Assessment of MAPK4 and ERK5 as Novel diagnostic biochemical markers in systemic lupus erythematosus'

Abstract

Background: Although anti-double-stranded DNA (dsDNA) antibodies is diagnostic markers for systemic lupus erythematosus (SLE), their limitations necessitate the identification of supplementary biologically relevant biomarkers to enhance diagnostic accuracy.

Objective: To evaluate serum levels of mitogen-activated protein kinase 4 (MAPK4) and extracellular signal-regulated kinase 5 (ERK5) in patients with active SLE and determine their individual and combined diagnostic performance relative to anti-dsDNA antibodies.

Patients and methods: This study involved 119 women with active SLE and 61 healthy female controls. Serum concentrations of MAPK4, ERK5 and the anti-dsDNA index were quantified using enzyme-linked immunosorbent assays (ELISA). Diagnostic performance was analyzed using receiver operating characteristic (ROC) curves, principal component analysis (PCA), and multivariate logistic regression.

Results: SLE patients showed a significant increase in MAPK4, ERK5 and anti-ds DNA levels compared with the controls (all $P < 0.001$). The ROC curves for all have AUCs of 0.99. PCA demonstrated that the three biomarkers loaded onto a single dominant component that explained most of the variance. A logistic model combining the three markers achieved classification accuracy of 99.4 %. The three biomarkers showed consistent behavior within the composite statistical model between them, suggesting that they are involved in a shared immunological pathway.

Conclusion: Serum MAPK4 and ERK5 levels were significantly elevated in active SLE, demonstrating exceptional diagnostic performance comparable to that of anti-dsDNA antibodies. Integrating these novel kinases into a multi-marker serological panel substantially enhances the diagnostic capability for SLE, particularly when conventional markers yield inconclusive results.

Keywords: Autoimmunity; Signal Transduction; ERK5; MAPK4; SLE.

Introduction

SLE is a chronic, multi-systemic autoimmune disease characterized by unpredictable episodes of exacerbation and remission. Can happen in any sex and age but more common in women of child-bearing-age. Epidemiological studies reveal that SLE is a rare disease with world-wide occurrence and there seems to be a clear disparity between different ethnic groups and geographical areas regarding incidence and prevalence [1,2]. The excess burden of SLE in young females presenting to rheumatology centers has been reported, at least in hospital-based studies from Iraq and a few other Middle Eastern countries, but reliable population-based incidence or prevalence data are sparse [3,4].

The clinical presentation of SLE is due to the deposition of auto antibodies and immune complexes in various organs, leading to systemic inflammation among several organ systems developing different pathological events contributing toward a chronic disease that remains difficult to manage. Hyperactivation of lymphocytes is the best-studied mechanism in SLE pathogenesis. Besides genetic and environmental factors, epigenetic mechanisms associated with immune dysregulation have been recognized since a long time [4], including DNA methylation, histone modification or non-coding RNA regulation [5, 6]. CD4+ T cell DNA hypomethylation and the dysfunctional ERK kinase signaling associated with it have also attracted considerable interest, as these changes are thought to break self-tolerance and lead to autoantibody production. Therefore, returning ERK signaling and re-establishing the abnormal methylation patterns in T cells have been postulated as achievable novel therapies for SLE [7].

Anti-dsDNA antibodies are among the oldest and most important immunological markers used in the diagnosis and classification of SLE. Their role has been consolidated by the 2019 EULAR/ACR criteria, which list them as a major classification item provided that highly specific assays are used[8,9]. Nevertheless, variability in antibody specificity, complex conditions under which these antibodies arise, and the wide range of self-antigens they can recognize imply that anti-dsDNA alone is not

In Press

sufficient as a universal standard for all patients, as pathogenic dsDNA antibodies may drive apoptosis, inflammation, and tissue fibrosis by targeting different self-molecules [10,11].
Central to the regulation of intracellular signaling cascades are MAPKs, which control gene expression, cell differentiation, proliferation, and survival. MAPK4 has recently been identified as an atypical kinase in this cascade, with roles depending on the context of its involvement: inflammation and immune responses. Experimental studies also showed MAPK4 plays a role in the modulation of pro-inflammatory signaling pathways and cytokine production; changes in its activity have implications for acute lung injury models and B cell activation[12,13]. Taken together, these results suggest a role for MAPK4 in immune dysregulation relevant to chronic inflammatory and autoimmune diseases[14].
ERK5 is a member of the ERK family network. Such as with many transcriptional cofactors, its long C-terminal tail may confer transcriptional regulatory functions that mediate a direct action of active ERK5 on gene expression[15]. The MEK5–ERK5 cascade is a critical pathway regulating cell proliferation and survival, and aberrant expression or hyperactivation of ERK5 has been observed in various human malignancies [16]. In addition to cancer, ERK5 activation in endothelial and monocyte cells has been implicated in promoting inflammatory responses and vascular leak. In fact, pharmacological blockade of this pathway decreases cytokine production and improves survival in models of acute inflammation, implicating ERK5 to be a player during vascular[17].
As there is no studies have yet been conducted that measure serum concentrations of MAPK4 and ERK5 in patients with active lupus or compare their ability to differentiate between patients and healthy individuals with the performance of established markers such as anti-dsDNA, leaving a clear gap regarding the potential for adopting these two kinases as novel serological biomarkers in SLE [18].
The present study measured serum MAPK4 and ERK5 levels in patients with active SLE compared to healthy individuals, along with anti-dsDNA antibody levels, and evaluated the diagnostic performance of MAPK4, ERK5, and anti-dsDNA tests individually, and examined whether combining these markers in a single statistical model improves the differentiation between SLE patients and healthy individuals.

Patients and Methods

Study design

This case-control investigation was done at the Rheumatology Department of the Baghdad Teaching Hospital, Medical City, Baghdad, Iraq. Participants were enrolled between January 2024 and September 2024. The study included 119 women newly diagnosed with active SLE and 61 healthy women in the control group, with an approximate age range of 18 to 48 years. All participants were non-diabetic and had no history of alcohol abuse.

Diagnosis, inclusion and exclusion criteria

SLE was diagnosed according to the American College of Rheumatology criteria updated in 1982 for the categorization of SLE[19]. The sample included newly diagnosed SLE patients with a SLEDAI-2K disease activity score of six or higher at sample collecting time [20]. Healthy volunteers of a similar sex and age were selected as the control group. A rheumatologist confirmed the diagnosis and classification of each lupus case based on clinical examination supported by relevant laboratory tests.

Blood sample collection and biochemical assay

Approximately 5 mL of venous blood was taken and allowed to clot. Serum was then centrifuged for 10 min. Serological tests included the measurement of human MAPK4, ERK5, and anti-dsDNA IgG antibodies using several commercially available sandwich ELISA assays and in accordance with the manufacturer's instructions. Serum MAPK4 concentration was measured using a human MAPK ELISA kit (FineTest, Wuhan Fine Biotech Co., Ltd., China; catalog no. EH3325) with an analytical range of 1.563–100 ng/mL. Serum ERK5 levels were measured using a human ERK5 protein ELISA kit (SunLong Biotech Co., Ltd., Hangzhou, China; catalog no. SL3338Hu) with an assay range of 0.6–20 pmol/L.
Anti-dsDNA IgG levels were estimated using a double-stranded DNA antibody ELISA kit (SunLong Biotech Co., Ltd., China; catalog no. SL2252Hu). As per the manufacturer's instructions, the initial photo absorptivity readings were converted to quantitative values and expressed as a dsDNA index by correlating each sample measurement with the assay cutoff value, such that higher index values reflected a higher degree of anti-dsDNA reactivity. This index, rather than absolute concentration in a specific unit, was used as the anti-dsDNA variable in all statistical analyses.

In Press

Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics software. Quantitative variables are described as means and SDs and using descriptive statistics and histograms to assess distribution. We used independent t-test for comparison of dsDNA, MAPK4 and ERK5 levels in/among SLE patients and healthy controls. Simultaneous effects of the markers were assessed using a one-way generalized linear multivariate model (GLM/MANOVA). Receiver operating characteristic (ROC) curves were done to detect the diagnostic value of dsDNA, MAPK4 and ERK5 for separating SLE patients from healthy controls including AUC (95% confidence interval), optimal cutoff values, sensitivity and specificity. A binary logistic regression model (lupus patient's vs controls) in combination with PCA and Pearson correlation analysis was used to study the concatenated performance of the three markers and their relative contribution to case classification; statistical significance for all analyses was set at $p < 0.05$.

Results

The study included 119 female patients with active SLE and 61 apparently healthy women as controls, with a comparable age distribution; the age was 33.19±8.25 years in patients vs. 32.33±9.09 years in controls ($P > 0.05$). All SLE patients fulfilled the 1982 revised ACR classification criteria and had active disease at the time of sampling, defined by a SLEDAI-2K score ≥ 6. The mean body mass index (BMI) of the patient group was 28.292±5.726 kg/m^2. BMI data were not collected for the control group.

As in Table 1, serum levels of all three biomarkers were markedly higher in patients with SLE than in healthy controls. The dsDNA index was significantly elevated in patients (1.87 ± 0.45) compared with controls (0.70 ± 0.27; $P < 0.001$). Likewise, mean serum MAPK4 concentrations were substantially higher in the SLE group (52.44 ± 5.82 ng/ml vs. 23.10 ± 6.17 ng/ml; $P < 0.001$), and ERK5 levels showed a similar pattern (7.59 ± 1.97 pmol/L vs. 2.13 ± 0.73 pmol/L; $P < 0.001$).

Table 1. Serum levels of dsDNA index, MAPK4 and ERK5 in SLE patients and healthy controls

Biomarker	SLE Patients (n=119)	Healthy Controls (n=61)	P-value
dsDNA index	1.87 ± 0.45	0.70 ± 0.27	<0.001
MAPK4 (ng/ml)	52.44 ± 5.82	23.10 ± 6.17	<0.001
ERK5 (pmol/L)	7.59 ± 1.97	2.13 ± 0.73	<0.001

In a multivariate general linear model including the three biomarkers simultaneously, the overall effect of group (SLE vs. control) was highly significant (Wilks' lambda = 0.117, F = 442.17, $P < 0.001$); furthermore, the between-subject F-values confirmed strong group differences for the dsDNA index, MAPK4, and ERK5 (all $P < 0.001$).

Each of the three biomarkers showed excellent diagnostic performance in distinguishing SLE patients from healthy controls. The area under the ROC curve (AUC) was 0.989 (95% confidence interval [CI] 0.968–1.000) for the dsDNA index, indicating very high accuracy. MAPK4 and ERK5 performed similarly, with AUC values of 0.994 (95% CI 0.981–1.000) and 0.994 (95% CI 0.982–1.000), respectively (Table 2).

Table 2. Receiver operating characteristic analysis of serum biomarkers for discriminating SLE patients from healthy controls

Biomarker	AUC	95% CI Lower	95% CI Upper	Cut-off Value	Sensitivity %	Specificity %	P-value
dsDNA index	0.989	0.968	1.000	1.200	98.4	95.0	<0.001
MAPK4 (ng/ml)	0.994	0.981	1.000	36.506	98.4	100.0	<0.001
ERK5 (pmol/L)	0.994	0.982	1.000	3.251	98.4	100.0	<0.001

AUC = Area Under the Curve; CI = Confidence Interval. Cut-off values were determined by maximizing Youden index (23).

In Press

PCA was utilized to investigate the latent structure of the three serum biomarkers across the whole sample. A single component explaining around 85% of the total variance from the dsDNA index, MAPK4 and ERK5 (eigenvalue = 2.55) was extracted (Figure 2). All three markers clustered quite closely around one common axis (0.906 for dsDNA index, 0.949 for MAPK4 and 0.91 ERK5), confirming that none is opposite on contrary axes given the high factor loadings (Table 3).

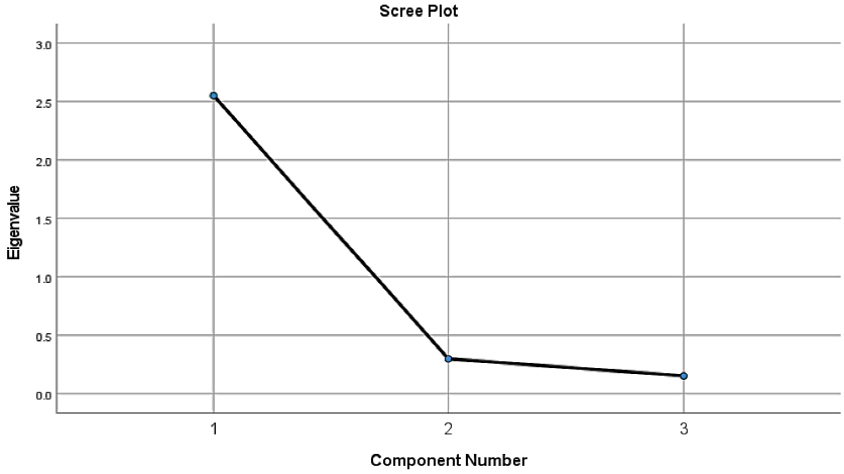

Figure 2. Scree plot of principal component analysis for serum dsDNA index, MAPK4 and ERK5

Table 3. Component matrix (factor loadings) of principal component analysis for serum dsDNA index, MAPK4 and ERK5

Biomarker	**Component Loading** 1
dsDNA index	0.906
MAPK4 (ng/ml)	0.949
ERK5 (pmol/L)	0.910

When the first principal component (FAC1_1) was entered into the ROC analysis, it showed high discrimination between the patients and healthy controls, with an AUC of 0.994, sensitivity of 98.4%, and specificity of 100%.

Analysis of the Pearson's correlation coefficients among overall sample also indicated a positive correlation between three biomarkers. MAPK4 correlated with ERK5 ($r = 0.155$, $P < 0.001$), and the dsDNA index positively correlated with MAPK4 ($r = 0.165$, $P < 0.001$) and ERK5 ($r = 0.006$, $P < 0.001$). A multivariate logistic regression model was employed to assess the combined performance of the dsDNA index, MAPK4 and ERK5 in distinguishing SLE patients from healthy controls. Group (SLE vs. control) was entered as the dependent variable, and the three biomarkers were entered as independent predictors. The global model was an excellent fit ($\chi^2 = 216.53$, df = 3, $P < 0.001$); the Nagelkerke R^2 indicated that ≈97% of variance in group classification could be explained by the three biomarkers together (Nagelkerke R^2: 0.969). Model calibration was acceptable, as demonstrated by the Hosmer–Lemeshow goodness-of-fit test showing non-significant result ($\chi^2 = 9.97$, df = 8, $P = 0.267$). For classification performance, we achieved an overall accuracy of 99.4% with sensitivity and specificity scores of 100.0% and 98.4%, respectively by correctly classifying all SLE patients while misclassifying only one control sample.
In multivariate logistic regression models, however, none of the individual biomarkers retained independent statistical significance despite this very high overall discrimination. The regression coefficients were, dsDNA index (B = −0.278, P = 0.888, OR = 0.76, 95% CI 0.02–36.09), MAPK4 (B = −0.347; P = 0.061; OR= 0.71;95%CI(OR) : 032-24–1; TableS4) and ERK5 (B=−0434,P=0433,OR=065;95%CI:022-192).

Table 4. Multivariable logistic regression model

In Press

Biomarker	B	S.E.	Wald	df	P value	OR (Exp(B))	95% CI for OR
dsDNA index	-0.278	1.972	0.020	1	0.888	0.757	0.016 – 36.091
MAPK4 (ng/ml)	-0.347	0.185	3.516	1	0.061	0.707	0.492 – 1.016
ERK5 (pmol/L)	-0.434	0.555	0.614	1	0.433	0.648	0.218 – 1.920

Discussion

The present study demonstrated that serum concentrations of MAPK4 and ERK5 were significantly elevated in active patients SLE. Furthermore, these biomarkers exhibited exceptional diagnostic performance, with a sensitivity of 98.4% and a specificity of 100%, yielding an AUC of 0.994. This diagnostic accuracy was near to that of the well-established anti-dsDNA index (AUC = 0.989) within our cohort.

The significantly increased serum levels of MAPK4 and ERK5 in patients with lupus are biologically plausible, given the mounting evidence implicating aberrant MAPK/ERK signaling pathways in the pathogenesis of SLE and other autoimmune disorders[14,21]. Experimental studies have established that MAPK4, an atypical member of the MAPK family, plays a role in regulation of inflammatory responses and activating immune cells through different models. For instance, Huang *et al*. demonstrated that MAPK4 regulates immune cell signaling and inflammatory responses, with context-dependent regulation, particularly evident in autoimmune conditions, such as rheumatoid arthritis[13]. Similarly, Mao *et al*. reported that MAPK4 deficiency significantly reduces the expression of pro-inflammatory cytokines in macrophages, underscoring its involvement in propagating inflammatory signaling cascades[12].

ERK5 has an extremely long C-terminus tail, which gives transcriptional regulatory functions distinct from other ERKs [17]. Endothelial cells and monocytes activation due to MEK5-ERK5 pathway has been highly associated with increased production of several proinflammatory cytokines, vasculitis, as well as failure of vascular permeability [22]. Wilhelmsen et al. That ERK5 selectively induces secretion of proinflammatory cytokines such as IL-6 and IL-8 by human monocytes and endothelial cells, while pharmacologic blockage of the ERK5 pathway efficiently ameliorates acute cellular and systemic inflammation in animal models [23].

Importantly, we have established ERK signaling pathways leading to the epigenetic dysregulation in lupus. Signaling through the ERK pathway is impaired in CD4+ T cells from SLE patients, leading to global DNA hypomethylation and consequent aberrant gene expression, loss of self-tolerance and the development of lupus-like autoimmunity in mice [24]. Extensive recent studies also support the notion that abnormal epigenetic changes, such as DNA hypomethylation conditioned by impaired MAPK/ERK pathway activity throughout SLE pathogenesis [25,26]. Conclusions MAPK4 and ERK5 have significant potential in the development of novel serological biomarkers for SLE as standalone markers or to be used in combination with a panel of other non-specific inflammatory mediators.

Although there are a range of limitations to using anti-dsDNA antibodies as an individual marker, they remain among the pillars of SLE classification and diagnosis because of their high specificity[9]. When comparing different methodologies for anti-dsDNA detection using a strict definition of specificity as 95%, the sensitivity dropped substantially to values around only 40% in some instances [27]. In addition, both scoping reviews of lupus biomarkers [29,30] have observed that a large population with active disease is anti-dsDNA negative or only transiently positive and therefore caution against the utility of this marker alone to explain the diversity in clinical manifestations of SLE.

Considering these inherent limitations, the addition of other serological markers to complement anti-dsDNA testing is a natural next step in SLE diagnostics [18]. We showed that the diagnostic of MAPK4 and ERK5 was powerful as single gene. In order to increase diagnostic robustness, we developed a composite statistical model with the dsDNA index, MAPK4 and ERK5 together. The composite model demonstrated a very good overall fit, explaining ~97% of the variance in groups classification (Nagelkerke R^2 = 0.969) with spectacular overall accuracy (99.4%) when considered within a single binary logistic regression framework on data prior to October 2023.Interestingly, despite the high

In Press

predictive power of the full model, none of the individual biomarkers retained independent statistical significance. This phenomenon is consistent with the presence of strong multicollinearity among the predictive variables, a common occurrence in multi-marker diagnostic panels, in which biological indicators share underlying pathological pathways[30]. As described by Elkahwagy *et al.* in their review of statistical tools for diagnostic biomarker studies, high correlation coefficients between explanatory variables in logistic regression models lead to inflated standard errors and widened confidence intervals, which can weaken the significance of individual coefficients without diminishing the model's overall predictive efficacy[31].

The PCA showed that dsDNA, MAPK4, and ERK5 clustered closely together on a single dominant component (85% of the total variance explained), with uniformly high (>0.90) factor loadings for all three markers. This tight clustering suggests that these biomarkers share a considerable amount of common variance, probably reflecting some shared immunological/inflammatory pathological element driving SLE[32]. This reduced dimensionality reveals why the composite model achieves strong predictive validity despite there being no independent significance of any single marker individually, which harmonizes directly with methodological guidance that biomarker panels should be treated as systems rather than individual tests[33].

The findings of our investigation are strongly aligned with the current paradigm shift in lupus research, which emphasizes that traditional serology does not adequately capture the broad and heterogeneous spectrum of clinical and biological variations inherent to SLE[10,34]. Recent systematic and scoping reviews have consistently concluded that combining multiple emerging biomarkers into mathematical models or serological panels yields more robust and reliable diagnostic outputs than single-indicator approaches [29,35]. The serological panel proposed in this study ,combining dsDNA, MAPK4, and ERK5 ,serves as a compelling example of this multi-indicator strategy. The composite indicator derived from these markers offers significant discriminatory power, supporting its potential utility as an adjunctive diagnostic tool, particularly in clinical scenarios in which traditional indicators yield inconclusive results or in cases in which a diagnosis remains uncertain[36].

Limitations and Future Directions

While the results are encouraging, there are a number of limitations that should be recognized. There are limitations to this study; first the cohort only included women, thus limiting extrapolation of these findings to male SLE patients and those with different demographics. Second, the cross-sectional design prevents assessment of longitudinal dynamics and so we are unable to ascertain whether MAPK4 and ERK5 levels change with dynamic changes in disease activity, flares or specific therapeutic interventions. These findings should be confirmed by future multicenter and longitudinal studies, which may enable the evaluation of biomarker assumption as reliable tools for monitoring treatment response. Furthermore, to conclusively establish the utility of MAPK4 and ERK5 in clinical practice for the management of SLE, the performance of the proposed serological panel should be also assessed amongst different clinical subgroups including patients with active lupus nephritis or distinct autoantibody profiles.

Conclusions

This study demonstrated that serum levels of MAPK4 and ERK5 were significantly elevated in active systemic lupus erythematosus patients compared to healthy individuals. Both markers exhibited excellent diagnostic performance, with sensitivity and specificity comparable to those of well-established anti-dsDNA antibodies. When combined with anti-dsDNA in a composite statistical model, the three-marker panel achieved a classification accuracy, supporting the utility of a multi-biomarker approach. These results proposed that MAPK4 and ERK5 may be useful as promising complementary serological biomarkers, enhancing diagnostic capability in SLE, particularly when conventional markers yield inconclusive results. Further multicenter longitudinal validation is warranted.

In Press