.05$) and histological activity index ($r = 0.35$, $p < 0.05$) (Table 4).

Table 2
Patient characteristic at baseline.

	Active LN
	n = 35
Age, years[+]	31.9 (±12.3)
Female, n (%)	29 (82.9)
Duration of SLE, months[++]	48 (12–96)
SLEDAI-2K index[++]	15 (8–21)
Immunological parameters, n (%)	
Anti-dsDNA (IFI), dilutions[++]	320 (10–1280)
Anti-dsDNA (ELISA)	32 (91.4)
Low C3	26 (74.3)
Low C4	21 (60)
Renal parameters	
Serum creatinine, mg/dL[++]	0.95 (0.66–2.07)
Blood urea nitrogen, mg/dL[++]	19.8 (14–37.9)
Proteinuria/creatinuria ratio[++]	2.3 (1.3–5.5)
Urinary sediment, n (%)	
Epithelial cell casts	26 (74.3)
Granular casts	8 (22.9)
Hyaline casts	17 (48.6)
Haematuria	16 (45.7)
Histological parameters, n (%)	
Class I LN	1 (2.9)
Class II LN	2 (5.7)
Class III LN	5 (14.3)
Class IV LN	10 (28.6)
Class V LN	2 (5.7)
Class VI LN	2 (5.7)
Class III-IV LN	1 (2.9)
Class III-V LN	6 (17.1)
Class IV-V LN	6 (17.1)
Histological Indexes	
Activity index[++]	4 (2–11)
Chronicity index[++]	3 (0–6)

Data are expressed as [+]mean and standard error (SE), [++]median and interquartile range (IR), or as number (percentage). LN, Lupus Nephritis; SLE, Systemic Lupus Erythematous; SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000); Anti-dsDNA, Anti-double stranded DNA; IFI, Indirect Immunofluorescence; ELISA, Enzyme-Linked ImmunoSorbent Assay.

4. Discussion

Lupus nephritis is one of the most common and severe manifestations of SLE, and BAFF/APRIL signaling appears central to SLE pathogenesis. Mackay et al. reported that overexpression of BAFF in mice was sufficient to initiate the expansion of mature B-cells and induce lupus-like autoimmune manifestations such as high dsDNA antibody titer, circulating immune complexes, and glomerulonephritis with immunoglobulin deposits [18].

Renal biopsy is currently the gold standard for diagnosis, prognosis, and treatment guidance of LN. However, renal biopsy cannot be performed sequentially to assess disease course or therapeutic response due to its invasive nature. Therefore, it is necessary to identify non-invasive biomarkers that can be easily obtained and reflect current renal inflammation [34]. Over the last two decades, many clinical studies have demonstrated the presence of molecules associated with B-cell activation in the serum [29,35–37] and kidney tissues [24,26,28,38] of active LN patients. One study demonstrated that serum BAFF level was lower in SLE patients with nephrotic-range proteinuria than in SLE patients with less severe proteinuria. Therefore, it has been suggested that urinary excretion of cytokines may reduce serum concentrations in renal disease patients [39].

Urinary cytokines may reflect the current status of kidney inflammation and tissue damage, so we evaluated the mRNA expression levels of uBAFF, uAPRIL, and cognate receptors as possible urinary biomarkers of LN. Urinary BAFF mRNA was detectable in only a minority of patients (28.57%), mainly of class III and IV, and showed no correlation with other parameters of lupus activity or renal function, consistent with previous studies [25,30]. In contrast, we found that BAFF was detectable

Table 3
Histopathological findings in the urinary sediment of 35 patients with LN.

Patients	LN Class	Activity index	Chronicity index	mRNA uBAFF	mRNA uAPRIL	mRNA uBR3	mRNA uTACI	mRNA uBCMA
1	I	0	0	0	0	X	X	0
2	II	2	0	0	X	0	0	0
3	II	2	0	0	X	0	0	0
4	III	2	3	0	0	0	0	0
5	III	4	0	0	0	0	0	0
6	III	4	0	X	X	X	X	0
7	III	1	6	0	X	0	0	0
8	III	1	6	X	X	X	X	0
9	IV	6	9	0	0	0	0	0
10	IV	7	3	0	X	X	X	0
11	IV	11	1	X	X	X	X	0
12	IV	12	3	0	0	0	0	0
13	IV	15	3	0	0	X	X	0
14	IV	12	0	0	X	0	0	0
15	IV	10	2	0	0	X	0	0
16	IV	10	2	0	0	X	0	0
17	IV	1	8	X	0	0	0	0
18	IV	13	6	X	X	X	X	0
19	V	0	2	0	0	0	0	0
20	V	2	0	0	0	0	0	0
21	VI	0	12	0	0	0	0	0
22	VI	3	10	X	X	X	X	0
23	III-IV	8	1	X	X	X	X	0
24	III-V	5	3	X	0	X	X	0
25	III-V	4	4	X	0	X	X	0
26	III-V	5	0	0	0	0	0	0
27	III-V	2	6	0	0	0	0	0
28	III-V	4	2	0	0	X	X	0
29	III-V	3	0	X	X	X	X	0
30	IV-V	9	3	0	X	0	0	0
31	IV-V	14	8	0	0	0	0	0
32	IV-V	1	5	0	0	0	0	0
33	IV-V	13	2	0	X	X	X	0
34	IV-V	12	4	0	0	0	0	0
35	IV-V	12	2	0	X	X	X	0

LN, Lupus Nephritis; uBAFF, urinary B-cell activating factor of the tumor necrosis factor family; uAPRIL, urinary a proliferation-inducing ligand; uBR3, urinary BLyS receptor 3; uTACI, urinary transmembrane activator and cyclophilin ligand interactor; uBCMA, urinary Bcell maturation antigen.

by immunohistochemistry in the majority of 52 kidney biopsy samples from LN patients [26]. Furthermore, patients with class IV LN exhibited the highest glomerular BAFF expression, and expression was correlated with activity index ($r = 0.4647$, $p = 0.0001$). The discrepancy between urinary and glomerular BAFF may be explained by renal retention of membrane-expressed BAFF.

Like BAFF, APRIL has been investigated as a non-invasive prognostic biomarker for severe LN [29]. Expression levels of APRIL mRNA were

Table 4
Correlations of gene expression of BAFF and APRIL cytokines, and their receptors (BR3 and TACI), with lupus activity parameters and renal parameters in the urinary sediment of patients with LN.

	uBAFF		uAPRIL		uBR3		uTACI	
	Spearman's rank	*p*-value	Spearman's rank	*p*-value	Spearman's rank	*p*-value	Spearman's rank	*p*-value
Lupus activity parameters								
Anti-dsDNA (IFI)	0.02	*0.936*	−0.16	*0.396*	0.32	*0.086*	0.20	*0.295*
Anti-dsDNA (ELISA)	−0.03	*0.860*	−0.10	*0.598*	0.48	***0.005***	0.40	***0.024***
Low C3	0.13	*0.536*	0.32	*0.115*	−0.12	*0.563*	0.02	*0.990*
Low C4	0.27	*0.233*	0.11	*0.635*	0.21	*0.357*	0.49	***0.023***
SLEDAI-2K index	−0.07	*0.710*	−0.20	*0.242*	0.33	***0.049***	0.10	*0.579*
Renal parameters								
Serum creatinine	0.29	*0.089*	0.10	*0.569*	0.20	*0.242*	0.12	*0.478*
Blood urea nitrogen	0.15	*0.380*	0.22	*0.197*	0.21	*0.226*	0.14	*0.422*
Proteinuria/creatinuria ratio	0.05	*0.798*	−0.02	*0.906*	−0.20	*0.253*	−0.12	*0.493*
Epithelial cell casts	−0.12	*0.487*	0.51	***0.002***	0.11	*0.536*	−0.10	*0.556*
Granular casts	0.06	*0.713*	0.07	*0.675*	0.50	***0.002***	0.45	***0.007***
Hyaline casts	−0.14	*0.419*	−0.20	*0.243*	0.28	*0.098*	0.04	*0.806*
Haematuria	0.23	*0.188*	0.28	*0.099*	0.31	*0.066*	0.16	*0.357*
Activity index	−0.02	*0.888*	0.13	*0.461*	0.35	***0.039***	0.24	*0.165*
Chronicity index	0.11	*0.548*	−0.23	*0.192*	−0.19	*0.276*	−0.17	*0.322*

uBAFF, urinary B-cell activating factor of the tumor necrosis factor family; uAPRIL, urinary a proliferation-inducing ligand; uBR3, urinary BLyS receptor 3; uTACI, urinary transmembrane activator and cyclophilin ligand interactor; uBCMA, urinary B-cell maturation antigen; Anti-dsDNA, Anti-double stranded DNA; IFI, Indirect Immunofluorescence; ELISA, Enzyme-Linked ImmunoSorbent Assay, SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000).

point out the significant values obtained from the correlation analysis, it means, all the p-values <0.05.

Are all the values obtained from the correlation analysis, both the significant and the non-significant.

Are the headings of each column with the names of the parameters and the cytokines/cytokines receptors that where measured, with the statistical test carried out to obtain a p-value in each one.

significantly higher in glomeruli and the tubulointerstitial compartment of biopsy samples from patients with proliferative LN compared to controls as measured by real-time PCR [24]. Furthermore, uAPRIL levels correlated with renal SLEDAI ($r = 0.36$, $p = 0.033$) but not with complement levels, anti-dsDNA levels, non-renal SLEDAI, proteinuria, disease activity, or histological chronicity indexes [25]. In the present study, uAPRIL mRNA was detectable in 42.85% of all samples, mainly in class II, III and IV LN, and expression was moderately correlated with renal tubular epithelial cell casts ($r = 0.51$, $p \leq 0.0001$). Menke et al. [40] suggested that a rise in serum or urine CSF-1 produced by renal tubular epithelial cells may predict renal disease activity before clinical or paraclinical evidence of glomerular dysfunction, both on first occurrence and prior to recurrence. Similarly, APRIL is considered a crucial mediator of autoimmune kidney disease by stimulating autoimmune inflammation and macrophage influx. APRIL secretion can be stimulated with colony-stimulating factor-1 (CSF-1) in an inflammatory milieu [41], as has been demonstrated for BAFF in renal tubular epithelial cells [38]. These findings may explain the high frequency of APRIL detection in urine sediments.

Both BAFF and APRIL can interact with BCMA and TACI receptors, whereas BAFF also binds to BR3 in both mice and humans. Receptor expression patterns may reflect different disease phenotypes or severity, which could be valuable for guiding personalized treatment 14,15]. In our study, uBR3 and uTACI mRNAs were detected in 48.57% and 42.85% of patients, respectively, strongly suggesting involvement in LN pathophysiology. Further, uTACI expression was correlated with low urinary C4 and both receptors demonstrated moderate correlations with granular casts and anti-dsDNA. Zhao et al. reported that SLE patients with elevated anti-dsDNA antibody titers also demonstrated high TACI and BR3 mRNA expression in PBMCs (both $p < 0.01$) [42]. One study in 73 Chinese patients with SLE [43], found that TACI expression as measured by flow cytometry was strongly related to LN and indicated recurrence ($p = 0.002$), but there was no correlation with SLEDAI-2K, anti-dsDNA antibody, C3, or IgG. This is in contrast to glomerular expression, which was correlated with activity index ($r = 0.3462$; $p = 0.005$) in patients with class III and IV LN [26].

Additionality, we found positive correlations of uBR3 mRNA level with SLEDAI-2K ($r = 0.33$, $p < 0.05$) and histological activity index ($r = 0.35$, $p < 0.05$). However, this elevation in urinary uBR3 is at odds with the low immunohistochemical expression in kidney biopsy tissue of LN patients [24.26]. We suggest that the low BR3 expression in kidney tissue indicates over-activation of the BAFF-BR3 axis and concomitant receptor internalization, but the mechanisms leading to uBR3 mRNA expression are unclear. In contrast, uBCMA mRNA was not detected in the urinary sediment. The presence of these receptors likely reflects the specific cellularity of the urinary sediment (granular cast or renal tubular epithelial cells cast). Schwarting et al. [37] observed high BR3 expression and lower but still substantial BCMA expression in cultured human tubular epithelial cells and a renal tubular epithelial cell line (HK2), whereas no TACI mRNA was detected. However, all three receptors were overexpressed following stimulation with BAFF, CSF-1, and lipopolysaccharide. These discrepancies may stem from differences in study design or population but also underscore the complexity and lack of knowledge about the BAFF/APRIL signaling system.

This is the first study to assess the utility of the cytokines BAFF and APRIL as well as their main receptors as urinary biomarkers for LN. A major strength of this study is that urinary samples were obtained the same day as kidney biopsy, so these biomarkers reflect the current state of kidney inflammation and tissue damage. Further, collection on the same day mitigates potential interference by eventual haematuria. On the other hand, our study has several limitations, such as the small sample size from a single medical center. Further larger-scale studies are required to confirm the general validity of these findings. Moreover, SLE is clinically heterogeneous and mechanistically complex, so it is likely the multiple additional biomarkers must be identified for accurate diagnosis, prognosis, and treatment guidance.

In conclusion, despite evidence for high immunohistochemical expression of BAFF in the glomeruli of SLE patients, BAFF gene expression was detected in only a minority of urinary sediment samples. However, uAPRIL, uBR3, and uTACI were detected with greater frequency and expression levels were correlated with other disease parameters.

5. Study limitations

Patient enrollment was carried out for a period of time of three years, although the cross-sectional design, in addition to the inclusion criteria of the study made it difficult to achieve a bigger study sample, reason why the power of the study could be compromised. Additionally, sequential urine sampling was not performed; this could be part of further studies.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article. This work was funded by an institutional grant from Fundación Valle del Lili and Universidad Icesi.

Declarations of competing interset

None.

Acknowledgements

The researchers wish to thank Fundación Valle del Lili and Universidad Icesi for funding, Dr. Harry Pachajoa from Laboratory of Genomic Medicine from Universidad Icesi and Fundación Valle del Lili, and Adolfo Abadía for editorial assistance.

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