Use of Biomarkers as Predictors of Disease Flares and Kidney Damage in Patients with Systemic Lupus Erythematosus

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Abstract: *Background*: Routine biomarkers have limited value predicting flares in Systemic Lupus Erythematosus (SLE). Recent evidence suggests that urinary biomarkers, such as Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL), can predict renal relapses, differentiating activity to those with ongoing nephritis.

Objective: To determine if NGAL is useful for evaluating kidney relapse and / or SLE activity, compared to conventional biomarkers.

Methods: Descriptive cross-sectional study. Samples were collected from 66 patients prospectively followed, who fulfilled ≥4 ACR 2012 revised criteria for the classification of SLE. Lupus activity was evaluated by SLEDAI-2K, serum biomarkers were measured: anti-dsDNA and complement by ELISA method; and urinary biomarkers: protein in urine / 24 hours, urinary sediment and NGAL measured by immunochemiluminescence.

Results: Samples from 66 patients, including 64 women (97%) and 2 men (3%) with a mean of 45.7 years, lupus activity was high in 61%. Among these, 6% they met lupus nephritis criteria. The NGAL was altered in 32%; in patients with proteinuria >500 mg/l/24 hrs. and urinary cast the NGAL was elevated in 100% and 75% ($p < 0.05$); homogeneous and speckled ANA patterns were related to a high NGAL ($p <0.05$). When evaluating markers of lupus activity, altered anti-dsDNA and complement were related to a high activity ($p<0.05$); likewise, the NGAL was found to be altered ($p<0.05$).

Conclusion: In this study, biomarkers were found to be useful to assess lupic activity and renal flares. The use of NGAL shows promise in identifying activity and renal relapse, as well as anti-dsDNA and complement.

Keywords: Lupus, Activity, Biomarkers, Nephritis, Urinary NGAL.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex multisystem autoimmune disease [1]. Heterogeneous presentation and course remain one of the greatest challenges to physicians [2] with a relapsing and recurrent course leads the patient to organic damage and renal involvement or lupus nephritis (LN).

Currently, there are validated tools to evaluate SLE's activity such as British Isles Lupus Assessment Group (BILAG) and Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), considering a score > 4 as high activity [3]. Although survival in Lupus has improved with greater than 90% 10-year survival in many cohorts [4], severe LN is an important cause of mortality, which maybe a result of the difficulty in recognizing a flare early.

The pathogenesis of LN is complex with genetic and environmental contributions leading to altered immune regulation and local inflammation, furthermore, renal biopsy is the diagnostic standard for LN, it is invasive, and only provides a single "snapshot" of a pathogenic process [5]. For an individual patient, one pathway may be contributing more to the disease process than another. Biomarkers identifying the pathways upregulated and may help for better assessment and treatment of SLE and LN [6]. Biomarkers have been defined as biological characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or response to intervention [7] and may be particularly useful in diagnosing and managing rheumatologic diseases that have varied symptomatology; in SLE routine biomarkers such as anti-dsDNA and complement have limited value in predicting relapse [8] and an ideal LN biomarkers will: identify those at risk, distinguish between active and chronic disease, orientation of therapy and be accessible. Novel biomarkers in LN include a variety of inflammatory cytokines/chemokines; however, these have not yet to be widely implemented in clinical practice and recent evidence suggests that urinary biomarkers, such as *Urinary Neutrophil Gelatinase Associated Lipocalin*

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(NGAL), can predict renal relapses, differentiating activity to those with ongoing nephritis [9].

Anti-dsDNA (double stranded), is included by the ACR [10] and SLICC [11] in their SLE classification criteria; however, its presence is not unique to SLE [12] and has been proposed to be a prognostic and predictive of organ disease, although results are inconsistent. There is an association of anti-dsDNA with LN, describe as immune complex formation and renal binding [13].

Complement is a key component of the innate immune system. Reduction of complement occurs in SLE and others as congenital complement deficiencies, infections, liver failure, acute pancreatitis, cryoglobulinemia and thermal burns [14]. Studies showed that SLE patients fixed complement, resulting in lower complement levels, this fixation is present in kidney, liver, spleen and heart tissue and co-localized with antigen antibody complexes [15].

NGAL (also known as lipocalin-2, siderocalin, uterocalin, and 24p3) belongs to the lipocalin family, is a small glycosylated protein produced in many tissues, but initially described in neutrophils [16]. NGAL have been studied extensively as a biomarker in acute kidney injury (AKI) [17]. Various studies have reported that levels of uNGAL are markedly increased in AKI [18], progression of chronic kidney disease [19] diabetic nephropathy [20], cardiorenal syndrome [21], hypoxia [22] and other disorders. In LN uNGAL was found to be a significant predictor of renal disease activity in all SLE patients in various studies, and a significant predictor of flare in patients with a history of biopsy-proven nephritis [9], and levels in LN patients are significantly higher than those in non-LN patients, and may result in earlier diagnosis [23,24]. The ability of uNGAL to predict future renal disease activity and flares would be extremely useful in determining its clinical utility as a biomarker, the aim of this study is show that NGAL is useful for evaluating kidney relapse and / or SLE activity, compared to conventional biomarkers.

MATERIALS AND METHODS

Descriptive cross-sectional study where 66 patients were recruited, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All of them informed consent prior to their inclusion in the study. Samples were collected from patients prospectively followed up at the AGAR clinic who fulfilled ≥4 ACR 2012 revised criteria for the classification of SLE. Lupus activity was evaluated by SLEDAI-2K. Blood samples were collected for serum biomarkers: anti-dsDNA and complement by ELISA method; and urinary samples biomarkers: protein in urine / 24 hours, urinary sediment and uNGAL measured by immunochemiluminescence. All statistical analysis was made using SPSS version 26 for Windows, data was expressed in frequency tables; t-test for means and 2x2 tables for calculation of p with statistical significance <0.05, odds ratio (OR), Confidence Interval 95% CI and cut-off points for sensitivity and specificity.

RESULTS

Sixty six patients who fulfilled SLE criteria (Table **1**), including 64 (97%) women and 2 men (3%) with a mean age of 45.7 years (± 21.3, range 15-79 yr), majority of patients were between 21-40 yr (32%) with OR 2.06 (95%CI 0.69-6.12) and 41-60 yr (35%) with OR 1.2 (95%CI 0.41-3-6). Lupus activity was high in 61% (40) (SLEDAI-2K≥5) and a median of 8 points (0-32). The uNGAL was elevated in 32% (21) with a mean of 185.35 ng/ml (Table **2**), age and sex were not related with elevated uNGAL, however lupus activity was related with elevation of uNGAL (p<0,05), especially in SLEDAI>5 with OR 3.7 (95%IC 1.07-12.8).

Laboratory tests revealed 89% of patients are ANA positive (Table **2**), homogeneous and speckled patterns were most frequent 64% and 15%; anti-dsDNA were elevated in 70% of patients at the moment of the evaluation, and only 14% have anti-Sm elevated; in general, low complement was present only in 20%; the frequency of positive antiphospholipid was low (12% for ACL and 10% Anti-B2GL); alterations in urine tests, in general, were low, and only 6% of patient fulfilled criteria for lupic nephritis, with urinary cast in 6%, protein in urine of 24 hours >500 mg/L in 4% of patients, with a mean of 138 mg/l/24 hrs. uNGAL was altered in 32% (21) with a mean of 185.35 ng/ml; in a multivariate analysis, we found that patients with low complement (OR 10.7 95%CI 1.99-57.8) and homogeneous (OR 0.36 95%IC 0.12-1.1) and Speckled (OR 4.1 95%CI 1.01-16.5) ANA patterns are associate with elevated uNGAL (p<0.05); as soon as patients who fulfilled criteria for LN with proteinuria >500 mg/l/24 hrs and urinary casts the uNGAL was elevated in 100% and 75% (p < 0.05) with a sensitivity (Sn) of 15% and 19%, and specificity (Sp) of 98% and 100%,

Table 1: Sociodemographic Features, Disease Activity Index (SLEDAI-2K) and Elevated uNGAL of Patients with SLE

		Total n=66	Elevated uNGAL n=21			
		%	%	p-value	OR	95%CI
Age	<20	8 (5)	5 (1)	>0.05	0.50	0.05-4.8
	21-40	32 (21)	43 (9)	>0.05	2.06	0.69-6.12
	41-60	35 (23)	38 (8)	>0.05	1.20	0.41-3-6
	>61	26 (17)	14 (3)	>0.05	0.36	0.09-1.46
	Mean (years) (15-79)	45,73	43,57			
Sex	Woman	97 (64)	100 (21)	>0.05	-	-
	Man	3 (2)		>0.05	-	-
SLEDAI 2-K	Low activity ≤4	39 (26)	15 (4)	<0,05	0,26	0.07-0.92
	High activity ≥5	61 (40)	43 (17)	<0,05	3.7	1.07-12.8
	Mean SLEDAI 8,14 (0-32) points					

SLEDAI-2K: Systemic lupus erythematous disease activity index-2000. uNGAL: Urinary Neutrophil Gelatinase Associated Lipocalin.

Table 2: Comparison of Immunological Biomarkers of Lupus Activity (n = 66) and Altered uNGAL (n = 21)

		n=66 %	Altered uNGAL (n=21) %	p-value <0,05	OR	95%CI
ANA	Positive	89 (59)	81 (17)	>0,05	0.30	0.06-1.5
ANA pattern	Homogeneous	64 (42)	24 (10)	<0.05	0.36	0.12-1.1
	Speckled	15 (10)	60 (6)	<0.05	4.1	1.01-16.5
	Centromere	5 (3)	0	>0.05	0.00	0.00
	Nucleolar	6 (4)	25 (1)	>0.05	0,7	0.06-7.1
Anti- dsDNA	> 20 UI/L	70 (46)	35 (16)	>0.05	1.6	0.49-5.2
Anti-Sm	> 25UI/L	14 (9)	45 (4)	>0.05	1.8	0.44-7.8
Low complement	C3	14 (9)	78 (7)	<0,05	10.7	1.99-57.8
	C4	20 (13)	62 (8)	<0.05	5	1.36-17.7
Positive APL	ACL IgM/IgG	12 (6)	33 (2)	>0.05	1,23	0.2-7.5
	Anti-B2GL IgM/IgG	10 (5)	60 (3)	>0.05	4,2	0.6-28.5
Urinary sediment	WBC 5/mm3xHPF	9 (6)	50 (3)	>0.05	2,3	0.42-12.6
	Urinary cast	6 (4)	75 (3)	<0.05	7.3 Sn:15 Sp:98	0.71-75.2
Protein in urine 24 hours mg/L	<150	73 (48)	25 (12)	<0.05	0,3 Sn:57 Sp:20	0.10-1.03
	150-499	21 (14)	35 (5)	>0.05	1,2 Sn:24 Sp:80	0.36-4.32
	>500	6 (4)	100 (4)	<0.05	- Sn:19 Sp:100	-
	Mean	138				
NGAL	>100 ng/ml	32 (21)	Mean 185,35			

NGAL: Urinary Neutrophil Gelatinase Associated Lipocalin. ANA: anti-nuclear antibody by immunofluorescence. dsDNA: double stranded DNA. Sm: Smith. APL: antiphospholipid. ACL: Anticardiolipin. Anti-B2GL: Anti B2-Glicoprotein. Sn: sensitivity. Sp: specificity.

Table 3: Description of Cut-Off Points, p-Value, Sensitivity and Specificity; for Standard Biomarkers and NGAL in Lupus Activity (SLEDAI 2K> 4)

	Cut-off	SLEDAI 2K>4 % (n=40)	p-value <0.05	OR	CI 95%	Sensitivity	Specificity
Anti-dsDNA	>20 UI/L	80 (32)	<0.05	3.4	1.14-10.2	80	46
Anti-Sm	>25 UI/L	13 (5)	>0.05	0.72	0.19-3.24	12	85
Low complement	C3 <88 mg/dl	33 (13)	<0.05	-	-	32	100
	C4 <16 mg/dl						
Positive APL	ACL	13 (5)	>0.05	4.8	0.51-44.26	18	95
	Anti-B2GL	10 (4)	>0.05	3.7	0.38-35.3	14	95
NGAL	>100 ng/L	43 (17)	<0,05	4.06	1.18-13.9	42	85
Protein in urine 24 hours mg/L	<150	70 (28)	>0.05	0.7	0.22-2.17	70	23
	150-499	20 (8)	>0.05	0.8	0.25-2.75	20	77
	>500	10 (4)	>0.05	-	-	10	100
Urinary sediment	Urinary cast	8 (3)	>0.05	-	-	8	100
	WBC 5/mm3xHPF	10 (4)	>0.05	1.3	0.22-7.8	10	92

NGAL: Urinary Neutrophil Gelatinase Associated Lipocalin. SLEDAI-2K: Systemic lupus erythematous disease activity index-2000. dsDNA: double stranded DNA. Sm: Smith. APL: antiphospholipid. ACL: Anticardiolipin. Anti-B2GL: Anti B2-Glicoprotein. WBC: White Blood Cell.

respectively, these results show that altered uNGAL is highly specific for renal relapse in lupus.

To evaluate lupus erythematous systemic disease activity index in patients through SLEDAI-2K tool (Table **3**), we found 61% (40) have high disease activity with SLEDAI-2K>5 (mean 8 pts), most of the patients have high lupic activity and more risk of organic damage, biomarkers related to high activity ($p<0.05$) were anti-dsDNA (80%-32) with OR 3.4 (95%CI 1.14-10.2) sensitivity of 80% and specificity of 46%; low complement (33%-13) with sensitivity of 32% and specificity of 100% and uNGAL (43%-17) with OR 4.06 (95%CI 1.18-13.9) and Sn 42% and Sp 85%; and to evaluate renal activity, urinary casts and proteinuria are highly specific, as soon as uNGAL.

DISCUSSION

Biomarkers are useful to assess lupic activity and renal flares in SLE. Anti-dsDNA and complement are useful for evaluating disease activity, being more specific low complement and more sensitive elevations in dsDNA, however, the use of uNGAL shows promise in identifying activity and renal relapse in combination with conventional biomarkers, even in the absence of proteinuria and active sediment. Evidence of a relationship between NGAL and the renal injury associated with lupus nephritis was detailed in *in-vitro* studies by Rubinstein *et al.* (2007) who found a dramatic increase in the expression of Lipocalin-2 in lupus derived mesangial cells treated by nephritogenic murine anti-dsDNA monoclonal antibodies [8], but not in mesangial cells treated by isotype matched non-pathogenic control antibodies, Qing *et al.* (2006) [25]. The first human study looked at nephritis of childhood-onset SLE compared to control was Brunner *et al*. (2006) who found that SLE patients had significantly higher levels of urinary Lipocalin-2, and demonstrate these laboratory measurements had comparable sensitivities or specificities in predicting either biopsy-proven nephritis or renal disease in childhood-onset SLE [26]. Another clinical study was developed by Pitashny *et al*. (2007) who compared SLE patients with and without lupus nephritis and compared them to healthy controls [27] finding NGAL significantly upregulated in patients with LN. Recent studies by Rubinstein *et al*. (2010) show uNGAL was found to be a significant predictor of renal disease activity in SLE patients; just like our results, being more sensitive and specific forecaster of renal flare in patients with a history of LN than anti-dsDNA antibody titers [9]; elevated uNGAL significantly correlated with proteinuria and measurement of urinary Lipocalin-2 may result in earlier diagnosis of LN [23] in our results proteinuria>500 mg/24 hrs is strongly associated to elevated uNGAL. We concluded that uNGAL is a promising method diagnosing and measurement of disease activity in SLE and is useful in renal relapse even in the absence of proteinuria and urinary casts, but when both are present there is a high specificity for

renal flare. In our daily practice, we do not have easy access to kidney biopsy; and uNGAL is accessible, noninvasive and trustworthy test, and this could be an option in early detection and treatment of LN, and prevent morbi-mortality in lupic population. Current evidence suggesting that NGAL may be an important biomarker in the management of LN justifies further scientific investigation and larger clinical trials, including onset, relapses and remissions of the SLE.

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