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Serum IL-27 and IL-35 Levels as Complementary Biomarkers of Immune Status in HIV-Positive Individuals

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Abstract

BACKGROUND/AIMS: Human immunodeficiency virus (HIV) infection remains a worldwide public health challenge, primarily due to its disruption of immune regulation and cytokine homeostasis. As members of the interleukin (IL)-12 family, IL-27 and IL-35 contribute to immune regulation, although they may have functionally opposing roles. The objective of this study was to explore the association between IL-27 and IL-35 levels, viral load, and immune parameters in individuals living with HIV compared with healthy subjects.

MATERIALS AND METHODS: A total of 45 HIV-positive individuals and 45 healthy controls, matched for age and sex, were enrolled in this cross-sectional study. Serum IL-27 and IL-35 concentrations were measured using enzyme-linked immunosorbent assay. Correlations between cytokine levels, HIV-ribonucleic acid (RNA), and T-cell subsets were assessed using Spearman's rho. Logistic regression and receiver operating characteristic (ROC) analyses were conducted to identify predictors of HIV-positivity and to assess diagnostic performance, while multivariate patterns were examined using partial least squares discriminant analysis (PLS-DA).

RESULTS: IL-27 levels were significantly lower and IL-35 levels were significantly higher in HIV-positive patients than in controls ($p<0.01$ for both). IL-27 was negatively correlated with HIV RNA ($r=-0.40$, $p=0.02$) and the neutrophil-to-lymphocyte ratio ($r=-0.33$, $p=0.03$), and positively correlated with total T-cell percentage ($r=0.34$, $p=0.04$). Conversely, IL-35 showed a strong positive correlation with HIV-RNA ($r=0.66$, $p<0.001$) and strong negative correlations with CD4 count ($r=-0.47$, $p=0.001$) and CD4 percentage ($r=-0.78$, $p=0.001$). In logistic regression, IL-35 was an independent positive predictor of HIV positivity [odds ratio (OR) =2.08, 95% confidence interval (CI) =1.29-3.34, $p=0.003$], whereas IL-27 was inversely associated with HIV positivity (OR =0.993, 95% CI =0.988-0.998, $p=0.004$). ROC analysis demonstrated high discriminative ability for IL-35 [area under the curve (AUC) =0.863] and moderate discriminative ability for IL-27 (AUC =0.717); their combination achieved the highest accuracy (AUC =0.898; sensitivity =80%; specificity =91%). PLS-DA confirmed that elevated IL-35 levels, decreased IL-27 levels, and CD4 depletion were the major contributors to HIV-related immunosuppression.

CONCLUSION: IL-27 and IL-35 exhibit opposing immunomodulatory patterns in HIV infection. Decreased IL-27 and elevated IL-35 levels reflect disease activity and immune dysregulation. While IL-35 serves as an independent biomarker of immunosuppression, combined assessment of IL-27 and IL-35 increases diagnostic accuracy in distinguishing HIV-positive individuals from healthy controls.

Keywords: HIV, interleukin-27, interleukin-35, AIDS, biomarkers

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INTRODUCTION

In 1981, the emergence of acquired immunodeficiency syndrome (AIDS), which initially affected young gay men, marked a turning point in medical history, and the scientific community began searching for a cure.[1] These investigations eventually identified human immunodeficiency virus type 1 (HIV-1) as the pathogen responsible for one of the most severe infectious diseases in human history.[2]

Human immunodeficiency virus (HIV) infection is characterized by a progressive decline in CD4 cell counts, immunosuppression, and altered cytokine levels. Dysregulation of cytokine signaling has been proposed as a key contributor to HIV-associated immune dysfunction. In the early phase of HIV infection, levels of cytokines, such as interleukin (IL)-2 and interferon, are elevated, reflecting a predominant T helper 1 (Th1) immune response. Conversely, in the advanced stage of infection, a shift toward a Th2-dominant profile occurs, characterized by increased production of cytokines, including IL-4 and IL-10. Recent investigations have identified an expanding array of cytokines implicated in AIDS pathogenesis, encompassing IL-7, IL-15, and IL-21, and members of the IL-17, IL-18, IL-19, IL-20, IL-23, and IL-27 families.[3,4] Within this group, IL-23 and IL-27, both belonging to the IL-12 cytokine family, hold distinct immunological significance. IL-23 contributes to the induction of the Th17 pathway, whereas IL-27 supports Th1 polarization and promotes the maintenance of naïve T and B cell populations.[5]

IL-27 functions as a key immunomodulatory molecule involved in regulating both innate and adaptive components of the immune response. This cytokine is primarily produced by antigen-presenting cells upon stimulation.[6] IL-27 exhibits primarily anti-inflammatory activity by modulating cytokines such as IL-10 and IL-21 and by influencing distinct CD4+ T-cell subsets, including regulatory T (Treg) cells and Th17 cells.[7,8] Evidence indicates that IL-27 displays significant antiviral activity against HIV in immature dendritic cells;[9] therefore, IL-27 exerts protective effects across the primary cell populations targeted by HIV-1. Owing to its broad and potent anti-HIV activity, IL-27 has been explored as a promising candidate for therapeutic intervention in HIV-1 infection.[10] One investigation assessing IL-27 expression among individuals infected with HIV-1 reported a slight negative association between IL-27 levels and viral burden, suggesting that viral mechanisms might downregulate IL-27 to enhance disease progression.[11] Another study found that IL-27 levels correlated positively with CD4+ T-cell counts, but the potential relationship with viral load remained unexamined.[12] Bhati et al.[13] reported that plasma concentrations of IL-23 and IL-27 were substantially higher in HIV-1-infected subjects than in uninfected controls. The study further identified a strong direct association between CD4+ T-cell numbers and the titers of IL-23 and IL-27 in patients with HIV infection.[13]

IL-35, a recently characterized member of the IL-12 cytokine family, is a heterodimer composed of the IL-27 β-chain, Epstein-Barr virus-induced gene 3, and the IL-12 α-chain p35 (IL-12p35). It is predominantly produced by Treg cells and regulatory B cells, both of which contribute to immune tolerance and viral persistence in chronic hepatitis B virus (HBV) infection.[14,15] Previous studies have indicated that IL-35 modulates the functional responses of CD4+ and CD8+ T lymphocytes, thereby contributing to immunosuppression in hepatocellular carcinoma arising from chronic HBV infection or nonviral etiologies.[16-18]

Given the relationships described above and the potential correlation, we aimed to investigate the associations of IL-27 and IL-35 levels with HIV positivity and with laboratory parameters (complete blood count, routine biochemistry tests, viral load, and T-cell subsets) in HIV-positive patients compared with a healthy control group.

MATERIALS AND METHODS

Study Setting, Study Population, and Laboratory Analysis

Approval for the study protocol was obtained on Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: AEŞH-BADEK2-2025-084, date: 13.05.2025).

In 2025, 45 patients aged 18-80 years who were diagnosed as HIV-positive and had this diagnosis confirmed at the Infectious Diseases and Clinical Microbiology outpatient clinics of* were included in the study. For comparative purposes, a control cohort comprising 45 healthy subjects was included. The control participants were free of chronic illnesses, were not taking any continuous medications, and were comparable to the patient group in age and sex distribution. To minimize confounding factors, individuals with chronic inflammatory conditions, malignancies, alcohol use disorder, or other comorbidities known to modulate immune function were excluded. All enrolled HIV-positive participants were newly diagnosed, treatment-naïve, and free of acute infection at the time of sampling. Information on lifestyle factors (e.g., smoking and alcohol use) and HIV-1 viral subtypes were not available in the medical records; therefore, these variables could not be included in the analyses. All HIV-positive participants were treatment-naive enrollment, and blood samples were obtained initial diagnosis before initiation of antiretroviral therapy (ART). None of the participants had received ART prior to sample collection. Signed consent forms were obtained from all individuals included in the study.

Venous blood samples were obtained from seated participants before noon, following a fasting period of at least eight hours. To obtain serum, blood samples were collected into one 5 mL BD Vacutainer SST II Advance Blood Collection Tube (gel-separated) (Becton Dickinson, Plymouth, United Kingdom), and for complete blood count into one 3 mL BD Vacutainer™ K2EDTA Blood Collection Tube (Becton Dickinson, Plymouth, United Kingdom). The serum-separating tube used to collect serum from blood samples was incubated for 30 min and then centrifuged at 1500× g for 10 min to separate the serum. Routine biochemical analyses were performed on the same day using a Roche Cobas 8000-c701 (Roche Diagnostics, Mannheim, Germany) analyzer. Complete blood counts were performed using a Sysmex XN-1000 device (Sysmex Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany), which employs fluorescence flow cytometry. Remaining samples from biochemical tests requested from patients were aliquoted and stored at -80 °C until the day of the study. The levels of IL-27 and IL-35 in serum were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BT-LAB, Shanghai, China) according to the manufacturer's instructions. According to the manufacturer's data, the intra-assay and inter-assay coefficients of variation for both IL-27 and IL-35 assays were <10% and <12%, respectively. These kits have been previously used in HIV and other viral infection cohorts and have demonstrated acceptable analytical reliability for low-abundance cytokine measurements.

Complete blood count parameters, routine biochemical tests, viral load results [HIV- ribonucleic acid (RNA)], T-cell and subtype ratios, demographic data (e.g., age and sex), HIV test results, and IL-27 and IL-35 levels were evaluated for all individuals.

Statistical Analysis

The distributions of continuous variables was assessed using the Shapiro-Wilk test and graphical methods (histograms and Q-Q plots), and homogeneity of variance was examined using Levene's test. Descriptive statistics are reported as mean ± standard deviation or median (interquartile range) according to the distribution. Comparisons between the two groups were conducted using the Independent samples t-test for variables with a normal distribution and the Mann-Whitney U test for those without. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Effect sizes for group differences are presented as Hedges' g.

In the correlation analyses, Spearman's rho was used because it accommodates nonlinearity and is robust to outliers; correlations were presented with 95% confidence intervals (CIs). For correlation heatmaps with multiple comparisons, the Benjamini-Hochberg (false discovery rate) correction was applied as an additional sensitivity analysis for exploratory purposes.

Binary logistic regression was used to evaluate the predictors of HIV positivity (0= healthy, 1= HIV+). Key covariates (age, sex, neutrophils, and lymphocytes) were included in the model; and IL-27 and IL-35 were tested separately (Models 1 and 2) and together (Model 3). The coefficients were reported as odds ratios (ORs) with 95% CIs. Multicollinearity was assessed using the variance inflation factor and the correlation matrix; model fit was evaluated with the Hosmer-Lemeshow test; and discriminatory power was assessed using the receiver operating characteristic (ROC)- area under the curve (AUC). The need to transform right-skewed predictors (e.g., IL-35) was assessed graphically. To preserve the interpretability of units, the final models were reported in raw units. For IL-27 observations with few missing values, missing data were imputed using the median to enable calculation of a combined score for all individuals, and the results were checked for consistency by complete-case analysis.

ROC curves and AUC values were calculated to assess diagnostic performance. The individual curves for IL-27 and IL-35 were compared with the combined curve, which was obtained using the probability score derived from logistic regression and represents the combined effect of the two markers. Differences between the AUCs were assessed using the DeLong test. The best threshold values were determined using Youden's J criterion, and the corresponding sensitivity, specificity, and 95% CI values (full binomial/bootstrap; 2,000 samples when necessary) were reported.

Partial least squares discriminant analysis (PLS-DA) was applied to characterize the multivariate patterns. Variables were scaled using autoscaling (z-score), the number of components was selected using cross-validation, and the risk of overfitting was assessed using permutation testing. Variable contributions were summarized with variable importance in projection (VIP) scores; VIP >1 was used as a practical threshold for significant contribution.

All analyses were two-sided with α=0.05 and were conducted using SPSS v23 (IBM Corp., Armonk, NY, USA), R (pROC, ggplot2, mixOmics/ropls; R Foundation), MedCalc v23.3.7 (MedCalc Software Ltd.), and the analyze-it add-in (Microsoft Excel).

Sample size was determined a priori with the aid of G*Power 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf). The test family consisted of two-tailed t-tests comparing the means of two independent groups with a 1:1 group ratio and a significance level of α=0.05. Cohen's d (d=1.39) was calculated using the IL-35 values reported in the literature.[19] Given this effect size, 13 subjects per group were deemed sufficient to achieve 90% statistical power; if 95% power is targeted with the same settings, 15 subjects per group are required. In our study, this minimum requirement was significantly exceeded by including 45 participants in each group, thus increasing the precision of the estimates (narrower 95% CI), supporting more robust secondary and multivariate analyses (e.g., logistic regression, ROC), and preserving statistical power against possible exclusions or dropouts.

RESULTS

A comparison of the demographic and clinical data between HIV-1-positive patients and healthy controls is shown in Table 1 and Figure 1.

Spearman correlation analysis was performed to evaluate the associations among clinical, hematologic, and immunologic variables (Table 2). The complete correlation matrix for all study variables is provided in the (Supplementary Material Figure S1). To visualize the distinct biological roles of the investigated cytokines, a targeted heatmap summarizing the key associations of IL-27 and IL-35 is presented in Figure 2.

Within the HIV-1 patient group, IL-27 exhibited a pattern consistent with immune preservation. As shown in Figure 2, IL-27 correlated positively with the CD4/CD8 ratio (r=0.402, p<0.05) and inversely with the CD8 percentage (r=-0.348, p<0.05). Additionally, IL-27 showed negative associations with HIV-1 RNA viral load (r=-0.40, p=0.02) and with neutrophil-to-lymphocyte ratio (NLR) (r=-0.33, p=0.03), further supporting its association with a lower inflammatory burden (Table 2).

In contrast, IL-35 displayed strong associations with markers of immunosuppression and disease progression. Figure 2 highlights significant negative correlations between IL-35 and key T-cell indices, including total T-cell percentage (r=-0.767, p<0.001), CD4 count (r=-0.678, p<0.001), CD4 percentage (r=-0.662, p<0.001), and the CD4/CD8 ratio (r=-0.604, p<0.001). Conversely, IL-35 correlated positively with the CD8 percentage (r=0.526, p<0.001) and with glucose levels (r=0.397, p<0.05). Consistent with these findings, IL-35 was strongly positively correlated with HIV-1 RNA load (r=0.66, p<0.001; Table 2).

Three distinct specifications of the logistic regression model are presented in Table 3. In Model 1 (adjusted for age, sex, neutrophils, lymphocytes, and IL-35), IL-35 was independently and positively associated with HIV-positivity (OR =2.08, 95% CI 1.29-3.34, p=0.003), whereas lymphocyte count was inversely associated with HIV-positivity (OR =0.014, 95% CI 0.001-0.185, p=0.001). In Model 2, which included age, sex, neutrophils, lymphocytes, and IL-27, IL-27 remained independently and negatively associated with HIV positivity (OR =0.993, 95% CI =0.988-0.998, p=0.004), while the inverse association of lymphocyte count persisted (OR =0.082, p<0.001). In Model 3 (both cytokines together), the positive association for IL-35 persisted (OR 10.07, 95% CI 1.59-63.59, p=0.014), whereas the effect of IL-27 decreased and became non-significant (OR 0.978, 95% CI 0.949-1.007, p=0.137).

Table 1. Comparison of demographic and clinical data between HIV-1 positive patient and healthy controls

		Healthy control group (n=45)	HIV+ patient group (n=45)	Effect size (Hedges g)	p-value
Gender	Female	7 (16)	6 (13)		
	Male	38 (84)	39 (87)		
Age (year)		43 (32-54)	40 (27-52)	-0.189	0.368
WBC (10^3/µL)		7.39±1.58	4.76±1.56	-1.661	0.001
RBC (10^6/µL)		4.5 (4.1-5)	3.5 (3-4.1)	-1.332	0.001
Neutrophil (10^3/µL)		4.47±1.72	3.42±1.43	-0.658	0.02
Lymphocyte (10^3/µL)		2 (1.3-2.6)	1 (0.6-1.4)	-1.199	0.001
Platelet (10^3/µL)		304±79.3	251±71.1	-0.698	0.003
NLR		2.3 (1.75-3)	3.75 (2.38-5.28)	0.871	0.001
Glucose (mg/dL)		86 (81-91)	97 (90-105)	1.19	0.001
Urea (mg/dL)		26 (21-31)	27 (19-31)	-0.04	0.868
Creatinine (mg/dL)		0.7 (0.6-0.9)	0.8 (0.7-1)	0.449	0.017
ALT (U/L)		22.9±9.89	28.9±12.7	0.523	0.021
AST (U/L)		24.7±10.5	27.9±11.7	0.285	0.230
HIV-1 RNA (copies/mL)		(-)	207000 (75700-818000)		
T-cell (%)		74.2±4.733	42.4±13.5	-3.117	0.001
CD4 count (cell/µL)		986 (812-1191)	310 (209-438)	-2.895	0.001
CD8 (%)		31.8±3.72	56.8±12.4	2.708	0.001
CD4/CD8		1.78 (1.46-1.93)	0.35 (0.22-0.51)	-4.673	0.001
CD4 (%)		53.3±5.12	20.1±7.91	-4.955	0.001
IL-27 (ng/L)		229.7 (152.1-310.1)	152 (56.8-212)	-1.29	0.009
IL-35 (ng/mL)		2.74 (2.04-3.92)	8.51 (5.53-16.1)	1.26	0.001

WBC: White blood cell, RBC: Red blood cell, NLR: Neutrophil-to-lymphocyte ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HIV-1: Human immunodeficiency virus type 1, RNA: Ribonucleic acid, IL: Interleukin.

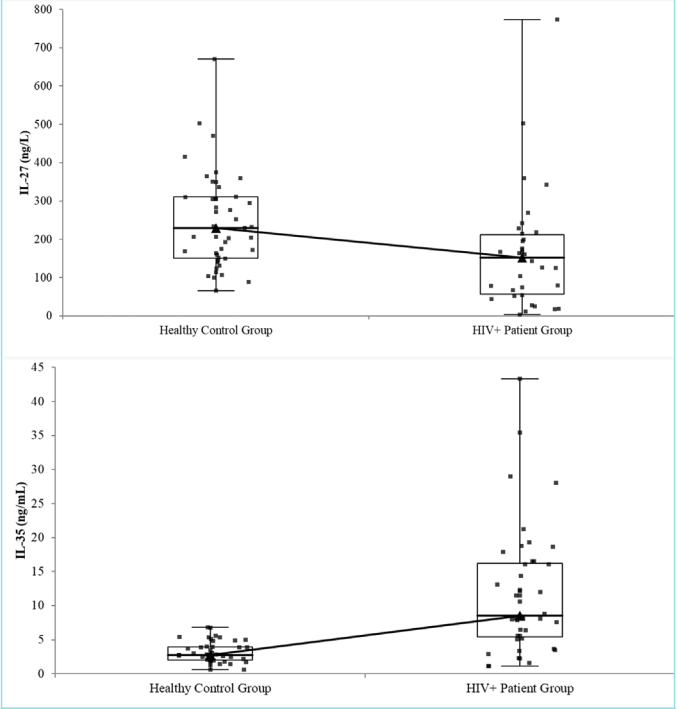

Figure 1. Distributions of IL-27 and IL-35 between the HIV+ and healthy control groups.

HIV: Human immunodeficiency virus, IL: Interleukin.

	IL-35	IL-27
Glucose	0.397*	-0.229
CD8	0.526*	-0.348*
NLR	0.051	-0.188
Creatinine	0.237	-0.195
AST	0.009	0.057
Lymphocyte	-0.328	0.175
CD4	-0.662*	0.390
CD4/CD8	-0.604*	0.402*
CD4 count	-0.678*	0.329
T-cell	-0.767*	0.359
WBC	-0.491*	0.115
RBC	-0.497*	0.157
Age	-0.151	-0.127
Neutrophil	-0.314	-0.030
Platelet	-0.212	0.219
Urea	0.067	-0.009

Figure 2. Correlation analysis of all variables. Significant correlation at *p<0.05 level.

WBC: White blood cell, RBC: Red blood cell, NLR: Neutrophil-to-lymphocyte ratio, AST: Aspartate aminotransferase, HIV-1: Human immunodeficiency virus type 1, RNA: Ribonucleic acid, IL: Interleukin.

This pattern is consistent with the expected effect of multicollinearity due to covariation among cytokines and the limited sample size. Age, sex, and neutrophil count were not significant in any of the models; lymphocyte count was independently and inversely associated with HIV positivity in all three models (p≤0.029).

In ROC analysis, IL-35 alone showed high discriminative ability (AUC =0.863, 95% CI, 0.774-0.926) and, at a threshold of >5.4 ng/mL, achieved a sensitivity of 75% (60-87) and a specificity of 93% (81-98). IL-27 showed more modest performance (AUC =0.717, 95% CI 0.605-0.812) and at ≤79.5 ng/L yielded a sensitivity of 38% (21-55%) and a specificity of 98% (88-99%) (Table 4).

The ROC curve for this combined score outperformed the single-marker curves (AUC 0.898, 95% CI 0.817-0.952). Using the Youden criterion, an optimal cut-off p-value of 0.49 yielded a sensitivity of 80% (65-90%) and a specificity of 91% (78-97%) (Table 4, Figure 3).

Table 2. Spearman correlations among clinical, hematological, and immunological variables within the HIV-1 patient group (n=45)

		Neutrophil	Lymphocyte	Platelet	NLR	Glucose	IL-27	IL-35	HIV-1 RNA	T-cell	CD4	CD4	CD8	CD4/CD8
Age (year)	r	0.149	0.202	0.026	-0.054	-0.312*	-0.130	-0.048	0.055	-0.021	-0.256	-0.300*	0.179	-0.237
	p	0.329	0.183	0.866	0.726	0.037	0.455	0.755	0.721	0.892	0.090	0.045	0.239	0.117
	N	45	45	45	45	45	35	45	45	45	45	45	45	45
Neutrophil (10^3/µL)	r	10.000	0.103	-0.042	0.587*	-0.047	-0.285	-0.30*	-0.031	0.103	-0.010	-0.039	0.026	-0.044
	p		0.502	0.786	0.000	0.757	0.097	0.044	0.842	0.501	0.949	0.800	0.868	0.772
	N		45	45	45	45	35	45	45	45	45	45	45	45
Lymphocyte (10^3/µL)	r		1.000	0.133	-0.71*	-0.158	-0.025	0.180	0.341*	-0.352*	-0.319*	-0.166	-0.004	-0.115
	p			0.384	0.000	0.299	0.885	0.237	0.022	0.018	0.033	0.275	0.982	0.453
	N			45	45	45	35	45	45	45	45	45	45	45
Platelet (10^3/µL)	r			1.000	-0.150	-0.042	0.177	0.062	0.244	-0.043	-0.331*	-0.344*	0.301*	-0.357*
	p				0.326	0.785	0.309	0.684	0.107	0.777	0.026	0.021	0.045	0.016
	N				45	45	35	45	45	45	45	45	45	45
NLR	r				1.000	0.152	-0.157	-0.33*	-0.263	0.317*	0.238	0.128	0.002	0.080
	p					0.319	0.366	0.026	0.081	0.034	0.115	0.401	0.990	0.602
	N					45	35	45	45	45	45	45	45	45
Glucose (mg/dL)	r					1.000	-0.131	-0.013	-0.003	0.042	-0.009	0.006	-0.031	0.025
	p						0.452	0.932	0.987	0.782	0.953	0.970	0.838	0.869
	N						35	45	45	45	45	45	45	45
IL-27 (ng/L)	**r**						1.000	-0.257	-0.397*	0.344*	0.297	0.251	-0.124	0.211
	p							0.137	0.018	0.043	0.084	0.147	0.478	0.224
	N							45	45	45	45	45	45	45
IL-35 (ng/mL)	**r**							1.000	0.660*	-0.702*	-0.47**	-0.249	0.205	-0.242
	p								0.000	0.000	0.001	0.100	0.177	0.108
	N								45	45	45	45	45	45
HIV-1 RNA (copies/mL)	r								1.000	-0.74**	-0.55**	-0.348*	0.132	-0.280
	p									0.000	0.000	0.019	0.387	0.062
	N									45	45	45	45	45
T-cell (%)	r									1.000	0.477**	0.376*	-0.192	0.341*
	p										0.001	0.011	0.206	0.022
	N										45	45	45	45
CD4 count (cell/µL)	r										1.000	0.584**	-0.342*	0.520*
	p											0.000	0.022	0.000
	N											45	45	45
CD4 (%)	r											1.000	-0.78**	0.968*
	p												0.000	0.000
	N												45	45
CD8 (%)	r												1.000	-0.897*
	p													0.000
	N													45

*Significant correlation, p<0.05.

**Highly significant correlation, p<0.01.

NLR: Neutrophil-to-lymphocyte ratio, HIV-1: Human immunodeficiency virus type 1, RNA: Ribonucleic acid, IL: Interleukin.

Table 3. Multivariable logistic regression models for HIV-1 positivity (0= healthy, 1= HIV+) included three specifications - IL-35 only, IL-27 only, and both cytokines - and were adjusted for age, sex, neutrophil count and lymphocyte count

		B	Wald	Sig.	Odds ratio [Exp(B)] Lower	95% CI for EXP(B) Upper	
Model 1	Gender	-0.524	0.131	0.718	0.592	0.035	10.114
	Age (year)	0.011	0.152	0.697	1.011	0.955	1.071
	Neutrophil (10^3/µL)	0.162	0.263	0.608	1.175	0.633	2.182
	Lymphocyte (10^3/µL)	-4.258	10.548	0.001	0.014	0.001	0.185
	IL-35 (ng/mL)	0.732	9.134	0.003	2.080	1.294	3.344
Model 2	Gender	-0.788	0.814	0.367	0.455	0.082	2.518
	Age (year)	-0.022	0.834	0.361	0.978	0.932	1.026
	Neutrophil (10^3/µL)	-0.331	2.292	0.130	0.718	0.468	1.103
	Lymphocyte (10^3/µL)	-2.505	14.290	0.001	0.082	0.022	0.299
	IL-27 (ng/L)	-0.007	8.404	0.004	0.993	0.988	0.998
Model 3	Gender	-1.069	0.145	0.703	0.343	0.001	84.188
	Age (year)	-0.011	0.051	0.821	0.989	0.899	1.088
	Neutrophil (10^3/µL)	0.044	0.006	0.938	1.045	0.340	3.218
	Lymphocyte (10^3/µL)	-9.264	4.778	0.029	0.001	0.001	0.384
	IL-27 (ng/L)	-0.022	2.212	0.137	0.978	0.949	1.007
	IL-35 (ng/mL)	2.309	6.031	0.014	10.068	1.594	63.589

HIV-1: Human immunodeficiency virus type 1, IL: Interleukin, EXP: Exponentiation of B, CI: Confidence interval.

Table 4. Diagnostic performance of IL-27, IL-35, and a combined logistic score for discriminating HIV-1 positivity: cut-offs, AUCs, sensitivity, and specificity (95% CIs)

	Cut-off	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
IL-27	≤79.5	0.717 (0.605-0.812)	38 (21-55)	98 (88-99)
IL-35	>5.4	0.863 (0.774-0.926)	75 (60-87)	93 (81-98)
Combined IL-27 and IL-35	>0.49	0.898 (0.817-0.952)	80 (65-90)	91 (78-97)

HIV-1: Human immunodeficiency virus type 1, IL: Interleukin, AUC: Area under the curve CI: Confidence interval.

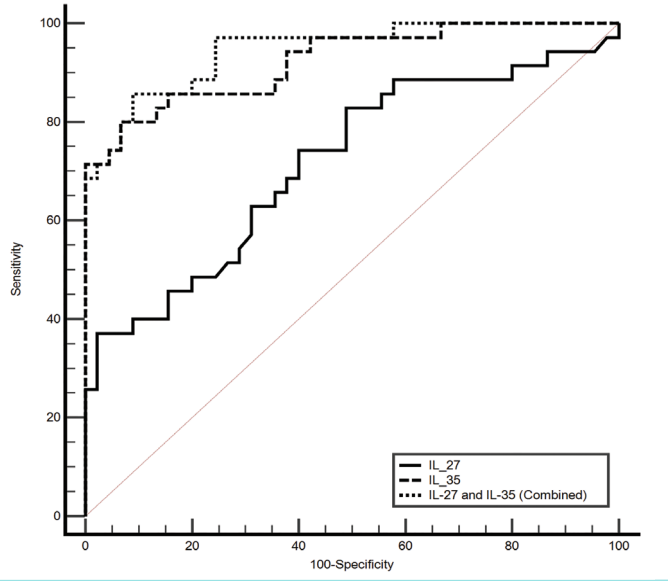

Figure 3. ROC curves for IL-27, IL-35, and their combined logistic scores.

ROC: Receiver operating characteristic, IL: Interleukin.

Multivariate modelling with PLS-DA separated the two groups primarily along Component 1, which accounted for nearly all modelled variance (Figure 4A). In the scores plot (Figure 4A), HIV-1-positive participants clustered on one side of Component 1, whereas healthy controls occupied the opposite side. The confidence ellipses showed limited overlap, indicating robust discrimination between the groups based on the analyzed biochemical and immunological profiles.

The contribution of individual variables to this separation was evaluated using VIP scores (Figure 4B). The analysis identified cytokines and cellular immunity indices as the principal contributors to the model. Specifically, IL-27 emerged as the most significant variable driving the projection, followed by IL-35 and CD4-related measures. This multivariate pattern highlights that the altered cytokine balance-characterized by the interplay of IL-27 and IL-35-together with CD4 status constitutes the dominant feature distinguishing HIV-1-positive patients from healthy controls.

DISCUSSION

In our study, we measured IL-27 and IL-35 levels in HIV+ individuals, examined their relationships with HIV-RNA, T lymphocyte subsets, and biochemical parameters, and compared them with those of healthy controls. We showed that in HIV+ individuals, IL-27 levels, known to have an anti-inflammatory effect, were lower, whereas IL-35 levels were

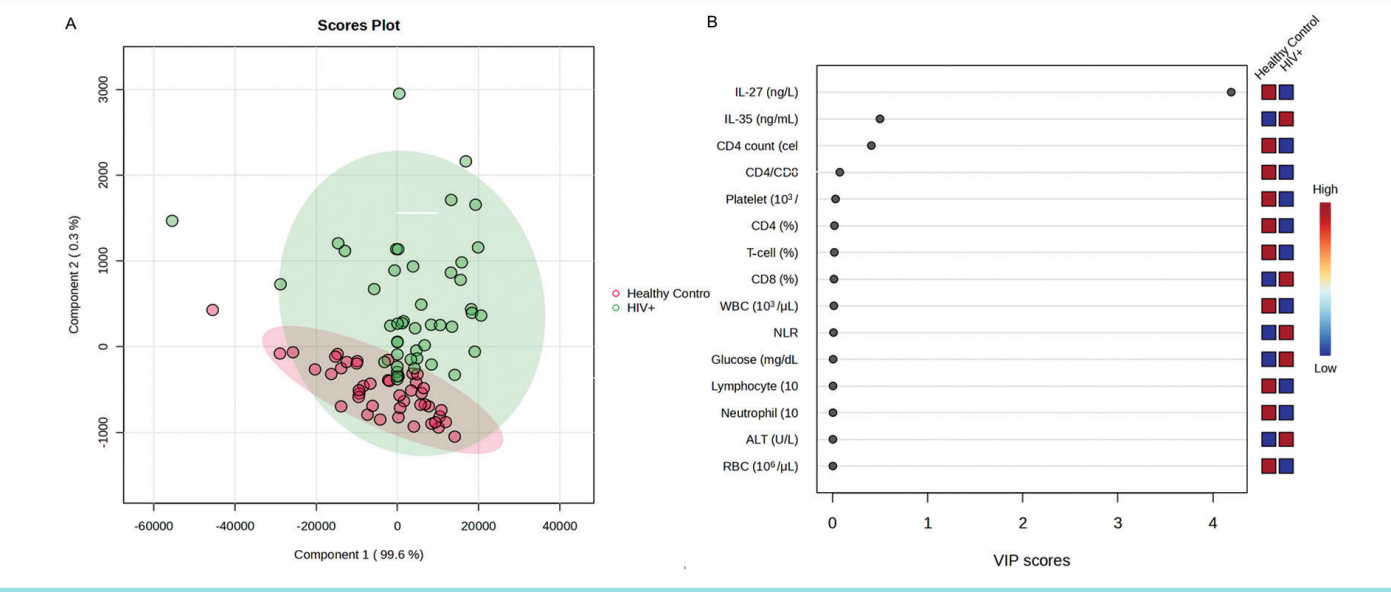

Figure 4. Multivariate discrimination of HIV-1-positive patients from healthy controls using PLS-DA. (A) Two-dimensional score plot with 95% confidence ellipses for each group. (B) Variable importance-in-projection (VIP) scores with a side heat bar indicating the relative direction of change in each group (z-scaled).

HIV-1: Human immunodeficiency virus type 1, PLS-DA: Partial least squares discriminant analysis, WBC: White blood cell, RBC: Red blood cell, NLR: Neutrophil-to-lymphocyte ratio, ALT: Alanine aminotransferase, IL: Interleukin.

higher. Because of HIV infection, we observed a significant decrease in all cell groups in HIV-positive individuals compared with those in the control group. Despite decreased cell numbers, the proportion of CD8+ cells was higher in HIV+ individuals. Because these patients were newly diagnosed, they were thought to be in the early stages of infection and to be dominated by CD8+ cells. HIV-RNA, which indicates viral load, was inversely correlated with IL-27 and positively correlated with IL-35. Taken together, IL-27 exhibited an anti-viremic/anti-inflammatory signature, whereas IL-35 was associated with higher viremia and CD4 T-cell depletion in the HIV-1 patient cohort. IL-27 receptors are broadly distributed across multiple immune cell populations, including dendritic cells, macrophages, monocytes, neutrophils, mast cells, eosinophils, and lymphocytes (T-cells, B cells, and natural killer (NK) cells). Functionally, IL-27 enhances Th1 polarization while inhibiting Th2- and Th17-mediated differentiation. Moreover, IL-27 mediates anti-inflammatory activity by inducing IL-10-secreting CD4+ T-cells, a subset of Treg cells crucial for immune homeostasis and control of inflammation.[20,21] In a review conducted by Kourko et al.,[22] the biological functions of IL-27 were analyzed alongside related cytokines such as IL-30 and IL-35. The interplay among these cytokines has been shown to suppress tumor progression through the activation of diverse immune effector cells, which can act on tumor cells either directly or via intermediary mechanisms. For instance, IL-27, together with these cytokines, enhances NK cell-mediated cytotoxicity, augments the effector function of CD8+ T lymphocytes, and supports the generation of CD8+ memory T-cells.[22] In our study, IL-27 levels were inversely correlated with HIV-1 RNA and positively correlated with T-cell and CD4 parameters, supporting the role of this cytokine in the antiviral response. Furthermore, the negative correlation between IL-27 and NLR, a marker of systemic inflammation, suggests that this cytokine may play a protective role in immune homeostasis.

IL-27 levels are typically elevated during infections; this elevation is believed to be linked to IL-10 expression, which may help regulate inflammation while enabling the persistence of the infectious agent.[20,23] Consistent with these data, a 2021 study observed that IL-27 levels in COVID-19 patients were increased in the acute phase compared with controls and decreased during the recovery period.[24] In our study, we observed lower IL-27 levels in HIV-positive individuals than in HIV-negative individuals. The significantly higher IL-35 levels in HIV-positive individuals and the strong positive correlations with viral load and negative correlations with CD4 count and percentage suggest that IL-35 may be an indicator of immune suppression and viremia. These findings suggest that IL-27 exhibits protective effects and IL-35 exhibits suppressive effects, and that they have opposing biological roles in HIV pathogenesis. IL-35 was first discovered in 2007. This cytokine, a member of the same family as IL-27, suppresses the differentiation and activity of Th1 and Th17 cells by inducing Treg proliferation and enhancing IL-10 secretion.

Despite belonging to the same IL-12 cytokine family, IL-27 and IL-35 exert distinct and often opposing effects on immune regulation through divergent downstream signaling pathways. IL-27 primarily activates STAT1 and STAT3 signaling, promoting Th1 differentiation and antiviral immune responses, while simultaneously limiting excessive inflammation. In contrast, IL-35 signals predominantly through STAT1/STAT4 heterodimers, leading to expansion of Treg cells and suppression of effector T-cell activity. This functional divergence provides a mechanistic framework for the inverse associations of IL-27 and IL-35 with viral load and CD4 depletion observed in the present study.

In tumor studies, IL-27 has been shown to have antitumor properties, whereas IL-35 promotes tumor development.[22,25] HIV infection is also known to increase the incidence of malignancies and the risk of mortality.[26-28] The results of our study are consistent with these findings

when considering the effects of IL-27 and IL-35 on tumor cells. In logistic regression analyses adjusting for potential confounders such as age, sex, and neutrophil and lymphocyte counts, IL-35 remained independently and positively associated with HIV positivity, whereas IL-27 was independently and negatively associated. When both cytokines were included in the same model, only IL-35 remained significant. This may be explained by the covariance between the two cytokines and by the small sample size. ROC analysis indicated that IL-35 alone had high discriminatory power (AUC =0.863), whereas IL-27 showed more modest discriminatory power. The model evaluating the two cytokines together provided the highest diagnostic accuracy (AUC =0.898), suggesting that their combined use has potential as a biomarker. The multivariate analysis (PLS-DA) results also supported this biological pattern. The distinction between the groups was largely driven by IL-35, CD4-related parameters, and NLR, with a limited contribution from routine biochemical variables. In the HIV+ group, high IL-35 levels, low CD4 measurements, and increased NLR, when considered together, revealed a predominant immunosuppressive and pro-inflammatory profile that may facilitate viral persistence and immune exhaustion. In healthy controls, higher IL-27 levels and preserved CD4 indices suggested continued immune integrity. This study demonstrates that IL-35 is a suppressive cytokine linked to disease activity in HIV infection, whereas IL-27 may be a protective factor supporting the antiviral response. Our results align with previous research indicating that IL-27 enhances antiviral immunity, whereas IL-35 contributes to immune exhaustion. Therefore, these two cytokines can be considered opposing markers of immune regulation in HIV infection. In conclusion, elevated IL-35 levels and decreased IL-27 levels in HIV-positive individuals reflect the opposing immunological roles of these two cytokines. Increased IL-35 levels have been linked to enhanced viral replication and immune suppression, whereas IL-27 is associated with a preserved immune response and reduced viremia. These contrasting cytokine profiles may provide valuable information for the development of new biomarkers and the identification of immunomodulatory targets in HIV pathogenesis.

The attenuation of the IL-27 effect in the multivariate model likely reflects multicollinearity among IL-27, IL-35, and CD4-related variables, which are highly correlated. When these markers were entered simultaneously, the shared variance among cytokine and lymphocyte indices attenuated the independent contribution of IL-27, a phenomenon commonly observed in models with biologically linked immunological parameters.

An important strength of the present study is that all cytokine measurements were performed at the time of diagnosis in ART-naïve individuals. Therefore, IL-27 and IL-35 values were not influenced by antiretroviral treatment, which is known to modulate cytokine responses. This allowed us to evaluate cytokine patterns that more accurately reflect the natural immunological state prior to therapy.

Study Limitations

Certain limitations of this study should be acknowledged. The cross-sectional design does not allow for causal conclusions, and the modest sample size may reduce the applicability of the results to broader populations. In addition, cytokine measurements were obtained at a single time point, and neither treatment response nor longitudinal variations were evaluated. Although demographic and clinical factors, such as lifestyle habits and viral subtypes, may influence cytokine responses, these variables were not systematically available in the patient records and, therefore, could not be incorporated into the analyses. However, key immune-modulating comorbidities were excluded, and all patients were treatment-naïve at the time of diagnosis, thereby reducing major sources of biological variability. While cytokine assays may vary across commercial ELISA platforms, the kits used in our study have shown low intra- and inter-assay variability and have been applied in previous HIV-related studies, supporting the reliability of our cytokine measurements. Future research involving larger cohorts and assessments before and after ART will be essential to better clarify the dynamic roles of IL-27 and IL-35.

CONCLUSION

Our study provides new evidence that IL-27 and IL-35 play opposing immunological roles in HIV pathogenesis. Serum IL-27 levels were significantly lower in HIV-positive individuals than in healthy controls, whereas IL-35 levels were significantly higher. IL-27 showed negative correlations with HIV-1 RNA and systemic inflammatory markers, and positive correlations with CD4-related parameters, suggesting a protective and antiviral role in supporting immune homeostasis. In contrast, IL-35 showed strong positive correlations with viral load and inverse associations with CD4 cell indices, demonstrating its association with immunosuppression and disease activity. In the multivariate and ROC analyses, IL-35 was identified as a robust and independent indicator of HIV positivity, whereas IL-27 exhibited a modest but biologically significant protective association. The combined use of the two cytokines improved diagnostic performance, highlighting their potential as complementary diagnostic markers. Multivariate modeling confirmed that high IL-35, low IL-27, and CD4 deficiency together defined the immunological profile of HIV-positive patients. Taken together, these findings demonstrate that IL-27 and IL-35 are essential yet antagonistic regulators of the immune response in HIV infection. Assessing these two factors together may improve the accuracy of early diagnosis and help identify novel immunomodulatory targets for therapeutic intervention. Future longitudinal studies incorporating ART initiation and follow-up are warranted to clarify the temporal dynamics of IL-27 and IL-35 and assess their potential value as biomarkers for disease monitoring and treatment response in HIV infection. Future longitudinal and treatment-based studies are needed to validate these cytokines as reliable biomarkers for disease monitoring and prognosis in HIV infection. Longitudinal and treatment-based studies incorporating ART are warranted to validate these cytokines as reliable biomarkers for disease monitoring and prognosis in HIV infection.

MAIN POINTS

- Serum interleukin (IL)-27 levels were significantly lower and IL-35 levels markedly higher in treatment-naïve human immunodeficiency virus (HIV)-positive individuals than in healthy controls.
- IL-27 showed inverse associations with human immunodeficiency virus (HIV) type 1 ribonucleic acid and inflammatory markers, whereas IL-35 was strongly correlated with higher viral load and CD4 T-cell depletion.

- IL-35 emerged as an independent predictor of HIV positivity in multivariable models and demonstrated high diagnostic performance in receiver operating characteristic analyses.
- The combined assessment of IL-27 and IL-35 improved discriminatory accuracy, highlighting their complementary value as biomarkers of immune dysregulation.
- Multivariate modeling confirmed that elevated IL-35, reduced IL-27, and impaired CD4-related parameters define the immunological profile of HIV infection.

ETHICS

Ethics Committee Approval: Approval for the study protocol was obtained on Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: AEŞH-BADEK2-2025-084, date: 13.05.2025).

Informed Consent: Signed consent forms were obtained from all individuals included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.M.Y., N.K., G.Ç.Ş., Concept: M.B.B., C.T., A.Ö., Design: M.B.B., A.Ö., Data Collection and/or Processing: M.B.B., B.M.Y., N.K., E.B., Analysis and/or Interpretation: A.Ö., Literature Search: M.B.B., A.Ö., E.B., Writing: M.B.B., A.Ö., E.B.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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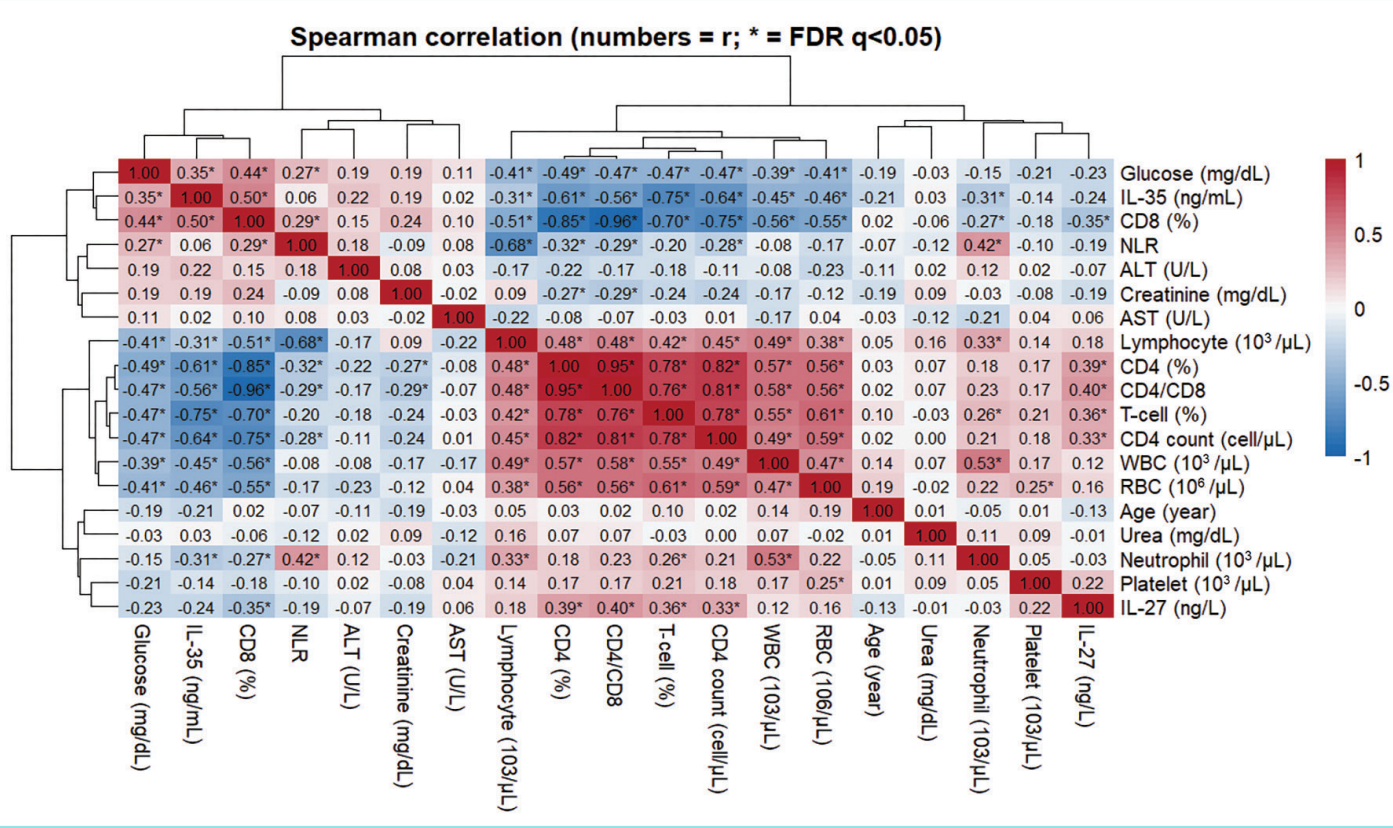

Supplementary Material Figure S1. The comprehensive correlation heatmap displaying associations among all clinical, hematologic, and immunologic variables. Spearman's rho coefficients are shown (significance levels: *p<0.05).

WBC: White blood cell, RBC: Red blood cell, NLR: Neutrophil-to-lymphocyte ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HIV-1: Human immunodeficiency virus type 1, RNA: Ribonucleic acid, IL: Interleukin, FDR: False discovery rate.