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Article

Intra-renal expression of B-cell activating factor (BAFF) in active proliferative lupus nephritis, a retrospective study

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Background: Systemic lupus erythematosus (SLE) is an auto-immune disease that affects various body systems. Approximately half of SLE patients develop lupus nephritis (LN). Lupus nephritis is classified into six classes, with classes III and IV being the most proliferative. Lupus nephritis severity and prognosis can be influenced by several biomarkers. Some of these biomarkers are used as therapeutic targets, including the B-cell activating factor (BAFF), which plays a fundamental role in SLE pathogenesis. A BAFF-targeted immunoglobulin G, Belimumab, has been approved by the FDA as an add-on treatment for active proliferative LN (pLN). The aim of the study was to explore the role of intra-renal BAFF expression as a potential severity stratifying tool and a predictor of response. ***Materials & Methods:*** Our study was retrospective, 40 patients who were diagnosed as active pLN were recruited after completion of induction therapy. All patients at baseline were clinically assessed by evaluating renal, serological and histopathological parameters. After 6 months of the start of induction therapy, follow-up response parameters were evaluated. Renal biopsies were extracted from the pathology archive and gene expression of BAFF was quantified using real-time PCR. ***Results:*** After follow-up, 21 patients were considered as early responders(eR) according to the reduction of urinary protein creatinine ratio and stabilization of the estimated glomerular filtration rate. Baseline intra-renal BAFF gene expression was higher in non-responders (eNR) than responders (1.7 vs 1.48, p=0.741), although that was statistically non-significant. We could not find a significant correlation between BAFF gene expression and pertinent baseline or follow-up response parameters. ***Conclusion:*** Although median BAFF expression was higher in eNR than eR, we could

not conclude a definite relation between intra-renal BAFF and other severity assessments or response parameters and could not be solely used as a stratifying biomarker.

Keywords: Lupus, lupus nephritis, B-cell activating factor.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects 43 per 100,000 individuals of the overall population.[1] Many organ systems could be affected by SLE notably, the skin, mucous membranes, musculoskeletal system, and kidneys. Approximately 40- 50% of SLE patients suffer from lupus nephritis (LN) throughout the course of the disease.[2] Lupus nephritis is considered a major morbidity affecting the life of lupus patients.[3, 4] In 2003, the International Society of Nephrology/Renal Pathology Society (ISN/RPS) categorized LN into 6 classes, of which classes III and IV (proliferative classes) are considered the most active.[5]

Many clinical as well as laboratory biomarkers are thought to reflect the severity and prognosis of LN. A lot of them are still investigational and some are considered therapeutic targets to be explored in clinical trials.[6] The role of the B-cell activating factor (BAFF), a cytokine belonging to the TNF family, is one of the most studied aspects in the immune-pathogenesis of SLE.[7] Initial studies investigated the role of belimumab in LN without enough encouraging results, however, recent studies reported increased efficacy when added to standard therapy. [8-10] Belimumab was recently approved by the Food and Drug Administration (FDA) as add-on treatments for the induction of active proliferative LN (pLN).

The purpose of our study was to explore whether intra-renal BAFF expression could serve as an early response predictor in patients who had been diagnosed with active pLN. Additionally, we aimed to determine the correlation, if any, between BAFF expression and pLN activity.

Patients and Methods

We conducted a retrospective study, A total of 40 LN patients, who had adequate renal biopsy samples- taken at the time of LN activity- available at the pathology department archive of Alexandria Main University Hospital, were included. The diagnosis of SLE was initially made by applying the classification criteria established by the Systemic Lupus International Collaborating Clinics (SLICC).[11] A diagnosis of LN was made following the detection of the urinary protein of more than 0.5 g/

mg creatinine by the urinary protein creatinine ratio (u-PCR) quantification method in addition to a renal biopsy. Renal biopsy samples were kept in paraffin blocks until used for analysis.

Data collection

Data collected at baseline included demographics, presence of hypertension (HTN) and edema, time between SLE onset and LN diagnosis, current and previous lupus activity, previous medications, routine laboratory results e.g., complete blood count (CBC), u-PCR, creatinine, urea, glomerular filtration rate estimation (eGFR) using the CKD-Epi (chronic kidney disease epidemiology) equation,[12] serum Albumin, and urine analysis. Abnormalities in serological parameters (Anti-double stranded deoxy-ribonucleic acid (anti-dsDNA), complement 3 and 4 (c3, c4), and renal histopathologic findings were recorded. SLE disease activity index (SLEDAI-2K)[13], renal SLEDAI (rSLEDAI) were evaluated as well.

Laboratory Analysis

Serum creatinine, urinary protein, and urinary creatinine were measured using chemistry analyzer Dimension RxL Max (Siemens Healthineers, Germany). The estimated glomerular filtration rate (eGFR) was recorded using the 2021 CKD-EPI equation. Complements 3 and 4 were measured using BN ProSpec system (Siemens Healthineers, Germany). Anti-dsDNA was measured using BIO-FLASH chemiluminescent analyzer (INOVA Diagnostics, USA).

Histopathological investigations

At initial analysis before treatment, 3 to 5 mm thick formalin-fixed, paraffin-embedded (FFPE) renal biopsy sections were prepared with hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff, and periodic acid-silver methenamine stains for light microscopy examination and were assessed by 2 pathologists.

Lupus nephritis classification, as well as chronicity and activity indices (out of 12 and 24, respectively), were determined in accordance to the revised 2018 ISN/RPS classification for LN; the activity index was measured by adding the scores of; "endocapillary proliferation (out of 3), fibrinoid necrosis (out of 6), cellular or fibro-cellular crescents (out of 6), neutrophilic infiltration and karyorrhexis (out of 3), wire looping/hyaline thrombi (out of 3) and interstitial inflammation (out of 3)". The index of chronicity was estimated by adding the semi-quantitative scores of; "glomerular sclerosis (out of 3), fibrous crescents (out of 3), interstitial fibrosis (out of 3), and tubular atrophy (out of 3)"[5]. For renal biopsies taken before 2019, a revised examination was done to adjust to the updated 2018 activity and chronicity indices criteria [5]. After recollection for BAFF expression analysis, for each paraffin block, three sections were discarded and then eight sections of 5 μm thicknesses were cut using a scalpel and transferred into a sterile microcentrifuge tube.

Treatment Received

All patients received standard induction protocols for treatment of active pLN which included pulse steroid therapy (500 mg-1 g daily for 3 days) followed by mycophenolate mofetil 2-3 g per day or monthly cyclophosphamide "INH protocol" (0.5–1 g/m2 for 6 months) or "Eurolupus protocol" (500 mg every 2 weeks for 6 doses). Maintenance therapy was constituted of either azathioprine 2-2.5 mg/kg daily or mycophenolate mofetil 1-2 mg daily.[14]

Assessment of Response

Patients continued their follow-up regularly in the lupus clinic. At 6-month follow-up, variations in the previously reported clinical findings, laboratory parameters, SLEDAI-2K, and rSLEDAI were recorded. Patients were subsequently divided according to renal response into early responders (eR) and early non-responders (eNR) as defined by "the reduction of u-PCR by 50% or more and a normal or nearly-normal GFR (or, if previously abnormal, within 10% of the range of normal GFR)". [15]

Renal biopsy sample re-collection

Retrospectively, 0 adequate (≥ 9 glomeruli) renal core biopsies which were formalin-fixed, paraffin-embedded (FFPE) from LN patients were extracted from the pathology department archives. Relative quantification intra-renal gene expression of BAFF using quantitative real-time polymerase chain reaction (qRT-PCR) was performed.

RNA extraction:

Total RNA was extracted from FFPE renal tissues, by using RNeasy FFPE Kit (Qiagen, USA), according to the manufacturer's manual. The RNA concentration and purity were assessed by Nano-drop 2000/2000c spectro-photometer (ThermoFisher Scientific, USA).

- **Reverse Transcription (RT)**

Genomic RNA was transformed into complementary DNA (cDNA) using Revert-Aid First Strand cDNA Synthesis kit (ThermoFisher Scientific, USA). One µg of total RNA was added to the RT reaction mix in a total volume of 20 µL. Twenty microliters of RT reaction mix were prepared by adding one µg of the total RNA. This mixture was incubated in Arktik thermal cycler (ThermoFisher Scientific, USA) at 25 °C for 5 minutes, 42 °C for 60 minutes, and finally 70 °C for 5 minutes.

- **Quantitative RT-PCR**

Relative quantification of renal tissue BAFF transcript was conducted by Maxima-SYBR Green qPCR Master-Mix (2X) (ThermoFisher Scientific, USA, Cat. No. K0251) using Rotor-Gene qRT-PCR System (QIAGEN, Germany) according to manufacturer instructions. The PCR reactions were carried out in duplicate with a final volume of 25 µL. The reaction mixture included 12.5 µL of Maxima-SYBR Green/ROX qPCR Master Mix (2X), 1 µL of forward primer (10 pmole), 1 µL of

reverse primer (10 pmole), 0.05 μL of 10x diluted ROX solution, 200 ng of cDNA and nuclease-free water to make up the final volume of 25 μL. The reaction protocol included initial denaturation for 10 min at 95°C (one cycle), subsequent denaturation for 15 seconds at 95°C (40 cycles), 30 second-annealing at 55°C and extension for another 30 seconds at 72°C. The process of amplifying the cDNA of both BAFF and GAPDH as a housekeeping gene involved using custom-made primers supplied by Applied Biosystems, USA. These primers specifically targeted the forward and reverse sequences of the cDNA strands. Four unlabeled sequence-specific primers were used to ensure accurate and efficient amplification of the desired cDNA sequences. The sequence of the primers was as follows: BAFF forward primer "5'-GGG-AGC-AGT-CAC-GCC-TTAC-3'" and reverse "5'-GAT-CGG-ACA-GAG-GGG-CTTT-3'"; GAPDH forward primer "5'-CCA-CTC-CTC-CAC-CTT-TGA-CG-3'" and reverse "5'-CCA-CCA-CCC-TGT-TGC-TGT-AG-3'". Relative expression was calculated using the 2–ΔΔCT method.[16]

Statistical analysis of the data:

The data collected was analyzed via the IBM-SPSS software package (version 25.0). The qualitative data were illustrated in terms of numbers and percentages to give a clear overview of the data set. To verify the normality of the data distribution, the Kolmogorov-Smirnov test was applied. The quantitative data was displayed using the median, and interquartile range (IQR), when normally non-distributed samples. A significant result was determined if p was equal to or less than 0.05, indicating a high level of confidence in the results.

The tests used were:

1.Mann Whitney test:

A non-parametric test employed to compare quantitative variables between two independent groups.

2. Chi-square

a. Fisher's exact significance was calculated when the expected count in more than 25% of cells is less than 5 for 2 X 2 tables.

b. For C X R tables, the Monte Carlo value of significance was calculated when the expected count in over 25% of cells is below 5.

3. Spearman Rho correlation coefficient

Non-parametric correlation between two quantitative variables.

Results

Baseline characteristics of the studied active pLN patients

There were 40 patients included in the retrospective analysis exploring intra-renal BAFF expression, 21 patients were considered eR and 19 patients eNR. Baseline relevant demographic, clinical, laboratory and activity indices are shown in Tables

1 and 2. There was no statistically significant difference between all of these parameters between early responders and non-responders except for baseline u-PCR, where higher percentage of eR had nephrotic range proteinuria more than eNR (p=0.032).

Table (1): Baseline demographic and pre-LN data.

	eR (n=21)		**eNR** (n=19)		**Total** (**n**=40)		**Test and significance**
Age (years) Median (IQR)	21 (18-27)		25 (19-26)		23		p=0.307 U=237.5
Sex	**N**	**%**	**N**	**%**	**N**	**%**	
Females	20	95.2	16	84.2	36	90	X^2=1.348
Males	1	4.8	3	15.8	4	10	p=0.331
Previous activity							
Musculocutaneous	8	38.1	10	52.6	18	45	X^2=3.130
Organ/serosal	1	4.8	3	15.8	4	10	P^{MC}=0.217
No activity before LN	12	57.1	6	31.6	18	45	
Previous steroid therapy							
No	4	19	3	15.8	7	17.5	X^2=0.073
Yes	17	81	16	84.2	33	82.5	p^{FE}=1.000
Time to diagnosis of LN (months)							
<12	12	57.1	12	63.15	22	55	X^2=0.150
≥12	9	42.9	7	36.8	18	45	P=0.698
Receiving IS before							
AZA	6	28.6	7	36.8	13	32.5	X^2=3.987
MMF	2	9.5	3	15.8	5	12.5	P^{MC}=0.300
MTX	0	0.0	2	10.5	2	5	

Legend: IQR: inter-quartile range; X^2: chi-square; p*: p-value is significant at level <0.05; p^{MC}: Monte Carlo significance of chi-square test; p^{FE}: Fisher's Exact significance of the chi-square test; U: Mann-Whitney U test

Adequate Renal biopsies were taken from all participants before induction therapy, the median glomerular number was 24 (min 11-max 55). Histopathological findings of eR and eNR are displayed in table 3. No statistically significant differences were found between eR and eNR regarding classes of LN or activity and chronicity indices.

Table (2): Baseline clinical and laboratory parameters at onset of pLN.

	eR (N=21)		**eNR** (N=19)		**Total** (N=40)		**Test and significance**
Edema	**N**	**%**	**N**	**%**	**N**	**%**	
No oedema	6	28.6	6	31.6	12	30	X2=3.910
mild	12	57.1	12	63.2	24	60	p^{MC}=0.335
moderate	0	0	1	5.3	1	2.5	
generalized	3	14.3	0	0	3	7.5	
Hypertension							
No	9	42.9	12	63.2	21	52.5	X2=1.648
Yes	12	57.1	7	36.8	19	47.5	p=0.199
Hb(g/dl)							
≤12	17	81	18	94.7	35	87.5	X2=1.733
>12	4	19	1	5.3	5	12.5	P^{FE}=0.345
Platelets (x10³/ul)							
≤100	2	9.5	0	0	2	5	X2=1.905
>100	19	90.5	19	100	38	95	P^{FE}=0.488
WBCs (x10³/ul)							
≤3	3	14.3	1	5.3	4	10	X2=0.902
>3	18	85.7	18	94.7	36	90	P^{FE}=0.607
u-PCR (g/mmol)							
<3.5	11	53.3	16	84.2	27	67.5	X2=4.61
≥ 3.5	10	47.6	3	15.7	13	32.5	p=0.032*
Urea (mg/dl)							
≤40	5	29.4	9	52.9	14	41.2	X2=1.943
>40	12	70.6	8	47.1	20	58.8	p=0.163
Creatinine (mg/dl)							
≤1	9	42.9	10	52.6	19	47.5	X2=0.382
>1	12	57.1	9	47.4	21	52.5	p=0.536
eGFR (mL/min/ 1.73m²)							
<90	13	61.9	9	47.36	22	55	X2=0.852
≥90	8	38.1	10	52.64	18	45	p=0.356
Albumin (g/dl)							
<3.5	5	23.8	3	15.8	8	20	X2=0.401
≥3.5	16	72.2	16	84.2	32	80	p=0.527
Anti-dsDNA							
negative	0	0	2	5.9	2	5	X2=4.343
< triple normal	11	52.4	5	29.4	17	40	p=0.094
≥ triple normal	10	47.6	12	64.7	21	55	
C3 (mg/dl)							
<90	3	14.3	1	5.3	4	7.9	X2=0.902
≥90	18	85.7	18	94.7	36	92.1	P^{FE}=0.603

C4 (mg/dl)							
<10	7	63.6	10	71.4	17	68	X2=0.172
≥10	4	36.4	4	28.6	8	32	P^{FE}=1.000
Cast							
Negative	16	76.2	10	55.6	26	66.7	X2=1.857
Positive	5	23.8	8	44.4	13	33.3	p=0.173
Pyuria Median (IQR)	9 (7-17)		11 (8-20)		11(7-18)		p=0.685 U=193
Negative	2	9.5	3	15.8	5	12.5	X2=0.358
Positive	19	90.5	16	84.2	35	87.5	p^{FE}=0.654
Hematuria Median (IQR)	4 (3-16)		7 (2-7)		6 (2-11)		p=0.281 U=141.5
Negative	9	42.9	11	57.9	20	50	X2=0.902
Positive	12	57.1	8	42.1	20	50	p=0.342
SLEDAI Median	16		20		19.5		p=0.252 U=42.5
rSLEDAI Median (IQR)	12 (8-12)		12 (8-14)		12 (8-12)		p=0.786 U=190

Legend: IQR: inter-quartile range; X^2: chi-square; p*: p-value is significant at level <0.05; p^{MC}: Monte Carlo significance of chi-square test; p^{FE}: Fisher's Exact significance of the chi-square test; U: Mann-Whitney U test

Table (3): Histopathologic characteristics of the study group.

Renal biopsy characteristics	**eR** (N=21)		**eNR** (N=19)		**Total** (N=40)		**Test and significance**
Class of LN	**N**	**%**	**N**	**%**		**%**	
III	4	19.0	3	15.7	7	17.5	X^2=0.955
III-IV	3	14.2	5	26.3	8	20	p^{MC}=0.812
IV	7	33.3	5	26.3	12	30	
IV-V	7	33.3	6	31.5	13	32.5	
Chronicity index Median (IQR)	3 (1-4)		3 (2-5)		3(1-5)		p=0.662 U=183
Glomerulosclerosis (out of 3)							
0	5	23.8	3	15.7	8	20	X^2=0.639
1	13	61.9	14	73.6	27	67.5	P=0.80
2	3	14.2	2	10.5	5	12.5	
Fibrous crescent (out of 3)							
0	18	45	18	45	36	90	X^2=0.902
1	3	7.5	1	2.5	4	10	p=0.342
Interstitial fibrosis (out of 3)							
0	7	33.3	6	31.5	13	32.5	X^2=3.228
1	6	28.5	7	36.8	13	32.5	p^{MC}=0.357

2	3	14.2	5	26.3	8	20	
3	5	23.8	1	5.2	6	15	
Tubular atrophy (out of 3)							
0	7	33.3	7	36.8	13	32.5	X^2= 0.951
1	8	38.1	4	21.05	11	27.5	p^{MC}= 0 .812
2	3	14.2	6	31.5	9	22.5	
3	2	9.5	1	5.2	2	5	
Activity index (out of 24) Median (IQR)	10 (8-10)		9.5 (7.5-11.5)		9 (7.75-11)		U=195 p=0.913
Endocapillary Proliferation (out of 3)							
1	5	23.8	7	36.8	12	30	X^2= 0.994
2	8	38.09	7	36.8	15	37.5	PMC= 0.608
3	8	38.09	5	26.3	13	32.5	
Cellular crescent (out of 6)							
0	9	42.8	7	36.8	16	40	
2	9	42.8	8	42.1	17	42.5	X^2=0.352
4	3	14.2	4	21.1	7	17.5	P^{MC}= 0.838
Segmental necrosis (out of 6)							
0	0	0.0	2	10.5	2	5	X^2=5.797
2	17	80.9	14	73.6	31	77.5	P^{MC}=0.237
4	4	19.1	3	15.78	7	17.5	
Wire looping/hyaline thrombi (out of 3)							
0	7	33.3	4	21.05	11	27.5	X^2=1.811
1	8	38.09	10	52.6	18	45	P^{MC}=0.612
2	4	19.04	2	10.5	6	15	
3	2	9.5	3	15.7	5	12.5	
Neutrophil infiltration (out of 3)							
0	3	14.2	2	10.5	5	12.5	X^2=2.6489
1	10	47.6	13	68.4	23	57.5	P^{MC}=0.265
2	8	38.09	3	15.7	11	27.5	
3	0	0	1	5.2	1	2.5	
Interstitial inflammation (out of 3)							
0	3	14.2	4	21.05	7	17.5	X^2=2.269
1	16	76.2	10	52.63	26	65	P^{MC}=0.314
2	2	9.5	5	26.31	7	17.5	
GBM thickening							
<50%	11	52.3	8	42.1	19	47.5	X^2=0.422
≥50%	10	47.6	11	57.9	21	52.5	P=0.516

Legend: IQR: inter-quartile range; X^2: chi-square; p*: p-value is significant at level <0.05; p^{MC}: Monte Carlo significance of chi-square test; p^{FE}: Fisher's Exact significance of the chi-square test; U: Mann-Whitney U test

After 6 months of initiation of induction therapy (table 4), it was found that the edema resolved in most of the early responders (90.4%) as compared to more than half of the early non-responders (66.7%). Fifty percent of the eNR had persistent or de-novo hypertension as compared to 19% of responders (p=0.041). In the non-responder group, 1 patient died of rapidly progressive GN, 7 patients had a temporary rise of creatinine level (1 case required Hemodialysis (HD)), and 2 patients had a persistent reduction in eGFR progressing to CKD. In the responders, 8 patients had a temporary rise in creatinine (1 of them required HD).

The median 6 months-u-PCR in the responders (0.520 g/mmol) was significantly lower than the non-responders (2.03g/mmol); whereas the median u-PCR percent reduction in the responders was significantly higher than non-responders (83.9% vs. 9.05%, p<0.001). Normalization of C3 level to ≥90 mg/dl was more significant in the responders than in non-responders (66.7% vs. 27.8%, p=0.015). Follow-up rSLEDAI was also significantly lower in eR than eNR (4 vs 8, p=0.045).

Table (4): Distribution of the studied LN patients (n=39, 1 died) according to post-induction follow-up characteristics.

Post-induction Data	**eR** (N=21)		**eNR** (N=19)		**Total** (N=40)		**Test and significance**
Edema	**N**	**%**	**N**	**%**	**N**	**%**	
0	19	90.4	12	66.7	31	79.4	X2=6.052
1	1	4.8	5	27.8	6	15.4	p=0.109
2	1	4.8	0	0	1	2.6	
3	0	0	1	5.6	1	2.6	
Hypertension							
No	17	81	9	50	25	67.6	X2=4.179
Yes	4	19	9	50	12	32.4	p=0.041*
FU-Creatinine (mg/dl)							
≤1	13	61.9	12	66.7	25	64.1	X2=0.096
>1	8	38.1	6	33.3	14	35.9	p=0.757
FU-u-PCR (g/mmol) Median (IQR)	0.620 (0.21-1.2)		2.03 (1.6-3.13		1.5 (0.6-2.3)		p<0.001 U=62.5
%Reduction in u-PCR	83.9 (70-91.6)		9.05 (-34.6-28.4)		57.5 (14.2-84.8)		p<0.001 U=0.00
FU-eGFR (mL/min/ 1.73m²)							
<90	4	19	4	22.2	8	20.5	X2=0.060
≥90	17	81	14	77.8	31	79.5	P^{FE}=1.000

FU-Anti-dsDNA							
negative	7	33.3	4	22.2	11	28.2	X2=2.073
< triple normal	11	52.4	8	44.4	19	48.7	p^{MC}=0.249
≥ triple normal	3	14.3	6	33.3	9	23.1	
FU-C3							
<90	7	33.3	13	72.2	20	51.3	X2=5.867
≥90	14	66.7	5	27.8	19	48.7	p=0.015*
FU-C4							
<10	1	4.8	1	5.6	2	5.1	X2=0.013
≥10	20	95.2	17	94.4	37	94.9	p^{FE}=1.000
FU-pyuria Median (IQR)	4 (2-8)		7 (4.25-15.5)		6 (3-10)		p=0.165 U=238.5
Negative	1 2	57.1	5	27.8	17	43.6	X2=3.399
Positive	9	42.9	13	72.2	22	56.4	p^{FE}=0.065
FU-Hematuria Median (IQR)	2 (0-6)	2.5 (0.5-5.5)		2 (0-6)			p=0.707 U=203
Negative	13	61.9	14	77.8	27	69.2	X2=1.146
Positive	8	38.1	4	22.2	12	30.8	p=0.284
FU-SLEDAI Median	4		8		8		p=0.053 U=258
FU-rSLEDAI Median (IQR)	4(5-11)		8(0-8)		8(4-8)		p=0.045 U=120

Legend: IQR: inter-quartile range; X^2: chi-square; p*: p-value is significant at level <0.05; p^{MC}: Monte Carlo significance of chi-square test; p^{FE}: Fisher's Exact significance of the chi-square test; U: Mann-Whitney U test

Baseline Intra-renal BAFF tissue expression

Higher intra-renal BAFF mRNA quantification was observed in eNR more than eR (1.7 vs 1.48). However, the difference in expression was not statistically significant (p=0.741).

Table (5): Difference in intra-renal BAFF expression between eR and eNR.

Intra-renal BAFF	**eR** (n=21)	**eNR** (n=19)	**Total** (**n=40**)	**Test of significance**
Median (IQR)	1.48 (0.54-2.42)	1.7 (0.25-3.81)	1.48 (0.332-2.58)	P=0.741 U=187
Min-max	0.07-6.3	0.06-6.1	0.06-6.3	

Legend: IQR: inter-quartile range; U: Mann-Whitney U test; p*: p-value is significant at level <0.05.

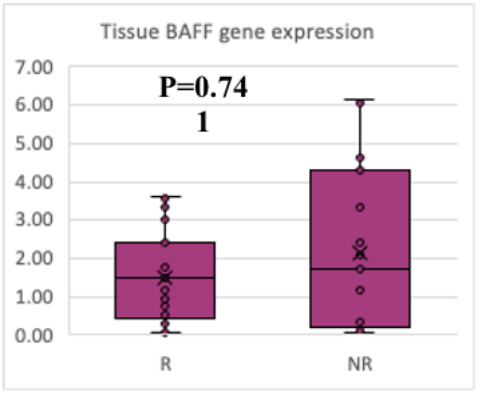

Figure 1: The difference in peripheral and renal tissue BAFF expression between responders and non-responders.

Higher tissue BAFF expression was only positively correlated with the longer time between diagnosis of SLE and LN (r=0.462, p=0.003), otherwise, no significant correlation was found between tissue BAFF and other baseline or follow-up diameters.

Similarly, No correlation was found between other baseline assessment parameters and follow-up response parameters, except for a negative significant correlation between Baseline SLEDAI and reduction of proteinuria after 6 months (r=-0.379, p= 0.017)

Table (6): Correlation between baseline intra-renal BAFF expression and 6-month response parameters

	Baseline intra-renal BAFF expression	
	r_s	**p-value**
6-month u-PCR	0.167	0.311
6-month Creatinine	0.125	0.447
6-month eGFR	-0.128	0.428
6-month SLEDAI	-0.262	0.103
6-month rSLEDAI	0.005	0.979

Legend: r_s: Spearman rho correlation coefficient; p*: p-value is significant at level <0.05.

Discussion

Over the last decade, mortality and morbidity rates from LN have significantly decreased due to the continuous advancements in therapeutic options. However, the selection and timing of certain treatments and combinations are not yet properly guided by validated severity stratifying markers or outcome predictors, which may result in missing the window period to prevent kidney damage and chronicity.

Till now, renal biopsy has been considered the gold standard for early diagnosis, classification of LN, and assessment of kidney inflammation and damage

based on activity and chronicity features. Baseline renal histopathological features have been found to be helpful in choosing the treatment protocol.[17] Nevertheless, renal biopsy is invasive, sometimes under-representative, and may lack correlation with clinical findings such as urinary proteinuria and decline in renal function, limiting its ability to accurately correlate with the classification or activity of LN.

A study by Ishizaki et al. found that 17 % of the SLE patients, included in their study, who had silent LN without active urinary sediment or decline in renal function had histo-pathologically proven active class III or IV LN [18]. Another study by Malvar et al. showed that proteinuria of more than 0.5 g/day persisted in 29% of study patients who had histopathological remission following treatment [19]. These observations underline the insufficiency of renal biopsy alone or the inadequacy of the in-use classification to reflect the actual severity and predict the outcome of therapy. Hence, lots of additional genetic, serological, urinary, and tissue biomarkers have been investigated for their ability to predict severity and early and late therapeutic response before deterioration in kidney functions occurs. Unfortunately, there is no established unanimous, reproducible predictor or severity stratification tool for LN.[17] Therefore, most of the treatment decisions and prognostic parameters are still dependent on renal biopsy results combined with basal clinical and laboratory parameters.

In 1999, as B-cell activating factor or B-lymphocyte stimulator, was discovered. [20] B-cell activating factor is a type II transmembrane protein that can also be modified into a soluble form.[21] The soluble form of BAFF promotes B cell survival, maturation, activity, and antibody class switching.[22] The role of BAFF and A proliferation-inducing ligand (APRIL) in the development of SLE is well determined in different studies [22-27]. However, there has been a controversy about the potential use of BAFF or APRIL as markers for SLE and LN disease activity or prognosis. While some studies showed associations and correlations between BAFF and disease activity, other studies could not prove an association. In previous studies, levels of serum APRIL and BAFF proteins were shown to be elevated in lupus mouse models with more renal responsiveness to therapies targeting APRIL/BAFF system.[28-30] It was also observed that BAFF-deficient mice could develop LN and interstitial inflammation but did not progress to renal failure due to less endothelial and tubular injury. These findings shed light on the role of BAFF in the severity and progression of LN [31]. BAFF level was thought to also be more altered by the extent of proteinuria. [32] That is the reason our work tried to explore the potential of BAFF-mRNA (particularly the intra-renal expression), not BAFF protein level, as a baseline predictive biomarker of severity and response, selectively in active pLN patients.

Using Immunohistochemical staining, earlier studies showed an increase in intrarenal BAFF expression in proliferative LN.[33-36] Neusser et al detected high BAFF and APRIL-mRNA expression levels in patients with active proliferative LN relative to membranous LN and healthy kidney biopsies obtained from living donors. [33] Two other studies found high immune-histochemical expression of BAFF

and BAFF receptors, particularly in proliferative LN relative to class II and V LN. [34, 37] A work by Schwarting et al revealed a correlation between high kidney tubular BAFF expression and severity of LN in both mice and human kidney biopsies with proliferative LN. [35] A comparable study from Thailand by Treamtrakanpon et al found that baseline intrarenal APRIL and BAFF-mRNA expression measures -using RT-PCR- were higher in the non-responder group compared to patients who showed an early response, although they used response endpoints different from our study endpoints. [26]

On the other side, a recent study found that good prognosis was associated with early-stage BAFF expression in renal tissue and reduced LN activity, although it included only 17 patients who were grouped according to the BAFF expression. [38] In our study, despite the higher intra-renal expression of BAFF in non-responders, the difference was statistically insignificant relative to the BAFF expression measures in responders. Furthermore, an analysis of correlation between intra-renal BAFF gene expression and baseline parameters as well as response parameters revealed no statistically significant correlation, except for a positive correlation between the BAFF expression and the time between diagnosis of SLE and LN. The published results concerning the correlation between BAFF biomarkers and SLE disease activity indices are inconclusive. Our research found no correlation between or intra-renal BAFF expression and either SLEDAI-2K or rSLEDAI in the studied proliferative LN sample, contradicting Zollar et al findings in SLE patients. [27]

Limitations of the study

The relatively small sample size and retrospective data collection constituted the main limitations of our study as well as previously published comparable research. Additionally, patients were included at the first discovery of LN without knowing the exact onset since not all the patients were compliant with regular clinic visits. A percentage of patients received immunosuppression for other prior lupus activities which may have affected the assay results.

Conclusion:

Investigating the potential use of BAFF expression as a marker of early proliferative LN prognosis, our study could not prove an association between intrarenal BAFF gene expression and early response to the conventional induction in an Egyptian sample of patients with active pLN. It is possible that variations in research populations, disease activity scores, or test sensitivity are the reasons for the disparities observed in the studies. Also, the sample sizes of all these studies, including ours, were not large enough, which may explain the inconsistent results and the lack of reproducibility.

So far, identifying an ideal predictive marker for treatment has been exceedingly difficult, mainly due to the inability to consistently demonstrate a relationship between treatment and different cytokines concentrations, which are known to be responsible for the heterogeneous pathogenesis of LN. In light of this, the latest management guidelines issued by the European League Against Rheumatism recommend the consideration of add-on therapies such as Belimumab,

targeting BAFF, to all patients with class III or IV to intensify initial therapy and prevent progressive damage.[39]

Ethics

The investigation was authorized by the Ethical Committee of the Faculty of Medicine, Alexandria University on April 21st, 2021 (Approval no. 0201495, IRB no. 00012098), and the experiment was employed per the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All participants gave their informed consent.

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Availability of Data and Materials

Please contact author for any data requests.

Competing Interests

The authors declare that there is no conflict of interest.

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