RESEARCH PAPER

Dysregulation of Immune Co-stimulation and Vascular Pathways: sCD40L and Lp-PLA2 as Key Biomarkers for Depression in Rheumatoid Arthritis

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Received: 25th June 2026; **Revised:** 28th July 2026; **Accepted:** 10th October 2026; **Available Online:** 15th October 2026

ABSTRACT

Introduction:

Depression is a common comorbidity in rheumatoid arthritis (RA) and is linked to inflammatory pathways. However, overlapping symptoms between RA and depression complicate diagnosis. This study assessed a novel panel of serum biomarkers, soluble CD40 Ligand (sCD40L), lipoprotein-associated phospholipase A2 (Lp-PLA2), neurofilament light chain (NEFL), and relaxin-2, to characterize and diagnose depression in RA.

Methods:

In a case-control study, 60 RA patients were categorized into 35 without depression (RA) and 25 with depression (RA+Dep; HAM-D score ≥14), alongside 30 healthy controls. Serum biomarker levels were quantified via ELISA. Statistical analysis included partial correlation, binary logistic regression, and receiver operating characteristic (ROC) curve analysis to evaluate diagnostic performance.

Results:

The RA+Dep group had significantly higher sCD40L and Lp-PLA2 levels than the RA and control groups ($p<0.001$). NEFL and Relaxin-2 did not differ. Depression severity correlated positively with sCD40L ($r=0.377$, $p<0.05$) and Lp-PLA2 ($r=0.407$, $p<0.01$). Logistic regression identified Lp-PLA2 (OR=26.07, 95% CI: 3.78-179.81, $p=0.001$) and sCD40L (OR=3.30, 95% CI: 1.10-9.88, $p=0.033$) as independent predictors of depression. ROC analysis showed sCD40L had the highest diagnostic accuracy for differentiating RA+Dep from RA (AUC=0.76, 95% CI: 0.66-0.86).

Conclusion:

Depression in RA is characterized by elevated sCD40L and Lp-PLA2, implicating specific immune and vascular-inflammatory pathways. These biomarkers, particularly sCD40L, show potential as objective diagnostic tools, supporting immune-inflammatory and vascular hypotheses of depression in RA and guiding future research.

Keywords: Rheumatoid Arthritis; Depression; Biomarkers; sCD40L; Lp-PLA2; Inflammation.

How to cite this article: Jasim ZA, Al-Issa AAR, Abdulridha GAO, Hadi HH, Al-Naqeeb TH, Al-Hakeim HK., Dysregulation of Immune Co-stimulation and Vascular Pathways: sCD40L and Lp-PLA2 as Key Biomarkers for Depression in Rheumatoid Arthritis. Int J Drug Deliv Technol. 2026;16(88s): 1903-1912; DOI: 10.25258/ijddt.16.88s.193

Source of support: *Nil.*

Conflict of interest: *None*

depressive symptoms via shared vascular-inflammatory pathways, suggesting it may serve as a marker of the vascular depression phenotype prevalent in chronic inflammatory states [17,18]. Neurofilament Light Chain (NEFL) is a marker of neuroaxonal damage [19] that may reflect the neurotoxic effects of chronic inflammation. Previous studies in non-RA populations have reported associations between elevated serum NEFL and depressive symptom severity, leading to the proposal of NEFL as a potential blood-based marker of inflammation-related neuroaxonal effects [20,21].Relaxin-2 has anti-inflammatory properties that could mitigate inflammatory burden [22,23]. Preclinical models suggest its potential to mitigate inflammatory tissue damage. A relative deficiency in such endogenous protective signaling could theoretically predispose patients to a more severe inflammatory burden, encompassing both articular and neuropsychiatric symptoms.

While these biomarkers have been studied in isolation in either RA or depression, a concurrent analysis of this specific quartet, sCD40L, Lp-PLA2, NEFL, and Relaxin-2, has never been performed in the context of RA-associated depression. This integrated approach is crucial, as depression in RA likely arises from a confluence of these distinct yet interacting pathways: immune hyperactivation (sCD40L), vascular inflammation (Lp-PLA2), neuronal injury (NEFL), and inadequate anti-inflammatory response (Relaxin-2). Therefore, this study aims to determine the serum profile of sCD40L, Lp-PLA2, NEFL, and Relaxin-2 in RA patients with depression (RA+Dep) compared to those without depression (RA) and healthy controls. Also, to evaluate the diagnostic utility of these biomarkers, individually and in combination, to differentiate RA+Dep from RA, and to investigate their correlation with clinical measures of disease activity and depression severity. We hypothesize that RA+Dep patients will exhibit a distinct biomarker signature characterized by elevated sCD40L, Lp-PLA2, and NEFL, coupled with lower Relaxin-2 levels, and that this multi-analyte panel will provide superior discriminatory power for detecting depression in RA compared to conventional clinical assessments alone.

Subjects and Methods

Participants

This case-control study was conducted at the Rheumatology Department at Rizgary Teaching Hospital in Hawler (Erbil) Province,

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovial inflammation leading to joint destruction and significant extra-articular morbidity [1]. Depression affects an estimated 16.8-50% of RA patients, a prevalence 2-3 times higher than in the general population [2,3]. This depression is not merely a reactive psychological burden but is mechanistically intertwined with RA pathophysiology [4]. A bidirectional relationship exists in which systemic inflammatory mediators, such as TNF-α, IL-1β, and IL-6, can breach the blood-brain barrier, disrupt neuroendocrine axes (e.g., the HPA axis), and alter monoaminergic neurotransmission, thereby directly contributing to the development and severity of depressive symptoms [5,6]. Consequently, depression in RA is associated with worse clinical outcomes, including heightened pain perception, increased fatigue, poorer treatment adherence, and accelerated functional decline [7,8].

Despite this established link, the clinical differentiation of depressed from non-depressed RA patients remains challenging. Current reliance on subjective patient-reported questionnaires (e.g., HAM-D17) is confounded by the somatic symptom overlap between RA (fatigue, sleep disturbance, pain) and depression. This diagnostic ambiguity underscores a pressing need for objective, blood-based biomarkers that reflect the distinct neuro-immune-inflammatory dysregulation underlying RA-associated depression [9,10]. An ideal biomarker panel would capture key pathological dimensions, including immune system hyperactivation, vascular inflammation, neuronal injury, and endogenous anti-inflammatory counter-regulation. Soluble CD40 Ligand (sCD40L) is a regulator of adaptive immunity that drives proinflammatory cytokine production [11,12] and is elevated in both RA and depression [13], where it is implicated in a proinflammatory and prothrombotic state that may exacerbate neuroinflammation [14,15]. Its role as a direct effector at the immune-CNS interface makes it a prime candidate for differentiating inflammatory subtypes of depression in RA.

Lipoprotein-associated phospholipase A2 (Lp-PLA2), an enzyme expressed by inflammatory cells within atherosclerotic plaques, is a validated biomarker of vascular inflammation [16] and linked to cognitive impairment and depressive symptoms. In RA, Lp-PLA2 activity is dysregulated and is increasingly linked to cognitive impairment and

No. E-EL-H6174; sensitivity: 0.94 ng/ml; detection range: 1.56-100 ng/ml; intra-assay CV: 5.2%; inter-assay CV: 7.8%). sCD40L (Catalog No. E-EL-H2346; sensitivity: 9.38 pg/ml; detection range: 0.156-10 ng/ml; intra-assay CV: 4.9%; inter-assay CV: 7.2%). NEFL (Catalog No. E-EL-H2158; sensitivity: 11.72 pg/ml; detection range: 23.44-1500 pg/ml; intra-assay CV: 5.5%; inter-assay CV: 8.1%). Relaxin-2 (Catalog No. E-EL-H2305; sensitivity: 8.56 pg/ml; detection range: 15.63-1000 pg/ml; intra-assay CV: 5.0%; inter-assay CV: 7.6%). ACPA (Catalog No. E-EL-H6075; sensitivity: 0.19 ng/ml; detection range: 0.31-20 ng/ml; intra-assay CV: 4.8%; inter-assay CV: 7.5%). The sera were diluted to meet the detection range and limitations of the kits. Serum CRP and RF were measured using semi-quantitative latex slide agglutination tests (Spinreact®, Barcelona, Spain). Serum CRP concentration (mg/L) was calculated as 6 × the CRP titer, where the titer represents the reciprocal of the highest sample dilution showing positive agglutination. RF levels (U/L) were determined as 8 × the corresponding titer**.**

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDLc) were assessed using commercial kits (Biolabo®, Maizy, France). Very-low-density lipoprotein cholesterol (VLDLc) and low-density lipoprotein cholesterol (LDLc) were calculated using Friedewald's formula. Atherogenic indices were derived as follows: Castelli's Risk Index-I (CRI-I)=TC/HDLc; Castelli's Risk Index-II (CRI-II)=LDLc/HDLc; and the Atherogenic Index of Plasma (AIP)=$\log_{10}$(TG/HDLc), as previously described [27,28].

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, N.Y., USA). We established statistical significance as a two-tailed p-value of less than 0.05**.** The Shapiro-Wilk test and Q-Q plots were used to assess the normality of continuous data. Data are presented as mean±SD for normally distributed variables and as median and interquartile range (IQR) for non-normally distributed variables. Frequency (n) and percentage (%) denote categorical variables. One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare the three cohorts (healthy controls (HC), RA without depression (RA), and RA+Dep) for normally distributed continuous variables. Kruskal-Wallis with Dunn's post-hoc correction was used for continuous variables that are not regularly distributed. We analyzed categorical variables using Chi-square (χ^2) or Fisher's exact

Iraq, between April and July 2025. Participants comprised 60 patients diagnosed with RA and 30 age-matched healthy controls. RA diagnosis was established according to the 2010 American College of Rheumatology/European League Against Rheumatism criteria [24], with each patient achieving a composite score >6 based on joint involvement, seropositivity (rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACPA)), elevated acute-phase reactants (C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR)), and symptom duration. Demographic data and RA clinical characteristics were collected for all patients. Disease activity was assessed using the Disease Activity Score-28 based on ESR (DAS28-ESR), calculated via an online calculator (https://www.mdcalc.com/disease-activity-score-28-rheumatoid-arthritis-esr-das28-esr). All patients exhibited moderate-to-high disease activity, as defined by a Clinical Disease Activity Index (CDAI) >10. The presence of clinically significant depression was determined using the 17-item Hamilton Depression Rating Scale (HAM-D17), with a score ≥14 indicating moderate depressive severity [25,26]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants with systemic conditions that could confound biomarker measurements, including hepatic or renal disease, active infection, diabetes mellitus, or cardiovascular events, were excluded from the study. Current smokers were also excluded. The study protocol received approval from the Institutional Review Board of the College of Pharmacy, University of Howler, Iraq (Approval No. HMU-EC-Ph 12052024-18). It was conducted in accordance with the Declaration of Helsinki and international guidelines for human research ethics.

Blood Sample Collection and Biochemical Analysis

Venous blood samples (5 mL) were collected from all participants following a 12-14 hours overnight fast. Of this volume, 3 mL were drawn into serum gel tubes and allowed to clot at room temperature. Samples were then centrifuged at 1500 ×g for 10 minutes, and the resulting serum was aliquoted into three separate Eppendorf tubes and stored at -20 °C until analysis. We quantified serum concentrations of Lp-PLA2, ACPA, sCD40L, NEFL, and relaxin-2 using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Elabscience® Biotechnology Inc., USA). The manufacturers provided the following kit specifications: Lp-PLA2 (Catalog

hypothesis of AUC=0.5 (no discriminatory power).

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Results

Cohort Characteristics and Baseline Biomarker Levels

The study cohort comprised 30 healthy controls (HC), 35 RA patients without depression (RA), and 25 RA patients with comorbid depression (RA+Dep). Demographic and clinical characteristics are detailed in Table 1.

Parameters	Control [A]	RA [B]	RA+Dep [C]	p
Age yrs	47.13±8.32	46.34±6.19	48.24±6.63	0.595
Age of onset yrs	-	35.90±8.49	36.52±9.85	0.796
Duration yrs	-	10.44±6.95	11.72±7.60	0.501
BMI kg/m²	27.09±4.27	26.14±4.06	28.04±4.07	0.216
Family history No/Yes	29/1 [B, C]	19/16[A]	13/12[A]	0.001
Employment No/Yes	4/26[B, C]	12/23[A]	11/14[A]	0.037
Moderate/Severe	-	20/10	15/15	0.190
Female/Male	25/5	28/7	23/2	0.440
RF IU/mL	4.43±1.49 [B, C]	17.6±9.27 [A]	14.4±9.52 [A]	<0.001
CRP mg/L	3.49±1.27 [B, C]	19.71±14.01 [A]	17.04±13.60 [A]	<0.001
ACPA ng/ml	4.47(3.90-6.19)[B, C]	13.71(8.80-28.74)[A]	10.70(6.49-38.95)[A]	<0.001
ESR mm/hr	8.77±3.219 [B, C]	35.31±19.01 [A, C]	50.76±30.08 [A, B]	<0.001
DAS28	-	4.77(4.19-7.00)	5.85(4.56-6.50)	0.584
HAM-D17	4.83±1.82 [B, C]	8.57±3.22 [A, C]	21.16±5.37 [A, B]	<0.001
TG mM	1.31±0.25 [B, C]	1.73±0.35 [A]	1.74±0.36 [A]	<0.001
Cholesterol mM	5.07±0.54 [B, C]	5.61±0.57 [A]	5.52±0.56 [A]	0.001
HDLc mM	1.059±0.11 [C]	1.02±0.21	0.93±0.12 [A]	0.014
VLDLc mM	0.6±0.11 [B, C]	0.79±0.16 [A]	0.79±0.16 [A]	<0.001
LDLc mM	3.414±0.56 [B]	3.81±0.58 [A]	3.79±0.62	0.015
CRI-I	4.85±0.84 [B, C]	5.7±1.071 [A]	6.045±1.154 [A]	<0.001
CRI-II	3.27±0.79 [B, C]	3.899±0.951 [A]	4.182±1.062 [A]	0.001
AIP	0.088±0.09 [B, C]	0.229±0.119 [A]	0.264±0.107 [A]	<0.001
TJC	-	5(2-10)	13(5.5-25.5)	0.003
SJC	-	2(0-3)	2(0.5-2.5)	0.805
PDA	-	6(3-7)	8(5.5-10)	0.002
Lp-PLA2 ng/ml	214.35(93.55-30.79)[C]	223.62(162.01-304.10)[C]	467.22(282.8-531.45)[A,B]	<0.001
sCD4L ng/ml	1.41(0.70-2.51)[B, C]	2.20(1.62-3.31)[AC]	4.62(2.67-5.95)[A,B]	<0.001
NEFL pg/ml	3.20(1.93-6.01)	3.41(1.79-6.93)	3.52(2.08-6.39)	0.794
Relaxin-2 pg/ml	0.23(0.11-0.30)[B, C]	0.28(0.19-0.55)[A]	0.36(0.21-0.49)[A]	0.007

Table 1. Demographic, clinical data, and the biochemical levels of the healthy controls (HC) and RA patients with (RA+Dep) and without depression (RA)

Results are expressed as mean±standard deviation for normally distributed data. Binomial data were expressed as ratios and analyzed by the Chi-squared test, p: probability value, F/χ^2: F-statistic value or Chi-square statistic, df: degree of freedom, ACPA: Anti-Citrullinated Protein Antibodies, SJC: Swollen Joint Count, TJC: tender joint count, PDA: Patient Disease Activity, ESR: erythrocyte sedimentation rate, HDLc: High-Density Lipoprotein Cholesterol, LDLc: Low-DLc, VLDLc: Very low-DLc, CRI-I & II: Castelli's Risk Index I & II, AIP: Atherogenic Index of Plasma, Lp-PLA2: Lipoprotein-associated phospholipase A2, sCD40L: soluble CD40 ligand, NEFL: Neurofilament Light Chain, DAS28: Disease Activity Score-28, GHP: General Health Perception.

As anticipated, both patient groups exhibited elevated systemic inflammation (RF, CRP, ACPA, ESR), altered lipid profiles, and higher atherogenic indices compared to HC (all $p<0.05$). The RA+Dep and RA groups were well-matched for age, disease duration, and objective measures of disease activity, including SJC and DAS28. However, the RA+Dep group reported significantly higher TJC and patient PDA (both $p=0.001$). Critically, the HAM-D17 score was markedly higher in the RA+Dep group (21.16±5.37) compared to both tests, as appropriate. Partial correlation analysis was used to assess the bivariate correlations between important biomarkers (Lp-PLA2, sCD40L, NEFL, Relaxin-2, CRI-I, CRI-II, AIP), depression severity (HAM-D17), and clinical characteristics in the RA population (RA and RA+Dep). This investigation adjusted for age, gender, BMI, serum uric acid, creatinine, and urea.

To identify which biomarkers independently predicted depression status in RA patients, we performed binary logistic regression. The outcome (dependent variable) was depression status (RA+Dep = 1, RA = 0). The predictors (independent variables) were natural log-transformed values of Lp-PLA2, sCD40L, NEFL, Relaxin-2, AIP, and CRI-I. (CRI-II was excluded because statistical separation prevented model convergence, a technical issue indicating this variable did not contribute meaningful information. This was indicated by an excessively large odds ratio, an implausibly wide confidence interval, and a coefficient standard error inflated by several orders of magnitude, indicating no maximum likelihood convergence. The Hosmer-Lemeshow test analyzed model goodness-of-fit. Model performance was assessed using Nagelkerke R^2, classification accuracy, sensitivity, specificity, and adjusted odds ratios (ORs) with 95% CIs. Importantly, large odds ratios with wide confidence intervals should be interpreted cautiously, given the relatively small sample size.

ROC curve analysis was performed to evaluate the discriminatory ability of each biomarker for distinguishing RA+Dep patients from RA patients, with AUC and 95% CI reported. It is important to note that ROC analysis assesses the biomarker's ability to discriminate between groups in this specific study sample; it does not constitute clinical diagnostic validation, which would require prospective studies in independent cohorts. The Youden Index (J=sensitivity+specificity-1) optimizes sensitivity and specificity to obtain the best biomarker cut-off value. The Youden Index, sensitivity, and specificity at this cut-off are given. The sample size (n=60 RA patients, n=30 healthy controls) was determined based on feasibility considerations and similar published studies in the field [15,18]. A post-hoc power analysis using G*Power (Version 3.1.9.7) indicated that, with $\alpha=0.05$ and a sample size of 60 (35 RA and 25 RA+Dep), the study had 0.80 power to detect a large effect size (Cohen's $d \approx 0.80$) between groups for the primary biomarkers. Statistical significance of the AUC was assessed against the null

CRP, Dis. Duration, Rheumatoid factor, NEFL ACPA, Relaxin-2	
Control Variables	Age, Gender, BMI, Uric acid, Creatinine, Urea

*: p<0.05, #: p<0.01, $: p<0.001. *ACPA: Anti-Citrullinated Protein Antibodies, SJC: Swollen Joint Count, TJC: tender joint count, PDA: Patient Disease Activity, ESR: erythrocyte sedimentation rate, CRI-I & II: Castelli's Risk Index I & II, AIP: Atherogenic Index of Plasma, Lp-PLA2: Lipoprotein-associated phospholipase A2, sCD40L: soluble CD40 ligand, NEFL: Neurofilament Light Chain, DAS28: Disease Activity Score-28.*

Depression severity (HAM-D17 score) demonstrated significant positive correlations with the two key inflammatory biomarkers: Lp-PLA2 (r=0.407, p<0.01) and sCD40L (r=0.377, p<0.05). Furthermore, HAM-D17 scores showed strong positive correlations with markers of subjective disease burden, including TJC (r=0.510, p<0.01), and PDA (r=0.514, p<0.01), as well as with ESR (r=0.443, p<0.01). Notably, DAS28 scores were correlated with atherogenic indices (CRI-I, AIP) but not with Lp-PLA2 or sCD40L.

Lp-PLA2 and sCD40L are Independent Predictors of Depression Comorbidity in RA

A binary logistic regression model was constructed to identify biomarkers independently associated with depression comorbidity (RA+Dep *vs.* RA) (Table 3).

Table 3. Results of binary logistic regression analysis with RA+Dep as a dependent variable and RA as a reference group using the measured biomarkers as explanatory variables

Parameters	B(SE)	Wald	p	OR (95% CI)
Age of onset,	No significant correlation (p<0.05) with any of the measured biomarkers			

the RA (8.57±3.22) and HC (4.83±1.82) groups (p<0.001). Analysis of the primary candidate biomarkers revealed a distinct profile. Levels of Lp-PLA2 and sCD40L were significantly elevated in the RA+Dep group compared to both the RA and HC groups (Kruskal-Wallis test, p<0.001 for both) (Table 1). In contrast, levels of neurofilament light chain (NEFL) did not differ across groups (p=0.794). Relaxin-2 was elevated in RA patient groups compared to HC (p=0.007), but did not differ significantly between RA and RA+Dep groups.

Correlational Network Linking Depression, Specific Biomarkers, and Disease Features

To elucidate relationships within the RA population, partial correlation analysis was performed, controlling for age, gender, BMI, renal function, and uric acid (Table 2).

Table 2. Values of partial correlation coefficients for Lp-PLA2, sCD40L, NEFL, and Relaxin-2, in addition to the atherogenic indices, in relation to all other biomarkers in rheumatoid arthritis patients after controlling for all cofounders.

Parameters	HAM-D17	Lp-PLA2	sCD40L	CRI-I	CRI-II	AIP
TJC	0.510#	0.069	0.208	0.025	0.076	0.220
SJC	-	-	0.108	0.064	0.079	0.539#
PDA	0.014	0.079	0.054	1.000	0.983$	0.076
ESR	0.443#	0.022	0.000	0.028	0.085	*
DAS28	0.514#	0.107	0.044	0.033	0.005	0.180
HAM-D17	-	0.581	-	-	-	0.001
Lp-PLA2	0.029	0.079	0.008	0.050	0.054	0.411#
sCD40L	1.000	0.407#	0.377*	0.362	0.312*	0.257
CRI-I	0.407#	1.000	*	*	-	0.657
CRI-II	0.313	0.100	0.111	0.034	0.014	0.050
AIP	0.277#	0.111	1.000	-	-	0.219
	0.034	-	0.000	0.064	0.079	0.539#
	-	0.064	0.008	-	-	0.376#
	0.014	-	0.008	0.008	0.054	0.376
		0.079	-	1.000	0.983$	*
			0.054	0.983$	1.000	

Parameters	Cut-off	Sensitivity %	Specificity %	Youden J statistic	AUC (95 % CI)	p
Lp-PLA2 ng/ml	306.30	72.0	71.4	0.434	0.67(0.56-0.79)	**<0.001**
sCD40L ng/ml	3.18	52.0	51.4	0.200	0.76(0.66-0.86)	**0.049**
NEFL pg/ml	0.33	56.0	54.3	0.034	0.54(0.42-0.67)	0.982
Relaxin-2 pg/ml	5.79	56.0	57.1	0.034	0.71(0.60-0.81)	0.248
CRI-I	3.96			0.103	0.59(0.44-0.74)	0.370
CRI-II	0.27			0.170	0.58(0.43-0.73)	0.284
AIP				0.131	0.57(0.42-0.72)	

CRI-I & II: Castelli's Risk Index I & II, AIP: Atherogenic Index of Plasma, Lp-PLA2: Lipoprotein-associated phospholipase A2, sCD40L: soluble CD40 ligand, NEFL: Neurofilament Light Chain.

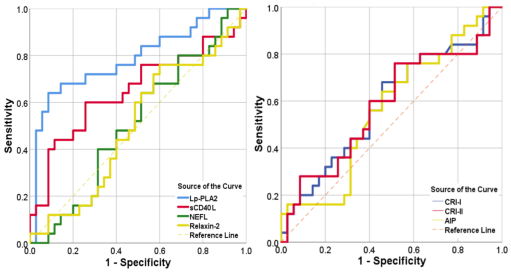


Figure 1. Receiver operating characteristic curves for differentiation between RA+Dep and RA by atherogenic indices (right) and other biomarkers (left).

sCD40L demonstrated the highest diagnostic accuracy, with an area under the curve (AUC) of 0.76 (95% CI: 0.66-0.86, p=0.049). At an optimal cut-off of 3177.3 pg/mL, it yielded sensitivities and specificities of 60%. Lp-PLA2 also showed significant discriminatory capacity (AUC=0.67, 95% CI: 0.56-0.79, p<0.001). The atherogenic indices (AIP, CRI-I, CRI-II) and the other candidate biomarkers (NEFL, Relaxin-2) did not demonstrate significant diagnostic utility, with AUC values not statistically different from 0.5 (all p > 0.05).

Lp-PLA2 ng/mL	3.261(0.985)	10.949	0.001	26.065(3.778-179.813)
sCD40L pg/mL	1.194(0.559)	4.564	0.033	3.301(1.104-9.876)
CRI-I	0.449(00.443)	1.028	0.311	1.566(0.658-3.729)
NEFL pg/ml	-0.205(0.391)	0.395	0.529	0.815(0.379-1.752)
AIP	0.508(0.808)	0.106	0.743	1.663(0.341-8.102)
Relaxin-2 pg/mL	-0.180(0.550)			0.835(0.284-2.452)

The parameters were entered as Ln-transformed parameters. CRI-II was excluded due to quasi-complete separation, as indicated by the odds ratio and confidence interval, which preclude reliable estimation. CRI-I & II: Castelli's Risk Index I & II, AIP: Atherogenic Index of Plasma, Lp-PLA2: Lipoprotein-associated phospholipase A2, sCD40L: soluble CD40 ligand, NEFL: Neurofilament Light Chain.

The model, which excluded CRI-II due to quasi-complete separation, was statistically significant (χ^2=30.208, df=6, p<0.001, Nagelkerke R^2=0.539) and correctly classified 85.3% of cases. The analysis identified Lp-PLA2 and sCD40L as significant independent predictors. Each unit increase in log-transformed Lp-PLA2 was associated with a markedly elevated odds of depression (Adjusted Odds Ratio, OR=26.07, 95% CI: 3.78-179.81, p=0.001). However, the wide confidence interval indicates substantial uncertainty around this point estimate, and the large OR should be interpreted cautiously given the relatively small sample size. This finding should be considered hypothesis-generating rather than definitive. Similarly, log-transformed sCD40L was associated with increased odds (OR=3.30, 95% CI: 1.10-9.88, p=0.033). Serum NEFL, Relaxin-2, and the atherogenic indices (AIP, CRI-I) were not significant predictors in this multivariate model.

Diagnostic Performance for Differentiating RA Patients with Depression

Table 4 and Figure 1 showed the receiver operating characteristic (ROC) curve analysis for discrimination between individuals with RA+Dep and RA using the measured biomarkers.

Table 4. Parameters of the receiver operating characteristic, area under the curve (AUC) analysis of the biomarkers in predicting RA+Dep versus RA. CI: Confidence interval.

problems. Our findings enhance the significance of neuropsychiatric comorbidity in RA. This is in line with the vascular depression concept, which says that cerebrovascular illness and inflammation cause depression in older adults by messing with circuits in the frontal and subcortical parts of the brain [31]. People with RA are far more likely to have atherosclerosis, and our findings imply that Lp-PLA2 may be a detectable blood-based mediator that connects this vascular risk to depression. In corroboration of this, increased Lp-PLA2 activity has been associated with cognitive decline and depressive symptoms in aged individuals and those with cerebral small vessel disease [18,32]. The ineffectiveness of generic atherogenic indices (AIP, CRI-I, CRI-/II) in our study underscores that Lp-PLA2 is a more precise indicator of active vascular inflammation than a static lipid profile, which seems more directly linked to depression comorbidity.

Disassociation between Neuronal Damage and the Depressive Score

The absence of significant differences in serum NEFL levels across our groups is a major adverse finding. NEFL serves as a very accurate marker of axonal damage, constantly elevated in neuroinflammatory and neurodegenerative conditions, such as multiple sclerosis and Alzheimer's disease [19,33]. The lack of increase in our RA+Dep group suggests that the depressive symptoms are not primarily attributable to widespread, chronic neuroaxonal degeneration seen in the peripheral region. This differentiates depression in RA from primary neurological disorders and indicates that mood disturbances may be more affected by the synaptic and neuroendocrine impacts of inflammatory mediators (e.g., cytokines, sCD40L) than by structural neuronal degeneration [34,35]. This corresponds with the discovery that depression in inflammatory disorders is often amenable to efficient anti-inflammatory treatment [36].

Subjective Burden and the Inflammation-Pain-Depression Triad

Our correlational study provides critical mechanistic insights. The strong correlations between depression severity (HAM-D17) and subjective measures: TJC and PDA, but not with the more objective SJC, illustrate the recognized link between depression and increased pain perception (pain sensitization) [37,38]. This relationship is likely bidirectional and occurs via shared neuroinflammatory pathways. Proinflammatory cytokines may increase the sensitivity of peripheral and central pain pathways, leading to

Discussion

This study provides novel evidence from a combined analysis of immune co-stimulation (sCD40L) and vascular inflammation (Lp-PLA2) in the context of RA-associated depression. Unlike previous studies that evaluated these biomarkers separately, our integrative approach highlights the potential interaction between immune and vascular pathways in shaping the depressive phenotype in RA.

The Role of Specific Inflammatory Pathways in RA-Associated Depression.

The significant increase of sCD40L in the RA+Dep group highlights the essential function of immunological co-stimulation and platelet activation in this comorbidity. sCD40L, primarily derived from activated platelets and T cells, functions as a key regulator of adaptive immunity. It controls how B cells change, how cytokines are made (such as TNF-α and IL-6), and how synovial fibroblasts are stimulated [12,13]. Our discovery that sCD40L levels were heightened and significantly associated with depression severity (HAM-D17) supports the immune-inflammatory concept of depression. This hypothesis suggests that proinflammatory cytokines may cross the blood-brain barrier, alter monoamine metabolism, and stimulate the hypothalamic-pituitary-adrenal (HPA) axis, thereby eliciting depressive symptoms [29,30]. In major depressive disorder (MDD) devoid of autoimmune disorders, elevated sCD40L has been previously identified, associating it with a proinflammatory and prothrombotic state that may exhibit neurotoxicity [15]. In RA, characterized by elevated baseline inflammation, an increased sCD40L response may signify a distinct immunological second hit that propels susceptible people into clinical depression. The discovery that sCD40L, unlike general disease activity (DAS28), predicts disease activity indicates that this route is specific to the depressed phenotype rather than merely indicative of overall RA severity.

The significance of Lp-PLA2 in vascular inflammation as a prevalent mechanism

The strong independent link between Lp-PLA2 and depression (OR=26.07) is a new and important discovery. Lp-PLA2 is an enzyme that comes from macrophages and is found in large amounts in atherosclerotic plaques. It breaks down oxidized phospholipids to generate potent proinflammatory substances, such as lysophosphatidylcholine and oxidized free fatty acids [16]. It is a known indicator for inflammation in blood vessels and future heart

We sincerely thank the medical staff at Rizgary Teaching Hospital, especially Dr. Azheen Barzanji, for their invaluable assistance and cooperation during the sample collection phase. We are also deeply grateful to the skilled team at the Asia Clinical Laboratory in Najaf City for their expertise and support in performing the ELISA measurements.

Author Contributions

All authors contributed equally to this work. Each author was involved in the study design, data analysis, manuscript drafting, and critical revision of the article, and has approved the final version for submission.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Funding Declaration

There is no specific funding for the present study.

Data Availability Statement

The datasets generated and analyzed during this study are available from the corresponding author (Al-Hakeim HK), upon reasonable request, once the authors have completed their primary research objectives.

Ethics Statements

The study protocol received approval from the Institutional Review Board of the College of Pharmacy, University of Howlar, Iraq (Approval No. HMU-EC-Ph 12052024-18). It was conducted in accordance with the Declaration of Helsinki and international guidelines for human research ethics.

Consent for Publication

Participants provided informed consent for the publication of anonymized data derived from their involvement in this research.

Informed Consent

Written informed consent was obtained from all individual participants included in the study.

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an intensified feeling of pain (fatigue, TJC), which in turn worsens mood [6]. The significant correlation between HAM-D17 and ESR, as opposed to DAS28, highlights that depression is linked to a specific aspect of systemic inflammation rather than just a composite clinical metric. The correlation between DAS28 and atherogenic indices suggests that total disease activity is linked to metabolic dysregulation, a separate but simultaneous consequence of chronic inflammation [39,40].

Diagnostic Potential and Clinical Implications.

The receiver operating characteristic (ROC) analysis has translational potential. The area under the curve (AUC) of 0.76 for sCD40L indicates a considerable accuracy in distinguishing between RA+Dep and RA [41]. A single biomarker is unlikely to serve as an independent diagnostic test. Nonetheless, sCD40L, in conjunction with Lp-PLA2 and several clinical variables (including TJC and PDA), might serve as the basis for a clinically significant risk-stratification or diagnostic support panel. This objective tool may alleviate the considerable problem of underdiagnosis, since the physical symptoms of RA often hinder traditional depression assessments [42,43]. Furthermore, recognizing patients with the high sCD40L/Lp-PLA2 inflammatory-depressive phenotype may enable more customized treatment strategies, including selecting therapies that provide both anti-inflammatory and potential neuropsychiatric benefits, or improving depression screening within this high-risk group.

Limitations of the study

This study has several limitations. First, the relatively small sample size may limit statistical power and precision of estimates. Second, the single-center design may restrict generalizability. Third, the cross-sectional nature precludes causal inference and longitudinal validation. Future multicenter and longitudinal studies are needed to confirm these findings.

Conclusion

RA-associated depression is associated with increased markers of immune and vascular inflammation, particularly sCD40L and Lp-PLA2. These findings provide exploratory evidence of their potential role in the underlying pathophysiology and warrant further investigation in larger, longitudinal studies.

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