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The Role of urinary kidney injury molecule 1 (KIM-1) in monitoring the treatment response in Egyptian Lupus Nephritis Patients

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Abstract

Lupus nephritis (LN) affects almost two-thirds of systemic lupus erythematosus (SLE) patients. Despite initial aggressive therapy, up to 25% of patients with LN will progress to permanent renal damage. Conventional serum markers for LN lack the sensitivity of an ideal biomarker. Urinary kidney injury molecule-1 (UKIM-1) is an excellent biomarker for early diagnosis of acute kidney injury and predicting renal outcomes. This study intended to determine the predictive performance of UKIM-1 among LN patients in response to induction therapy by assessing and correlating its levels with renal disease activity. The study included 60 SLE patients divided into 20 SLE patients with active lupus nephritis, 20 SLE patients with inactive lupus nephritis, and 20 lupus patients without nephritis as controls. The study was completed after six months from induction treatment. UKIM-1 was measured by an enzyme linked immunosorbent assay at baseline, three-month follow-up and after complete induction therapy. At baseline, the mean serum creatinine and mean UKIM-1 were 1.7 ±0.7 mg/dL and 10.3 ±1.2 ng/dL, respectively in active LN patients. The mean UKIM-1 levels of complete response and partial response groups were 9.8 ±0.9 ng/mL and 11.3 ±1.0 ng/mL respectively. Based on receiver operating characteristics curve analysis, we found a better diagnostic performance of UKIM-1 to predict response to induction treatment, outperforming conventional biomarkers. The sensitivity and specificity were 84.6% and 85.7 %, respectively at an area under the curve of 0.896 and the best cut-off level was ≤10.6 ng/mL. In conclusion, UKIM-1 performed better than conventional biomarkers in predicting response to treatment of active LN.

Keywords: Urinary kidney injury molecule (UKIM), systemic lupus erythematosus (SLE), lupus nephritis (LN).

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Introduction

Systemic lupus erythematosus (SLE) is a challenging condition that has an unpredictable course and presents unique issues in diagnosis and management.[1] It mostly affects women during the childbearing period, up to 20% of cases being affected in childhood. It is characterized by loss of self-tolerance and development of autoantibodies to nuclear self-antigens.[2]

SLE diagnosis is based on characteristic clinical manifestations affecting joints, skin, central nervous system, and the kidneys along with serological markers.[3] The basis for diagnosis was established by the American College of Rheumatology based on the presence of any four out of eleven criteria which was revised in 2015.[4.]

Lupus nephritis (LN) is a major risk factor for morbidity and mortality in SLE and 10% of patients with LN will develop end-stage renal disease.[5] Evaluation of LN is straightforward and should include urinalysis and measurement of kidney functions. Generally, it is based on serum creatinine concentration or estimated glomerular filtration rate.[6]

Renal biopsy is the gold standard in the diagnosis and classification of LN.[7] Renal biopsy, although considered to be a benign procedure, sometimes can have serious complications, even in expert hands, a reason why this cannot be performed repeatedly to assess renal status. Therefore, studying newer LN biomarkers is a target to overcome the drawbacks of the existing biomarkers and replace the need for renal biopsy.[8]

A biomarker refers to a biological, genetic, or chemical characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention.[9] Kidney injury molecule–1 (KIM-1) is a transmembrane protein with an immunoglobulin-like domain, as well as a mucin domain in the extracellular portion. KIM-1 is expressed at high levels in the proximal tubular epithelial cells in regeneration and is responsible for repairing and regenerating the damaged regions of the nephron after kidney injury, specifically in post-ischemic states and in response to nephrotoxicity.[10] Additionally, it has been related to acute tubular necrosis and renal cell carcinoma.[11] Regarding LN, KIM-1 levels are significantly higher in SLE patients with active kidney disease, compared with patients without renal activity. Elevated levels of this protein are significantly associated with tubular impairment and proteinuria. Also, it is very sensitive for detecting cellular glomerular crescents and highly specific for detecting interstitial infiltration.[12]

For the current study, we hypothesized that urinary **U**KIM-1 would significantly correlate with the severity of renal disease activity and might predict renal response to induction therapy. Therefore, the objective of this study was to determine the predictive value of UKIM-1 among LN patients in response to induction therapy by assessing and correlating its levels with the severity of renal disease activity.

Patients and Methods

Study population

Our study was conducted on 60 SLE patients who were admitted to the Rheumatology and Nephrology Units at Ain shams University Hospitals. They included 20 SLE patients with active lupus nephritis, 20 SLE patients with inactive lupus nephritis, and 20 lupus patients without nephritis as controls. Patients fulfilled at least four of the ACR 1982 revised criteria to diagnose SLE [19]. All patients were chosen based on a simple random selection from the Rheumatology and Nephrology Units at Ain shams University Hospitals during 2021 and 2022.

All patients received standard induction therapy. Patients were divided into two groups based on the renal response to treatment. Patients with complete response were defined by normal or ≤25% decline of Modification of Diet in Renal Disease glomerular filtration rate (MDRD-GFR) from baseline and a proteinuria of less than 0.5 g/day. Partial responses were defined by normal or ≤25% decline of MDRD-GFR from baseline and at least 50% reduction of proteinuria, with a level more than 0.5–3.0 g/day.

We excluded diabetics, patients with non-lupus related renal affection, patients on hemodialysis, renal transplant patients, drug-induced lupus patients, patients with overlapping syndromes, urinary tract infection patients, patients with active systemic infection, and active malignancies.

Treatment of lupus nephritis

Over the past decade, several randomized controlled trials have been conducted for Class III and IV LN both in the induction and in the maintenance phase. Consequently, the kidney disease Improving Global Outcomes (KDIGO) 2021 guidelines[20] are uniform in their recommendations for induction treatment. This includes intravenous Cyclophosphamide or Mycophenolate mofetil (2–3 g total daily dose) in combination with oral Glucocorticoids with or without three pulses of intravenous Methylprednisolone at the start of induction treatment. Although, in general, the use of both oral and intravenous Glucocorticoids has been proven effective, evidence is scarce concerning dose and duration of Glucocorticoids and recommendations are mainly based on expert opinion. In the guidelines, the initial dose varies from 0.5 to 1.0 mg/kg/day.[20]

Clinical and laboratory measurement

All participants were subjected to history taking including age, sex, duration of lupus, presence of hypertension or diabetes. Diagnosis of SLE was based on 4 out of 11 diagnostic criteria of lupus, including at least 1 clinical and 1 immunologic criterion, symptoms, and signs of LN as lower limb edema and hematuria. Also, Patients performed renal biopsy once at the beginning of diagnosis of active lupus nephritis with staging according to the International Society of Nephrology and the Renal Pathology Society criteria [21] as part of standard of care. Renal histologic features were evaluated by a renal pathologist. Activity index scores were calculated from the summing of individual scores. The range of activity index score is from 0 to 24 with higher scores representing higher activity. All of the patients were diagnosed according to the American College of Rheumatology diagnostic criteria. The disease activity was assessed by the SLEDAI-R score (Systemic Lupus Erythematosus Disease Activity Index renal score). If present, each of the four SLEDAI-R items receives a score of 4: proteinuria of > 0.5 gram/day, hematuria, and pyuria (both > 5 cells/high power field), and cellular casts. Thus, the score can range from 0 (non-active renal disease) to a maximal score of 16 [21].

Blood samples were obtained at baseline, three months after induction and after complete induction. Laboratory tests were analyzed using standard laboratory methods available for routine checkup at Ain Shams University Hospitals. They were used to determine complete blood cell count by microscopic examination of Leishman-stained peripheral blood smear using a hematology analyzer (XN-1000 SA-1, Sysmex, Japan). Serum creatinine and blood urea nitrogen (BUN) levels were obtained from hospital patient's records. Estimated Glomerular Filtration Rate (eGFR) was assessed by the Modification of Diet in Renal Disease (MDRD 4) [15]. Complete urine analysis of early morning midstream samples and urinary protein/creatinine ratio were performed.

UKIM-1 enzyme-linked immunosorbent assay

Urine samples were collected at baseline, three months and after 6 months from induction treatment. The samples were centrifuged at (716-2683 xg) for approximately 20 minutes to remove particular impurities then stored frozen at -80◦C until assayed. UKIM-1 level was measured by enzyme-linked immunosorbent assay (ELISA) Kits (Catalog No: YLA0724HU, Biont Human KIM-1 ELISA, SunRed biotechnology company, China Shanghai), according to the manufacturer's instructions. The ELISA reader (Tecan model, Sunrise company, Männedorf, Switzerland) was used to measure optical densities of the final ELISA products. All measurements were made in a triplicate and blind manner. Urinary KIM excretions were reported as the amount of urinary KIM in nanograms per milliliter (ng/ml).

Statistical Methods

The collected data were coded, tabulated, and statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 28.0 (IBM Corp., Chicago, USA, 2021). Quantitative data tested for normality using Shapiro-Wilk test, then described as mean±SD (standard deviation) as well as minimum and maximum of the range, and then compared using independent t-test (two independent groups) and ANOVA test (three independent groups). Qualitative data are described as

number and percentage and compared using Chi square test as well as Fisher's Exact test for variables with small, expected numbers. The receiver operating characteristics (ROC) curve was used to evaluate the performance of different tests to differentiate between certain groups. The Bonferroni test was used for post hoc comparisons. The level of significance was considered at *p* value ≤0.050.

Results

Our study included 60 SLE patients. Of these, 20 SLE patients were with active lupus nephritis, 20 SLE patients with inactive lupus nephritis, compared to 20 lupus patients without nephritis as controls. The active group included 20 patients with biopsy-proven active LN class III or class IV, with a mean age of 26.5±6.3years. Female patients were 16 (80.0%) and male patients 4 (20.0%). The duration of SLE ranged from recently diagnosed patients up to 10 years and the mean was 18.1±18.7 months, Table 1.

Table 1. Basic demographic, clinical and laboratory data in the study groups.

Variables		Active LN (Total=20)	Inactive LN (Total =20)	Control (Total =20)	*p* value
Age (years)	Mean±SD	26.5±6.3	29.1±7.7	27.7±8.1	^NS
Sex	Male	4 (20.0%)	3 (15.0%)	4 (20.0%)	§NS
	Female	16 (80.0%)	17 (85.0%)	16 (80.0%)	
Weight (kg)	Mean±SD	63.9±8.9	65.4±7.2	62.0±5.4	^NS
Co-morbid diseases	Hypertension	8 (40.0%)a	10 (50.0%) a	1 (5.0%)b	#0.006
	Diabetes mellitus	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Duration of SLE (months)	Mean±SD	18.1±18.7	21.3±15.8	19.5±11.9	^NS
Clinical manifestations	Lower limb edema	16 (80.0%)a	7 (35.0%) b	0 (0.0%)c	#<0.001
	Hematuria	9 (45.0%)a	0 (0.0%) b	0 (0.0%)c	§0.009
Immunosupressive treatment (steroid+)	Azathioprine	0 (0.0%)a	4 (20.0%) a	0 (0.0%)a	§<0.001
	Cyclophosphamide	15 (75.0%)a	0 (0.0%) b	0 (0.0%)b	
	Mycophenolate	5 (25.0%)a	16 (80.0%) b	0 (0.0%)a	
Class of LN ISN/RPS	III	11 (55.0%)	13 (65.0%)		#NS
	IV	9 (45.0%)	7 (35.0%)		
Activity index /24	Mean±SD	5.7±1.8	5.9±2.2		^NS
Chronicity index /12	Mean±SD	4.4±2.1	2.6±1.7		^0.006
SLEDAI-R score	Mean±SD	8.2±3.8			--

#Chi square test; §Fisher's Exact test; ^ANOVA test; *P* > 0.05 is not significant (NS).
Abbreviations: SLE: Systemic lupus erythematosus, rSLEDAI: renal SLE disease activity index, ISN/RPS International Society of Nephrology/Renal Pathology Society.

Basic Demographic, clinical and laboratory data between the three study groups:

There was no difference in age, sex, weight, and duration of SLE disease among the three studied groups (active Lupus nephritis, inactive Lupus nephritis and SLE without nephritis). Clinical manifestations such as lower limb edema and hematuria were significantly higher in active LN patients. Although no difference was observed in the class of LN and in activity index in renal biopsy between the first two groups of patients (active and inactive Lupus nephritis), however chronicity index was significantly higher in active LN patients (*p*=0.006). As regards blood picture, hemoglobin was statistically significantly higher in patients without LN (*p*=0.017). Albumin was lower in the active LN group, followed by inactive LN group (*p*=0.001). BUN and serum creatinine were higher in the active LN group (*p*=0.025 and *p*=0.001, respectively). eGFR was significantly lower in active LN group (Table 2).

Table 2. Comparison of the laboratory data between the three study groups.

Variables		Active LN (Total=20)	Inactive LN (Total =20)	Control (Total =20)	*p*-value
Hemoglobin gm/dL)	Mean±SD	9.1±2.3[a]	9.1±1.4[a]	10.6±1.6[b]	^0.017
TLC (x10^3/mL)	Mean±SD	7.3±4.0	7.0±4.5	6.5±2.8	^NS
Platelets (x10^3/mL)	Mean±SD	219.6±84.9	263.6±111.0	252.3±90.0	^NS
Albumin (gm/dL)	Mean±SD	2.7±0.4[a]	3.6±0.3[b]	3.9±0.4[c]	^<0.001
BUN (mg/dL)	Mean±SD	49.2±28.7[a]	17.2±6.3[a,b]	24.7±13.8[b]	^0.025
Creatinine (mg/dL)	Mean±SD	1.7±0.7[a]	0.8±0.1[b]	0.8±0.1[b]	^<0.001
eGFR (mL/min/1.73 m^2)	Mean±SD	62.6±26.3[a]	103.8±9.0[b]	102.9±8.5[b]	^<0.001

^ANOVA test; Homogenous groups had the same symbol "a, b, c" based on post hoc Bonferroni test.
P > 0.05 is not significant (NS).
Abbreviations: eGFR estimated glomerular filtration rate, BUN: blood urea nitrogen, TLC: total leucocytic count.

Baseline UKIM with patients' significant urinary findings:

Urinary abnormalities (i.e., sediments and casts) were exclusively observed in the active lupus group. Protein/creatinine ratio was significantly higher in the active LN group with no significant difference between inactive LN and control groups. The UKIM-1 level was higher in the active LN group, the differences were statistically significant between all the study groups (*p*<0.001) Table 3).

Table 3. Baseline UKIM-1 with patients' significant urinary findings.

Variables		Active LN (Total=20)	Inactive LN (Total =20)	Control (Total =20)	*p-value
		No (%)			
Active urinary sediment		9 (45.0%)	--	--	--
Casts (hyaline or granular)		11 (55.0%)	--	--	--
Protein/ Creatinine ratio (mg/gm)	Mean±SD	2.9±2.0a	0.4±0.1b	0.3±0.2b	^<0.001
UKIM-1 (ng/mL)	Mean±SD	10.3±1.2a	3.5±0.6b	1.3±0.4c	^<0.001

An active urine sediment was defined as the presence of pyuria (>5 white blood cell [WBC] per high power field [hpf]), hematuria (>5 red blood cell [RBC]/hpf), or bacteriuria.
^ANOVA test; Homogenous times had the same symbol "a, b" based on post hoc Bonferroni test. A post-hoc test was done to identify exactly which groups differ from each other. *$P \leq 0.05$ is significant.
Abbreviations: UKIM-1: Urinary kidney injury molecule.

Predictive performance of urinary KIM versus conventional biomarkers:

Of the 20 patients with active LN, 13 patients (65%) achieved complete response (normal or ≤20% decline of Modification of Diet in Renal Disease glomerular filtration rate (MDRD-GFR) from baseline and a proteinuria of less than 0.5 g/day), while 7 patients (35%) achieved partial response (normal or ≤20% decline of MDRD-GFR from baseline and at least 50% reduction of proteinuria, with a level more than 0.5–3.0 g/day) during the 6 months follow up period.

Serum Creatinine, urinary protein/creatinine ratio and UKIM-1 were significantly decreased at month-3 and month-6 follow-up from base line. However, there was no significant differences between month-3 and month-6. The eGFR was also significantly increased at 3 and 6 months compared to baseline value (Table 4).

Table 4. Performance of Urinary kidney injury molecule (UKIM-1) and other conventional biomarkers in the 20 active LN patients at different study periods.

Variable	Time	Mean±SD	Range	*p* value
Creatinine (mg/dL)	Baseline	1.7±0.7[a]	0.7–2.9	--
	Month-3	1.1±0.3[b]	0.6–1.8	^<0.001
	Month-6	1.0±0.3[b]	0.6–1.6	^<0.001
Protein/ Creatinine ratio (mg/gm)	Baseline	2.9±2.0[a]	0.7–7.5	--
	Month-3	0.8±0.7[b]	0.2–3.0	^<0.001
	Month-6	0.7±0.6[b]	0.0–2.5	^<0.001
eGFR (mL/min/1.73 m^2)	Baseline	62.6±26.3[a]	35.4–114.0	--
	Month-3	90.1±20.6[b]	52.4–119.4	^<0.001
	Month-6	93.4±21.2[b]	49.9–117.0	^<0.001
UKIM-1 (ng/mL)	Baseline	10.3±1.2[a]	8.2–12.8	--
	Month-3	4.9±1.8[b]	2.7–8.5	^<0.001
	Month-6	4.7±1.5[b]	2.9–7.4	^<0.001

^Paired t-test. Homogenous times had the same symbol "a,b,c" based on post hoc Bonferroni test. *p* value for comparison between baseline and times post treatment. *$p \leq 0.05$ is significant.
Abbreviations: eGFR: estimated glomerular filtration rate, UKIM-1: urinary kidney injury molecule.

The ROC curve analysis showed that the sensitivity and specificity for UKIM-1 level to predicting response to treatment were 84.6% and 85.7%, respectively at area under the curve of 0.896. The sensitivity and specificity were 84.6% and 85.7%, respectively for BUN with area under the curve of 0.890. The sensitivity and specificity were 53.8 % and 100 %, respectively for creatinine with area under the curve of 0.747. The sensitivity and specificity were 76.9% and 71.4 %, respectively for urinary protein/creatinine ratio with area under the curve of 0.769. The sensitivity and specificity were 53.8 % and 85.7 %, respectively for eGFR with area under the curve of 0.753. The Cutoff value for UKIM-1 was 10.6 ng/ml, Table 5, and Figure 1.

Table 5. Diagnostic performance and characteristics of renal function tests and UKIM-1 in predicting response to treatment.

Variables	AUC	*p* value	Cutoff	Sensitivity	Specificity	DA
Albumin	0.610	NS	≥2.7 gm/dl	76.9%	65.9%	73.1%
BUN	0.890	0.005	≤63.0mg/dl	84.6%	85.7%	85.0%
Serum Creatinine	0.747	NS	≤1.5mg/dl	53.8%	100%	70.0%
Protein/Creatinine ratio	0.769	0.052	≤2.6	76.9%	71.4%	75.0%
eGFR	0.753	NS	≥54.1ml/min/1.73m^2	53.8%	85.7%	65.0%
UKIM-1	0.896	0.004	≤10.6 ng/ml	84.6%	85.7%	85.0%

AUC: Area under curve. DA: Diagnostic accuracy. *P* > 0.05 is not significant (NS).
Abbreviations: BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, UKIM-1: urinary kidney injury molecule-1.

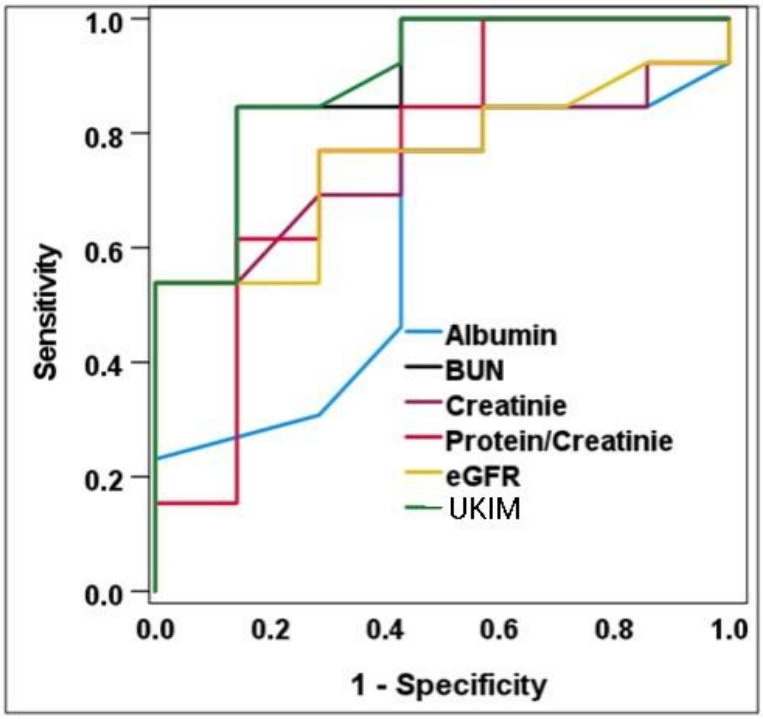

Figure 1. Receiver Operating Characteristics (ROC) curve for renal function tests and UKIM-1 in predicting response to treatment.

Discussion

In the present study, we aimed to investigate the performance of UKIM-1 in predicting treatment outcomes compared to conventional biomarkers of LN disease activity. Our study results indicated that UKIM-1 might be a novel biomarker of LN. Our study showed that baseline UKIM-1 level was higher among active LN group, the difference was statistically significant among all the three study groups (active lupus nephritis, inactive lupus nephritis and lupus patients without nephritis) (p<0.001). This observation agreed with the findings of a study by Samir et al., 2018.[18]

Our study revealed that there was significant positive correlation between baseline UKIM-1 and protein/creatinine ratio among the active LN group rather than the other two groups (p<0.001). This was consistent with the study of Samir et al., 2018, and Nozaki et al., 2022.[17,18] As regards the International Society of Nephrology/Renal Pathology Society (ISN/RPS) class[22] and baseline UKIM-1, we found no significant differences in UKIM-1 between different classes of LN. Conversely, Nozaki et al., 2022, found that UKIM-1 levels were elevated in SLE class IV and IV+V, which are considered to have high LN disease activity according to the ISN/RPS, 2018 classification.[17]

Our results showed that upon correlating between baseline UKIM-1 and various baseline renal parameters. (albumin, protein/creatinine, eGFR) and UKIM-1 had significant perfect diagnostic performance and characteristics in differentiating LN active group from LN inactive group (p=0.001). In our study creatinine, protein/creatinine and KIM-1 significantly decreased at month-3 and month-6 after treatment from baseline level. However, there was no significant differences between month-3 and month-6. Which confirms that KIM-1 may be a good indicator of regression and response to treatment. Similarly, eGFR showed a statistical significance increase in LN active patients at month-3 and month-6, compared to the baseline value. After 3 months and 6 months from induction of remission in the active group of LN patients, a positive correlation was observed between UKIM-1 and serum creatinine (<0.001). This comes in line with Nozaki et al., 2022, who stated that the UKIM-1 level after 6–8 months of treatment

was significantly correlated with serum creatinine (r = 0.53; p = 0.005).[16]

In conclusion, this study showed similar diagnostic performance of UKIM-1 compared to conventional markers for predicting disease severity and clinical response to treatment of active LN. Therefore, it may be proposed as one of the promising biomarkers in LN patients.

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Author Contributions

The study's principal investigators HE, HH, LK, RME and HR proposed the topic of this research and designed the study. HR, collected the data. All the authors contributed to preparing the final draft of the manuscript, revised the manuscript and critically reviewed the intellectual contents. In addition, they all read and approved the final manuscript and are responsible for its accuracy and integrity.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The research protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Ain Shams University (FMASU MD 284/2020).

Informed consent

Informed written consents were obtained from all patients who participated in this study.

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