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Altered LY6E and TRIM6 expression in PBMCs correlated with HBsAg clearance and response to Peg-IFN-α treatment in HBeAg-negative chronic hepatitis B patients

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Abstract

Background Pegylated interferon alpha (Peg-IFN-α) has the potential to eradicate hepatitis B surface antigen (HBsAg). This study aimed to investigate whether the expression levels of lymphocyte antigen 6 family member E (LY6E) and tripartite motif-containing protein 6 (TRIM6) mRNAs in peripheral blood mononuclear cells (PBMCs) of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B virus (HBV) patients is associated with the response to Peg-IFN-α treatment and HBsAg clearance.

Methods In this prospective study, HBeAg-negative chronic HBV patients treated with Peg-IFN-α were followed for 48 weeks. The participants were classified into two groups, the virological response (VR) group and nonvirological response (NVR) group, according to the changes in HBV DNA and HBsAg levels observed at week 48 of treatment. Furthermore, these patients were divided into a serological response (SR) group and a nonserological response (NSR) group, depending on whether they exhibited a loss of serum HBsAg or evidence of seroconversion. The expression levels of LY6E and TRIM6 mRNAs in PBMCs were evaluated using real-time quantitative PCR with fluorescence detection. The diagnostic performance of LY6E and TRIM6 was assessed by analyzing the receiver operating characteristic (ROC) curve and calculating the area under the ROC curve (AUC).

Results After the treatment period, the observed VR and SR rates were 44.64% and 28.57%, respectively. Dynamic changes in LY6E and TRIM6 mRNA levels were significantly different between the VR and NVR groups and between the SR and NSR groups. Multivariate analysis revealed that TRIM6 was independently associated with VR at weeks 12 and 24 of Peg-IFN-α therapy and with SR at week 12; in addition, LY6E was independently associated with VR at week 12 and SR at week 24. At week 24, the area under the curve (AUC) for LY6E in the prediction of VR was 0.6942, and the AUC for the prediction of SR was 0.7766; at week 12, TRIM6 had AUCs of 0.7600 for the prediction of VR and 0.8469 for the prediction of SR.

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Conclusions LY6E and TRIM6 are important biomarkers for early therapeutic responses to Peg-IFN-α and HBsAg clearance.

Trial registration Registration number: 2023 – 311. Date of registration: 1 October 2023.

Keywords Hepatitis B virus, Peg-IFN-α, LY6E, TRIM6

Background

Chronic hepatitis B (CHB) remains a major global health problem affecting millions of people worldwide. Despite existing prevention strategies, CHB remains a significant burden, with approximately 1.5 million new cases diagnosed each year [1]. According to current estimates, approximately 296 million people suffer from this disease [2, 3].

The treatment of chronic hepatitis B involves multiple therapeutic strategies, among which pegylated interferon alpha (Peg-IFN-α) and nucleos(t)ide analogs (NAs) are the most common treatment options [4, 5]. Both treatments are effective in inhibiting hepatitis B virus (HBV) replication, reducing liver inflammation, and lowering the risk of complications such as cirrhosis and liver cancer. However, only a small fraction of patients receiving treatment with Peg-IFN-α or NAs achieve seroclearance of hepatitis B surface antigen (HBsAg) [6]. Compared with nucleos(t)ide analogs (NAs), Peg-IFN-α therapy is associated with greater serological response rates and a reduced likelihood of posttreatment relapse. Notably, Peg-IFN-α is unique in its ability to promote viral eradication, which is not a feature of any other treatment option [7]. However, the factors that explain why patients differ in their response to treatment are not fully understood. Therefore, the early identification of responders would greatly assist clinicians by allowing them to initiate treatment specifically for those patients expected to respond positively and enabling therapy adjustment for individuals who may not respond favorably to Peg-IFN-α monotherapy.

The mechanism of Peg-IFN-α treatment begins with its attachment to specific receptors located on the surface of host cells, leading to activation of the Janus kinase (JAK) signaling pathway and relay of transcriptional activation signals (STATs) [8, 9]. The activation of this pathway results in the expression of a number of genes, collectively termed interferon-stimulated genes (ISGs), which play important roles in host defense against HBV infection [10, 11]. Recent studies have suggested that the mRNA expression levels of ISGs in hepatocytes or peripheral blood may play a critical role in Peg-IFN-α therapy [12, 13]. Key ISGs, such as TRIM25, MX2, and TRIM5γ, are believed to be crucial to the anti-HBV immune response and contribute to the broader antiviral immune environment [14–17]. Despite these advancements, reliable and definitive biomarkers to predict treatment outcomes are currently lacking. Identifying these biomarkers is crucial, as they could provide valuable insights into the therapeutic efficacy of Peg-IFN-α prior to treatment initiation. The identification of these biomarkers will facilitate the creation of more individualized treatment strategies, allowing health care providers to customize therapies according to the unique requirements of each patient.

Lymphocyte antigen 6 family member E (LY6E) is also referred to as retinoic acid-inducible gene E (RIG-E) [18]. Originally discovered through high-throughput screening, LY6E is an ISG [19, 20] that facilitates infection by flaviviruses, certain alphaviruses, and influenza A viruses [21–23]. In a recent study, increased LY6E expression was also observed in chimpanzee livers and in chronic hepatitis C patients following IFN-α treatment [22]. The tripartite motif-containing protein (TRIM) family comprises more than 70 proteins that are crucial for immune responses, with tripartite motif-containing protein 6 (TRIM6) being an important member [24, 25]. Recent research has indicated that TRIM6, identified as an ISG, plays a significant role in suppressing the replication of HBV [26, 27].

The purpose of this study was to measure the mRNA levels of LY6E and TRIM6 in peripheral blood mononuclear cells (PBMCs) from hepatitis B e antigen (HBeAg)-negative chronic HBV patients, as well as the associations of these mRNA levels with the response to Peg-IFN-α therapy and HBsAg clearance.

These findings are vital for understanding the antiviral mechanisms underlying Peg-IFN-α treatment and the elements that lead to interferon-α resistance. We found that TRIM6 may serve as a protective element for HBeAg-negative CHB patients, but the presence of LY6E could increase the risk of persistent HBsAg, indicating that LY6E may be a susceptibility gene for this condition. The differences in the expression and activation of LY6E and TRIM6 were significantly associated with the antiviral efficacy of Peg-IFN-α. These results provide a crucial understanding of the predictive value of LY6E and TRIM6 mRNA levels in PBMCs of HBeAg-negative chronic HBV patients, particularly how these mRNAs are related to the efficacy of Peg-IFN-α therapy and HBsAg clearance. This study may also contribute to the development of personalized treatment strategies utilizing Peg-IFN-α.

Methods

Patient recruitment

This study was a prospective observational cohort study involving individuals diagnosed with CHB. A total of 56 HBeAg-negative participants, aged between 18 and 65 years, received a minimum of 48 weeks of Peg-IFN-α therapy in the outpatient Department of Infectious Diseases of the First Affiliated Hospital of Chongqing Medical University, commencing in October 2023 and continuing until October 2024. The study design is illustrated in a flowchart in Supplementary Figure S1.

The diagnosis and treatment of HBeAg-negative CHB patients adhered to the 2018 guidelines issued by the American Association for the Study of Liver Diseases (AASLD) for the management of chronic hepatitis B infection [28]. The inclusion criteria were as follows: positive HBsAg, negative HBeAg, positive HBeAb and HBcAb, low HBV DNA load (< 2000 IU/ml), normal liver function, and no or mild liver inflammation or liver fibrosis. The exclusion criteria were as follows: (1) coinfection with other hepatitis viruses, such as hepatitis C virus (HCV) or hepatitis D virus (HDV); (2) coinfection with other viral pathogens, such as human immunodeficiency virus (HIV) and Epstein–Barr virus (EBV); (3) autoimmune liver disease; (4) severe liver damage or other liver diseases, such as alcoholic liver disease and fatty liver disease; (5) diagnosis of other malignant diseases; and (6) contraindications to interferon treatment.

This study included 40 individuals diagnosed with untreated CHB and 26 healthy control subjects, all of whom provided written informed consent. This investigation received approval from the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Reference number: 2023 – 311). We evaluated a variety of parameters, including baseline HBsAg levels and subsequent measurements every 12 weeks, in HBeAg-negative chronic HBV patients undergoing treatment with Peg-IFN-α. Peripheral blood samples were collected at baseline, week 12 and week 24. PBMCs were isolated, and the mRNA levels of intracellular LY6E and TRIM6 were measured. HBeAg-negative chronic HBV patients were classified into two groups according to the changes in HBV DNA and HBsAg levels after 48 weeks of Peg-IFN-α treatment. Patients with a reduction in HBV DNA of more than 2 log10 IU/ml or a significant reduction in HBsAg (more than 1 log10 IU/ml or HBsAg clearance) were classified into the virological response (VR) group. Patients who did not meet these criteria were classified into the nonvirological response (NVR) group. Serological response (SR) was defined as the disappearance of serum HBsAg or HBsAg seroconversion during Peg-IFN-α treatment; subjects without these serological changes were classified as having a nonserological response (NSR).

Clinical and laboratory measurements

Real-time quantitative PCR with fluorescence detection was employed to measure HBV DNA concentrations, with a minimum detection threshold of 20 IU/ml (Roche, Cobas TM48, Shanghai). Abbott Architect i2000 Detection Reagent (Abbott, Architect i2000) was utilized to evaluate the serum levels of HBsAg/HBsAb and HBeAg/HBeAb. Liver function was analyzed with an automated biochemical analysis detector (Roche, Cobas, Shanghai). Routine hematological evaluations were conducted using automated methodologies (Mairui, BC-6600, Shanghai).

RNA extraction and quantitative real-time PCR (qRT–PCR)

Two-step reverse transcription polymerase chain reaction (RT–PCR) was used to measure the mRNA levels of LY6E and TRIM6 in PBMCs. Total RNA was isolated from PBMCs with TRIzol reagent, and complementary first-strand DNA (cDNA) was generated using a cDNA synthesis kit. Next, real-time quantitative PCR was performed on the cDNA using a Bio-Rad CFX96 fluorescence quantitative PCR system (USA). The PCR conditions were as follows: predenaturation: 95 °C for 30 s → PCR: 40 cycles of 95 °C for 5 s and 60 °C for 30 s → melting: 95 °C for 5 s, 60 °C for 1 min → cooling: 50 °C for 30 s → reading the board (**see the Supplementary Methods for details**). The expression levels of the target genes were assessed through relative quantification, utilizing GAPDH as the reference gene. The primers used in the analysis are detailed in Table S1.

Statistical analysis

Statistical analysis and data visualization were performed using SPSS 27.0 and GraphPad Prism 10.1.2. The serum concentrations of HBsAg and HBV DNA were logarithmically transformed. Both univariable and multivariable logistic regression analyses were employed to evaluate the correlation between various variables and VR and SR to screen independent predictors, which were subsequently introduced into R version 4.4.2 to establish a nomogram prediction model. A receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was calculated to assess the discriminatory ability of the nomogram prediction model. The bootstrap method with 1000 resamplings was employed for validation. The concordance index (C-index) was computed, and a calibration curve was created to evaluate the consistency and predictive accuracy of the nomogram prediction model. Data conforming to a normal distribution are reported as the mean ± standard deviation, whereas data with a nonnormal distribution are reported as the median (interquartile range). The independent samples t test was applied to datasets with a normal distribution, whereas the Mann–Whitney U test was used for those with a nonnormal distribution. The diagnostic efficacy of

LY6E and TRIM6 was evaluated through receiver operating characteristic (ROC) curve analysis, which included calculating the area under the curve (AUC). All the statistical evaluations were performed as two-sided tests at a significance level of $P < 0.05$.

Results

Baseline characteristics of patients and treatment response

All participants were classified into three distinct groups on the basis of their disease status: 40 patients with untreated CHB, 56 HBeAg-negative CHB patients receiving Peg-IFN-α treatment, and 26 healthy controls. The baseline characteristics of all participants are presented in Table 1. Serum HBsAg levels were significantly lower in CHB patients receiving Peg-IFN-α treatment than in untreated CHB patients. There were no significant differences between the groups in terms of age, sex, ALT level, AST level, total bilirubin level, platelet count, or white blood cell count. A total of 56 HBeAg-negative CHB patients completed 48 weeks of Peg-IFN-α treatment. Among them, 16 patients (28.57%) achieved a serological response, and 25 patients (44.64%) achieved a virological response (Table S2 and Table S3).

LY6E and TRIM6 mRNA levels in PBMCs are associated with HBV infection

To explore the role of LY6E and TRIM6 in HBV infection, we analyzed the mRNA levels of these genes in PBMCs from both healthy individuals and CHB patients via quantitative reverse transcription PCR (qRT−PCR). The results revealed that LY6E mRNA levels were significantly greater in the PBMCs of untreated CHB patients than in those of healthy controls (***P* = 0.0045**, Fig. 1A). Conversely, TRIM6 mRNA levels in the PBMCs of untreated chronic hepatitis B patients were significantly lower (***P* = 0.0020**, Fig. 1B) than those in the PBMCs of healthy individuals. These findings suggest that HBV infection may affect the mRNA levels of LY6E and TRIM6 in PBMCs.

Comparison of characteristics of patients with and without virological or serological responses during Peg-INF-α treatment

According to the changes in HBV DNA and HBsAg levels observed at week 48 of Peg-IFN-α treatment, patients were classified into the virological response (VR) group and nonvirological response (NVR) group. Patients with virological response (VR) had significantly higher ALT levels at week 12 than those without virological response (NVR) did (***P* = 0.0107**, Fig. 2 and Table S4). Furthermore, compared with the NVR group, the VR group presented significantly elevated AST levels at week 0 (***P* = 0.0176**), week 12 (***P* = 0.0194**), and week 24 (***P* = 0.0496**). Conversely, HBsAg levels were lower at week 0 (***P* = 0.0242**), week 12 (***P* = 0.0055**) and week 24 (***P* = 0.0010**) in the VR group than in the NVR group, as illustrated in Fig. 2 and Table S4. No statistically significant differences were found in the HBV DNA or total bilirubin (TBil) levels between the two groups (Table S4). Interestingly, the white blood cell (WBC) count in the VR group was markedly lower than that in the NVR group at week 0 (***P* = 0.0224**, Fig. 2 and Table S4), whereas no significant differences were observed at weeks 12 and 24.

HBeAg-negative chronic HBV patients treated with Peg-IFN-α were also classified into two groups, the serological response (SR) group and the nonserological response (NSR) group, according to serum HBsAg loss or HBsAg seroconversion after 48 weeks of Peg-IFN-α therapy. Baseline HBsAg levels were significantly lower in the SR group than in the NSR group at weeks 0, 12, and 24 (***P* < 0.0001**, Fig. 3 and Table S5). No statistically significant differences were found in the HBV DNA, ALT or total bilirubin (TBil) levels between the two groups (Table S5). Interestingly, AST levels increased significantly in the SR group at week 12 (***P* = 0.0416**, Fig. 3 and Table S5), whereas white blood cell counts were significantly lower at baseline (***P* = 0.0278**).

Table 1 Baseline characteristics in the groups

Characteristics	HC	Untreated CHB	CHB treated with Peg-IFN-α	*P* value
Number(*n*)	26	40	56	
Age(year)	33.35 ± 7.990	41.1 ± 9.934	46(38.25,51.00)	0.0962
Gender(male/female)	14/12	17/23	36/20	
HBsAg (log10 IU/mL)	UD	3.138(2.957,3.347)	2.954(1.639,3.349)	**0.0174**
HBV DNA (log10 IU/mL)	UD	1.699(1.699,2.835)	1.699(1.699,3.000)	0.8152
ALT(IU/L)	25.12 ± 7.506	19(13.25,38.75)	25.5(18.00,37.00)	0.1910
AST(IU/L)	22.5 ± 5.616	24(20.00,33.75)	23.5(19.00,30.75)	0.5925
WBC(*10^9 /mL)	5.745 (4.408,7.158)	4.209 ± 1.315	4.704 ± 1.343	0.0957
Tbil(umol/L)	11.75(6.575,15.68)	10.45(8.300,14.55)	10.65(8.200,14.05)	0.9484
PLT(*10^9/L)	202.3 ± 50.45	168.7 ± 65.10	160.0 ± 46.73	0.4536

HBsAg: hepatitis B surface antigen; ALT: alanine aminotransferase; AST: aspartate aminotransferase; WBC: white blood cells; Tbil: total bilirubin; PLT: platelet. The results are presented as the median inter-quartile range or mean ± standard deviation. Bold value was statistically significant $P < 0.05$; Untreated CHB vs. CHB treated with Peg-IFN-α; UD: undetected

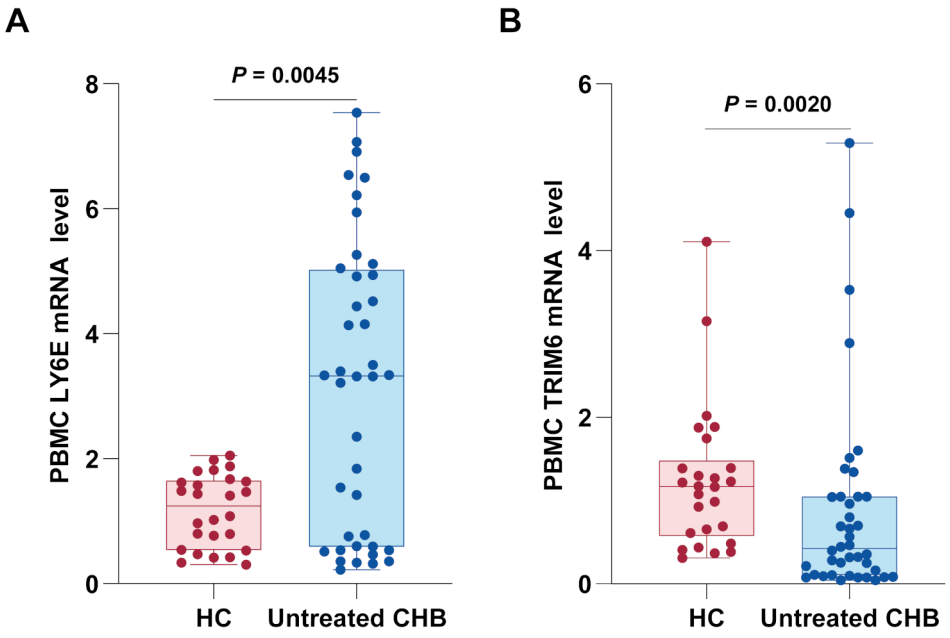

Fig. 1 LY6E and TRIM6 mRNA expressions in PBMCs of untreated CHB patients and healthy controls. LY6E (**A**) and TRIM6 (**B**) mRNA levels in PBMCs were analysed by qRT-PCR. The expression levels were calculated using the 2^-ΔΔct method (Livak method), with β-actin as the reference gene. $P < 0.05$ considered as significant

Dynamic changes in LY6E and TRIM6 mRNA levels during early antiviral therapy in patients treated with Peg-IFN-α

Increasing evidence suggests that Peg-IFN-α can increase the expression of LY6E and TRIM6 in in vitro cell culture models. However, the extent to which Peg-IFN-α stimulates these genes in PBMCs of HBeAg-negative CHB patients remains unclear. Therefore, we collected PBMCs from patients receiving Peg-IFN-α therapy and analyzed the changes in LY6E and TRIM6 mRNA levels in the VR group and NVR group. Our results revealed that in the early stage of Peg-IFN-α treatment, LY6E expression was significantly increased in the NVR group, whereas TRIM6 expression was increased in the VR group.

We compared the LY6E and TRIM6 mRNA levels in the VR/NVR groups with those in the SR/NSR groups before Peg-IFN-α treatment. The expression levels of both genes were comparable, with no statistically significant differences observed (Figure S2). LY6E mRNA expression increased at week 12 (***P*** **= 0.0088**) and week 24 (***P*** **= 0.0003**, Fig. 4B) in the NVR group. In contrast, TRIM6 mRNA levels did not significantly increase in the NVR group at either 12 or 24 weeks (***P*** **> 0.9999**, Fig. 4E). In the VR group, LY6E mRNA levels did not significantly change at either 12 weeks (***P*** **= 0.2004**) or 24 weeks (***P*** **= 0.9999;** Fig. 4A). In contrast, TRIM6 mRNA levels were significantly elevated at both 12 weeks (***P*** **= 0.0085**) and 24 weeks (***P*** **< 0.0001;** Fig. 4D) in the VR group. To further investigate the differences in LY6E and TRIM6 expression in PBMCs and their potential implications for early treatment response, we compared the mRNA levels of these genes between the VR and NVR groups. LY6E mRNA levels were significantly greater in the NVR group than in the VR group at both 12 weeks (***P*** **= 0.0231**) and 24 weeks (***P*** **= 0.0126;** Fig. 4C) of Peg-IFN-α treatment. In contrast, TRIM6 mRNA levels were significantly higher in the VR group than in the NVR group at both 12 weeks (***P*** **= 0.0007**) and 24 weeks (***P*** **= 0.0013;** Fig. 4F) of Peg-IFN-α treatment.

In the SR group, Peg-IFN-α treatment resulted in a significant increase in TRIM6 expression at both 12 weeks (***P*** **= 0.0021**) and 24 weeks (***P*** **= 0.0012;** Fig. 5D). In contrast, no significant change in TRIM6 expression was observed in the NSR group following Peg-IFN-α treatment at either 12 weeks (***P*** **= 0.9999**) or 24 weeks (***P*** **= 0.6824;** Fig. 5E). Moreover, in the NSR group, Peg-IFN-α treatment successfully induced LY6E expression at both 12 weeks (***P*** **= 0.0008**) and 24 weeks (***P*** **= 0.0002;** Fig. 5B). Conversely, Peg-IFN-α did not result in a significant increase in LY6E expression in the SR group at either 12 weeks (***P*** **= 0.9999**) or 24 weeks (***P*** **= 0.1697;** Fig. 5A). We also compared the mRNA levels of LY6E and TRIM6 between the SR and NSR groups. LY6E mRNA levels were significantly greater in the NSR group than in the SR group at both 12 weeks (***P*** **= 0.0017**) and 24 weeks (***P*** **= 0.0010;** Fig. 5C). In contrast, TRIM6 mRNA levels were significantly increased in the SR group at both 12 weeks (***P*** **< 0.0001**) and 24 weeks (***P*** **= 0.0128;** Fig. 5F).

Collectively, these findings suggest that the mRNA levels of TRIM6 and LY6E can be increased in distinct types of patients and that the expression levels of these genes

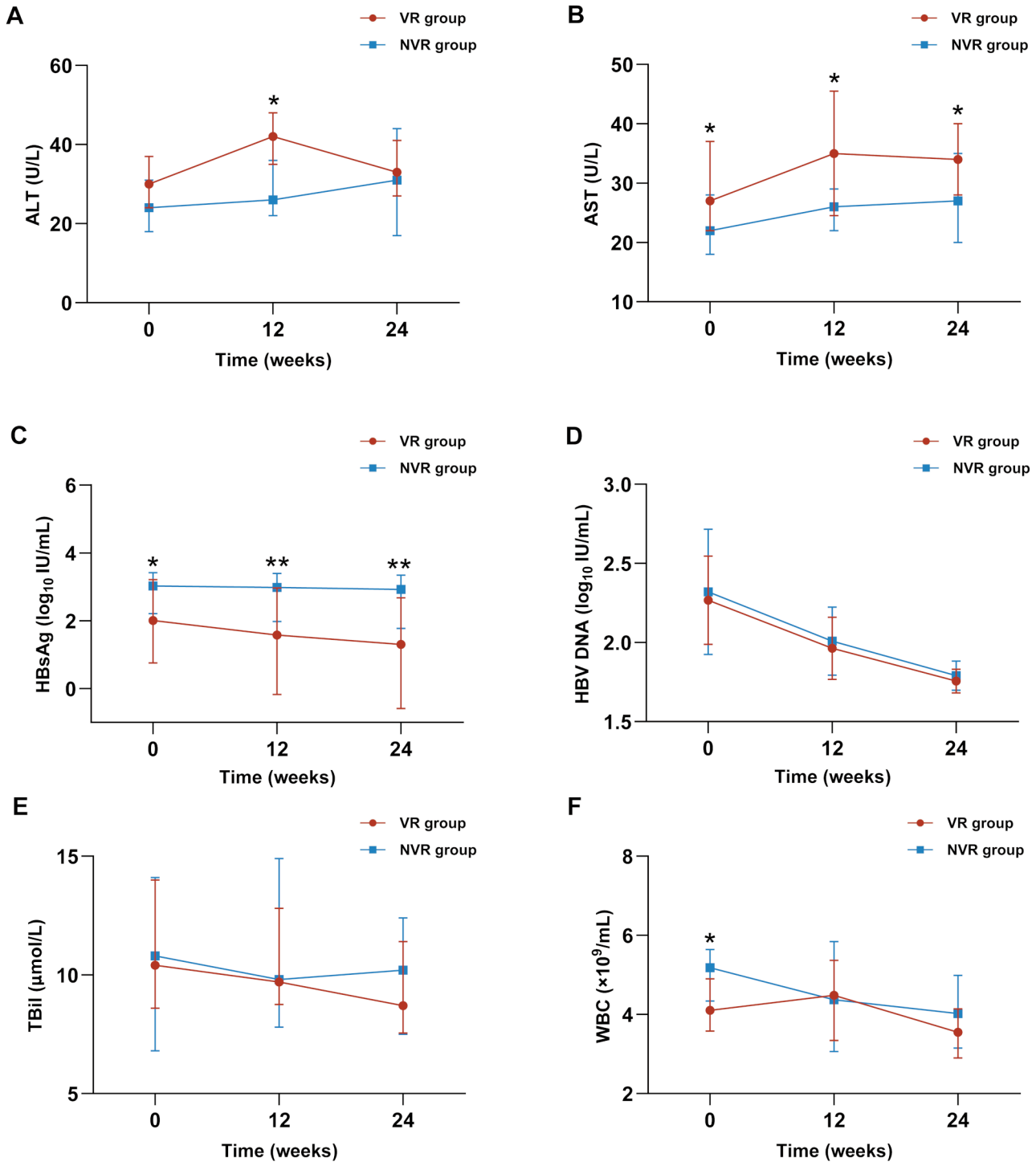

Fig. 2 The time change of various clinical characteristics in the VR and NVR group. ALT (**A**), AST (**B**), HBsAg (**C**), HBV-DNA (**D**), Tbil (**E**), and WBC (**F**) levels in VR and NVR patients received 48-week Peg-IFN-α treatment; *$P < 0.05$, **$P < 0.01$; ●, represents the median value of each parameter in the group with virological response; ■, represents the median value of each parameter in the group without virological response; the bottom and top of the whiskers represent 25 and 75 percentiles of each parameter

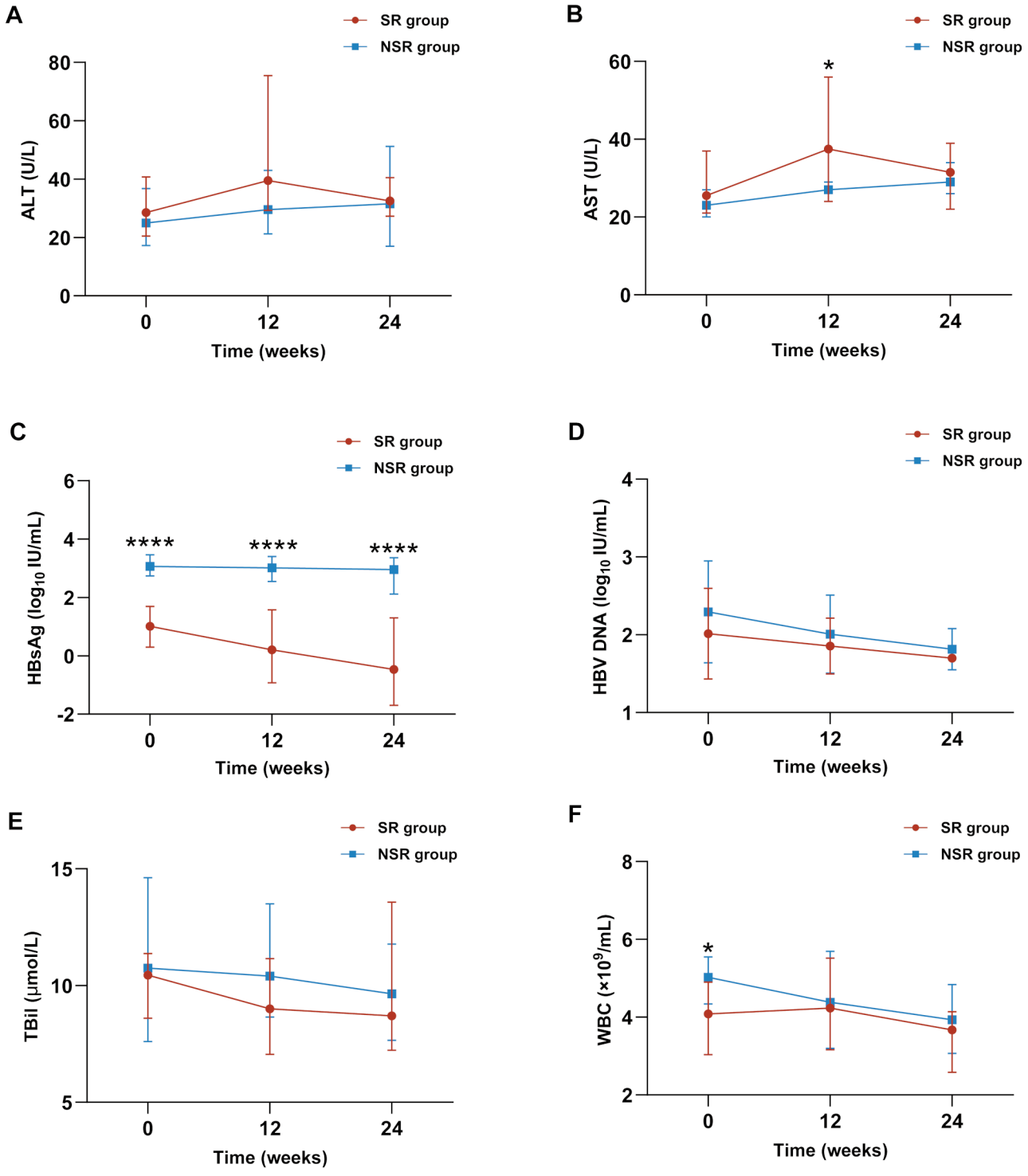

Fig. 3 The time change of various clinical characteristics in the SR and NSR group. ALT (**A**), AST (**B**), HBsAg (**C**), HBV-DNA (**D**), Tbil (**E**), and WBC (**F**) levels in SR and NSR patients received 48-week Peg-IFN-α treatment; **P* < 0.05, ***P* < 0.01; ●, represents the median value of each parameter in the group with virological response; ■, represents the median value of each parameter in the group without virological response; the bottom and top of the whiskers represent 25 and 75 percentiles of each parameter

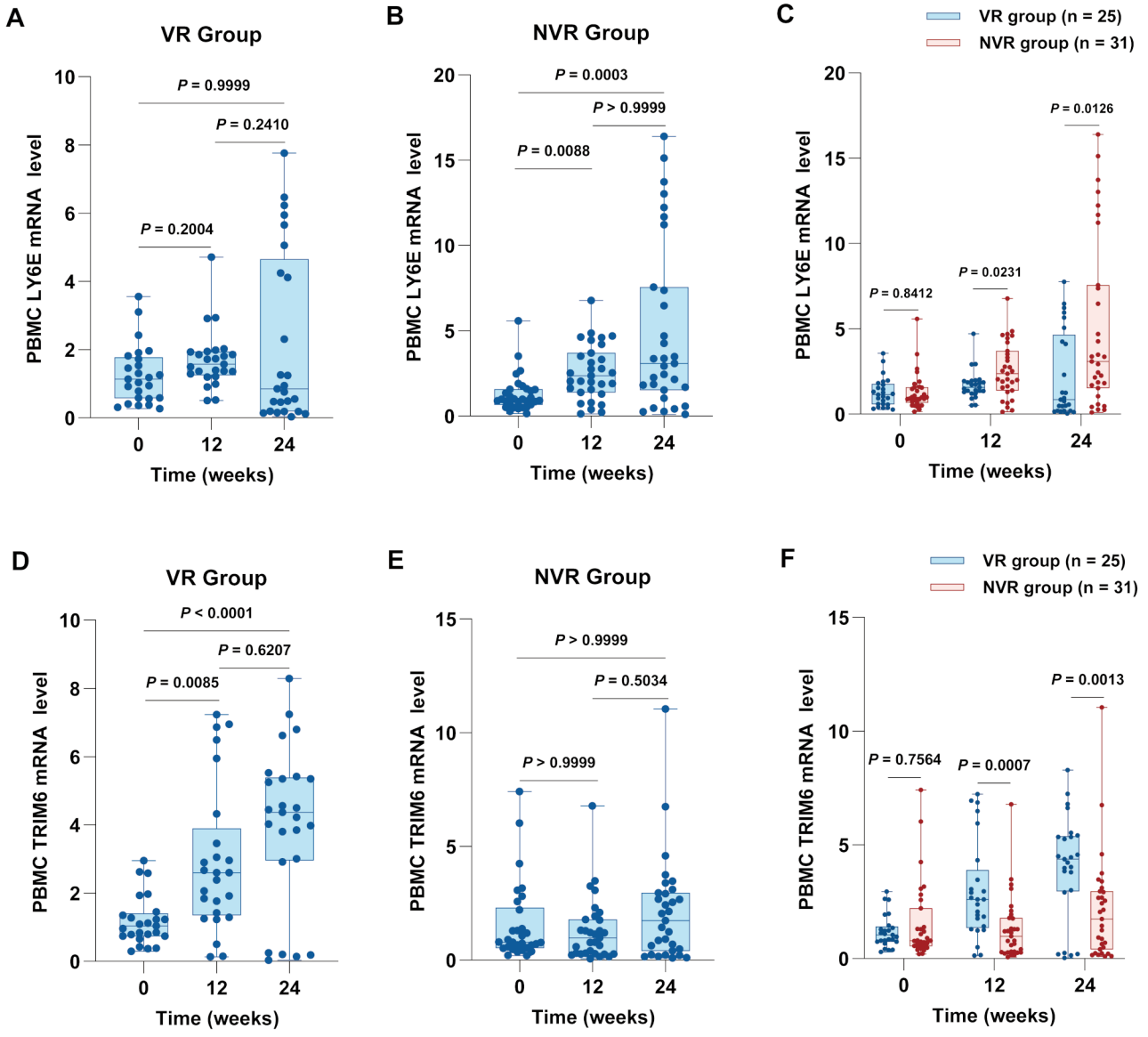

Fig. 4 Dynamic changes of LY6E and TRIM6 mRNA levels in the VR group and NVR group. (**A-B**) LY6E mRNA levels during therapy in the VR group and NVR group. (**D-E**) TRIM6 mRNA levels during therapy in the VR group and NVR group. (**C**, **F**) LY6E and TRIM6 mRNA levels in the VR group and NVR group were compared and at 0 weeks, 12 weeks and 24 weeks of therapy. LY6E and TRIM6 mRNA levels in PBMCs were measured by qRT-PCR. Values are described by median (interquartile range). $P < 0.05$ was statistically significant

are associated with the response to Peg-IFN-α therapy. Increased TRIM6 mRNA levels were associated with more favorable serological and virological responses to Peg-IFN-α treatment, but increased LY6E mRNA levels were linked to nonserological and nonvirological responses to the same therapy.

LY6E and TRIM6 strongly predict patient serological or virological response to Peg-IFN-α therapy

Univariate and multivariate analyses demonstrated that LY6E and TRIM6 could serve as reliable predictors of both VR and SR. Univariate analyses revealed significant correlations among LY6E, TRIM6, and both VR and SR at weeks 12 and 24 (Tables S6 and S7). After adjusting for several clinical parameters (including HBsAg, WBC count and ALT), multivariate analysis revealed that TRIM6 was independently associated with VR at weeks 12 and 24 and with SR at week 12 (Tables S6 and S7). Furthermore, multivariate analysis revealed that LY6E was independently associated with VR at week 12 and with SR at week 24 (Tables S6 and S7). Interestingly, univariate analyses revealed that HBsAg was significantly associated with both VR and SR at weeks 0, 12, and 24 (Tables S6 and S7). After adjusting for various ariables (including LY6E, TRIM6, and ALT), multivariate analysis confirmed

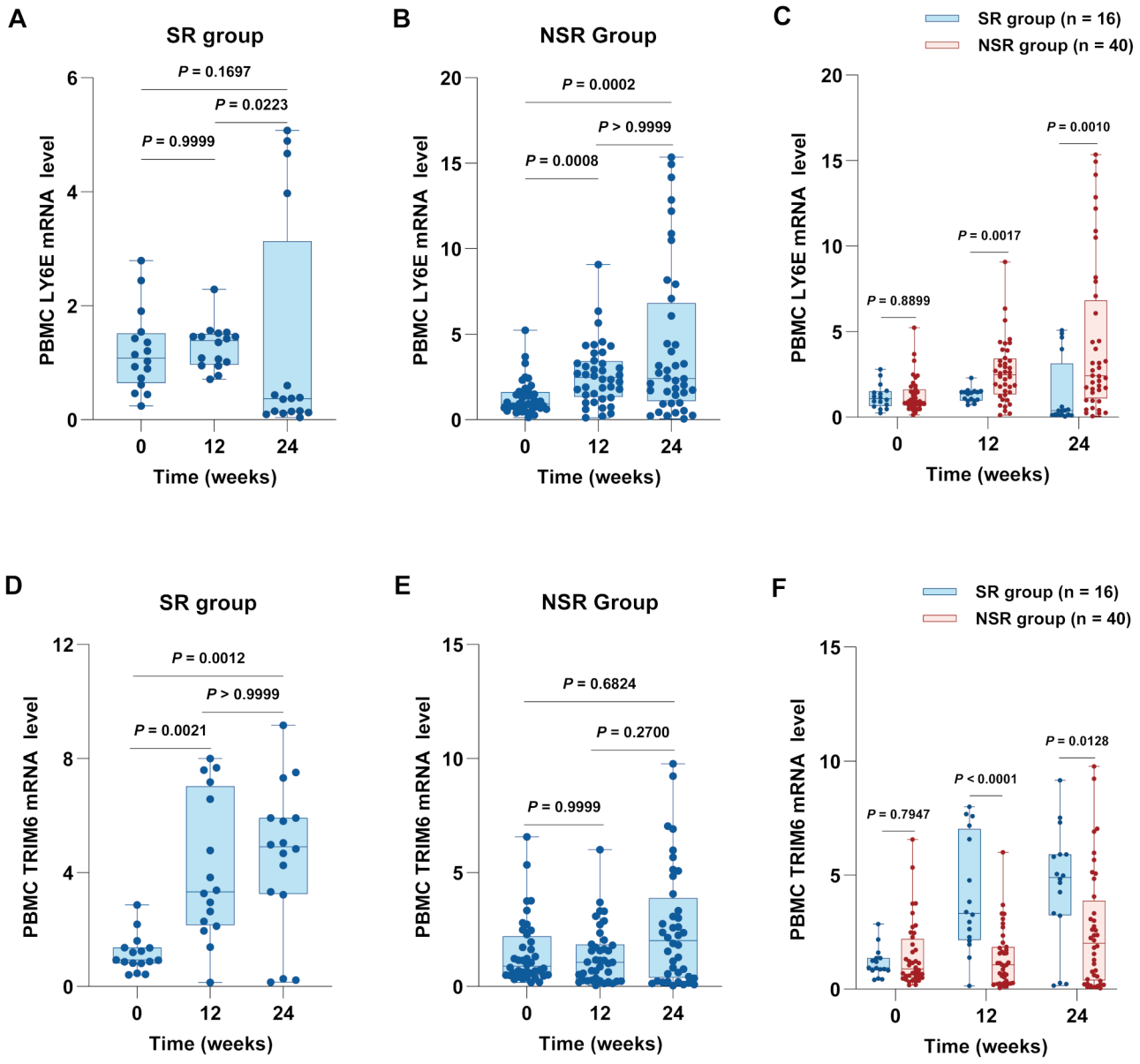

Fig. 5 Dynamic changes of LY6E and TRIM6 mRNA levels in the SR group and NSR group. (**A-B**) LY6E mRNA levels during therapy in the SR group and NSR group. (**D-E**) TRIM6 mRNA levels during therapy in the SR group and NSR group. (**C**, **F**) LY6E and TRIM6 mRNA levels in the SR group and NSR group were compared and at 0 weeks, 12 weeks and 24 weeks of therapy. LY6E and TRIM6 mRNA levels in PBMCs were measured by qRT-PCR. Values are described by median (interquartile range). $P < 0.05$ was statistically significant

that HBsAg remained significant at weeks 0, 12, and 24 (Tables S6 and S7).

Based on the results obtained from the multivariate analysis, the expression levels of HBsAg, LY6E, and TRIM6 were significantly associated with the virological response at 12 weeks. Consequently, these variables were introduced into the R software to establish a nomogram model for predicting the therapeutic efficacy of Peg-IFN-α treatment. In the nomogram, the values of HBsAg, LY6E, and TRIM6 were located on the variable axis, with a vertical line drawn upward to determine a score on the points axis (Fig. 6). A higher score, calculated as the sum of the specified scores for each predictor in the nomogram, indicates a greater probability of achieving a virological response. The discriminative power of the nomogram was evaluated using ROC curves, which indicated strong discrimination with an AUC of 0.887 (Figure S3). Furthermore, the C-index for the nomogram was calculated to be 0.888 (**95% CI = 0.803–0.971**), indicating a high level of consistency between the actual and predicted probabilities of the outcome. Additionally, the calibration curve for the nomogram's

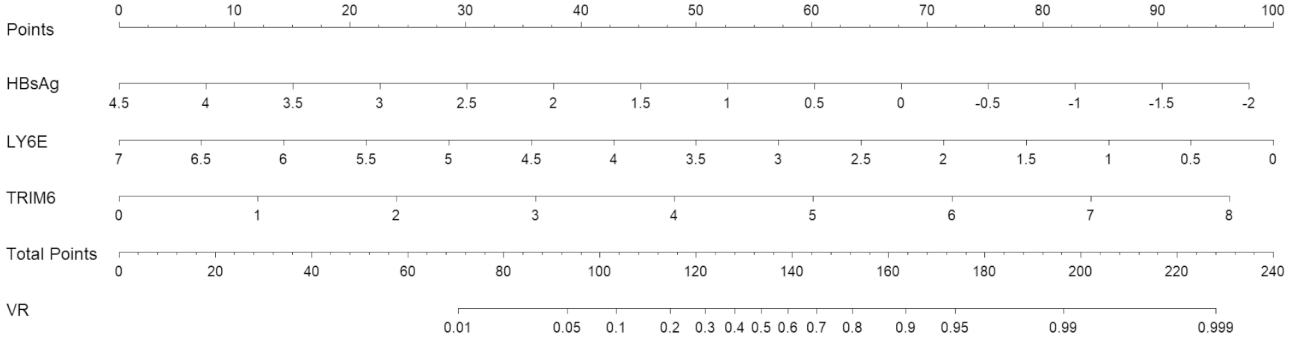

Fig. 6 Nomogram for predicting the virological response

prediction of virological response revealed a significant agreement (Figure S3) between the predicted and actual probabilities.

Receiver operating characteristic (ROC) curve analysis further underscored the predictive value of LY6E and TRIM6, particularly at weeks 12 and 24, for VR or SR, as evaluated through the area under the curve (AUC). In the ROC curve analysis for VR prediction, LY6E at week 24 emerged as the most reliable predictor (**AUC = 0.6942**, ***P*** **= 0.0131;** Fig. 7A and Table S8). The optimal cutoff value for LY6E at week 24 for predicting VR was **1.394**. In predicting the SR, LY6E at week 24 was the most reliable predictor (**AUC = 0.7766**, ***P*** **= 0.0013**; Fig. 7B and Table S8), with an optimal cutoff value of **0.7595**.

According to the receiver operating characteristic (ROC) curves, TRIM6 at week 12 was identified as the most effective predictor for VR (**AUC = 0.7600**, ***P*** **= 0.0009;** Fig. 7C and Table S8), with an optimal cutoff value of **1.224**. Similarly, TRIM6 at week 12 was the most reliable predictor for SR (**AUC = 0.8469**, ***P*** **= 0.0001;** Fig. 7D and Table S8), with an optimal cutoff value of **1.903**. Collectively, these results highlight the substantial potential of LY6E and TRIM6 as predictive biomarkers for both SR and VR.

Discussion

Peg-IFN-α treatment is a recognized approach for managing chronic HBV infections. However, the efficacy of interferon therapy in CHB patients varies significantly. This variability underscores the need for not only innovative combination therapies and enhancements to Peg-IFN-α treatment but also reliable new biomarkers to improve clinical management. Recent research has indicated that Peg-IFN-α has marked antiviral properties, including promoting the expression of interferon-stimulated genes (ISGs) and influencing the immune responses of the host [22]. At present, only a handful of ISGs, including SOCS3, STAT1, and MX, have been linked to antiviral actions against HBV, positioning them as potential indicators for predicting the clinical efficacy of IFN therapy [29]. However, the specific biological roles and antiviral functions of numerous ISGs remain inadequately understood.

In our research, we found a significant relationship between the expression levels of ISGs, including LY6E and TRIM6, in PBMCs and their antiviral responses. These results underscore the potential of LY6E and TRIM6 as early indicators for the response to Peg-IFN-α therapy. In this study, we explored the dynamic changes in LY6E, TRIM6, HBsAg, HBV DNA, ALT, and AST in HBsAg-negative chronic hepatitis B patients receiving 48 weeks of Peg-IFN-α treatment. Our findings suggest that LY6E and TRIM6 levels could serve as novel biomarkers for predicting VR and SR to Peg-IFN-α treatment in HBeAg-negative CHB patients. These insights may contribute to optimizing Peg-IFN-α treatment protocols for individuals with chronic hepatitis B.

We first examined LY6E and TRIM6 mRNA levels in PBMCs from healthy individuals and compared them with those in untreated CHB patients. The findings indicated that, in contrast with healthy controls, untreated CHB patients presented considerably increased LY6E mRNA levels but significantly decreased TRIM6 mRNA levels. This phenomenon may be connected to HBV, which may increase the expression of LY6E and decrease that of TRIM6. Recent investigations suggest that lower levels of HBsAg during Peg-IFN-α treatment correlate with improved antiviral response rates after treatment. Additionally, the loss of HBsAg is associated with a reduced likelihood of developing cirrhosis and transitioning to hepatocellular carcinoma (HCC), which is a preferable goal in the treatment of chronic hepatitis B. In our study, out of 56 patients who underwent 48 weeks of Peg-IFN-α treatment, 25 patients (44.64%) achieved a virological response, whereas 16 patients (28.57%) achieved a serological response. This response rate is comparable to previous research that highlights the efficacy of Peg-IFN-α therapy [30].

An early decrease in serum HBsAg levels during therapy has been recognized as a strong predictor of a sustained response to Peg-IFN-α [31]. In this study, we monitored the dynamic changes in serum HBsAg, HBV

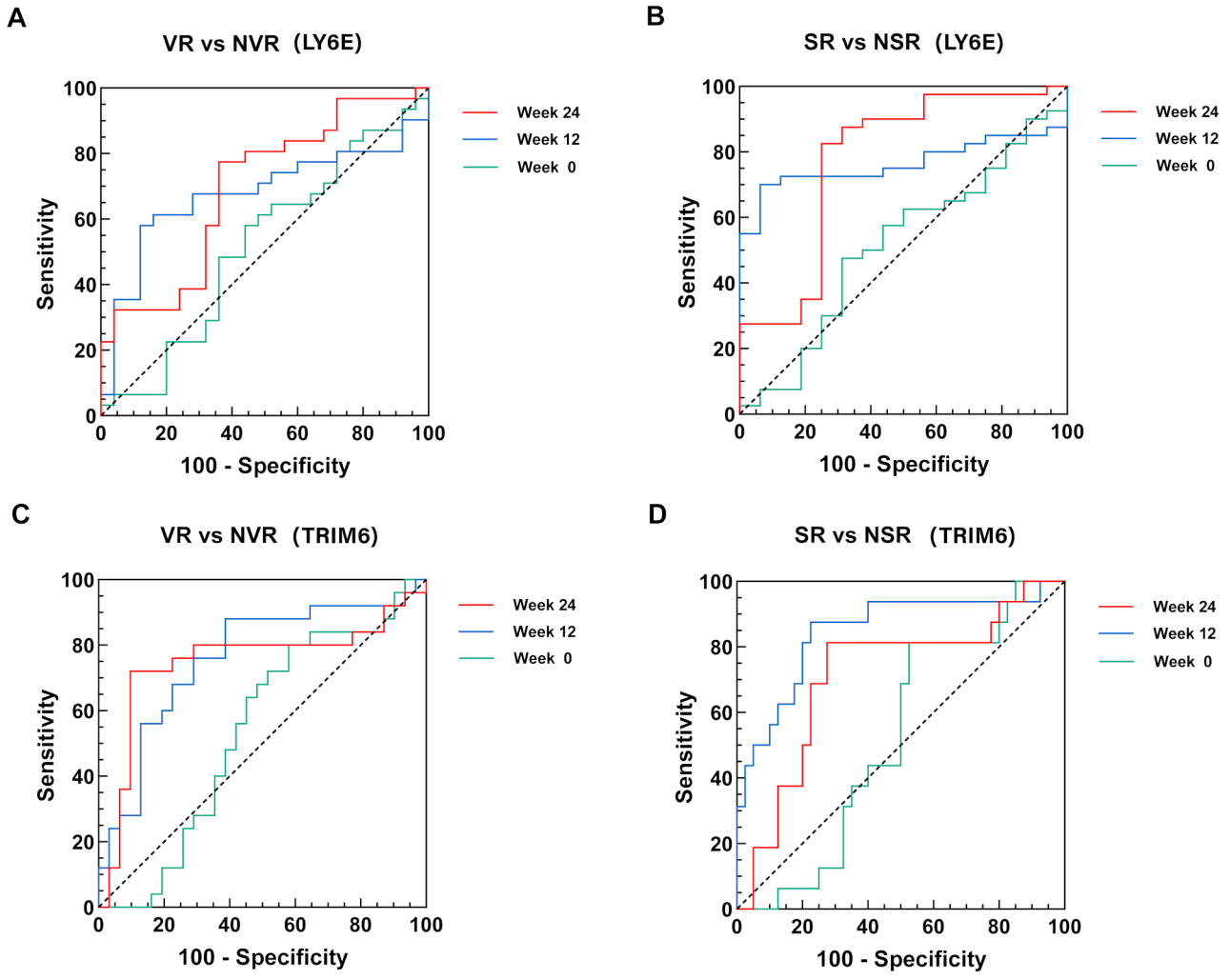

Fig. 7 Receiver operating characteristic curves predicting sustained virological and serological response after 48-week Peg-IFN-α treatment. (**A-B**) Receiver operating characteristic curves for LY6E at baseline, weeks 12, 24 as they predict sustained virological and serological response. (**C-D**) Receiver operating characteristic curves for TRIM6 at baseline weeks 12, 24 as they predict sustained virological and serological response. The Youden index was used to maximize the potential effectiveness of the biomarkers. AUC: area under the curve

DNA, ALT, and AST levels at different times throughout the treatment period. HBsAg levels decreased gradually over time, indicating the challenges associated with the complete clearance of HBsAg as HBsAg partially indicative of intrahepatic cccDNA levels [32]. Our results demonstrated that patients who responded to Peg-IFN-α therapy presented a greater reduction in HBsAg levels during treatment than nonresponders did, suggesting the efficacy of Peg-IFN-α therapy for CHB patients. Additionally, we observed a transient increase in ALT and AST levels at week 12 of treatment, which suggests that the efficacy of IFN-α therapy is closely linked to the immune clearance of HBs-positive hepatocytes. While the immune clearance of hepatocytes may lead to liver inflammation and a certain degree of hepatocyte damage, this phenomenon is generally transient and serves as an indicator of treatment effectiveness, ultimately contributing to an increased cure rate. We also examined the relationships between various factors, including ALT and HBsAg, and virological and serological responses after 48 weeks of Peg-IFN-α treatment. Our analysis identified HBsAg as a predictor of both virological and serological responses. However, the relationships between these clinical features and the SR or VR to Peg-IFN-α treatment remain unclear, and further studies are needed to establish their clinical relevance.

Studies have established a robust association between ISGs and the antiviral immune response [33–37]. We hypothesized that the activation of Peg-IFN-α could increase the mRNA expression of LY6E and TRIM6 in PBMCs from HBeAg-negative CHB patients, which was correlated with the efficacy of Peg-IFN-α treatment. Therefore, we compared the mRNA levels of LY6E and TRIM6 in the VR/NVR groups prior to Peg-IFN-α

treatment with those in the SR/NSR groups and observed that their expression levels were comparable. These findings establish a basis for further investigation into the dynamic changes in these markers and their correlation with the efficacy of Peg-IFN-α treatment. As anticipated, our results demonstrated that the changes in LY6E and TRIM6 mRNA expression levels in PBMCs differed between the VR group and the NVR group during the early stages of Peg-IFN-α therapy. Notably, TRIM6 mRNA levels were significantly higher in the VR group than in the NVR group, especially after 24 weeks of treatment. These findings suggest that the expression and activation of these ISGs may be potential indicators of antiviral efficacy and long-term outcomes of interferon therapy in clinical practice.

We confirmed that Peg-IFN-α treatment increases TRIM6 mRNA levels in PBMCs of patients with CHB, thereby contributing to the antiviral effects of interferon. Therefore, TRIM6 may play an active role in mediating potent interferon-induced antiviral responses. In contrast, LY6E mRNA levels were significantly greater in the NVR group than in the VR group. These findings suggest that LY6E triggers negative regulation of ISGs and leads to Peg-IFN-α nonresponse or resistance. Specifically, during viral infection, HBV uses its core protein and X protein to suppress interferon signaling [38]. TRIM6 may help overcome this suppression by promoting the activation of key molecules involved in the IFN pathway, such as STAT1, STAT2, and IRF9. This disruption of the immune evasion mechanisms of HBV can increase the susceptibility of the virus to immune clearance. Conversely, by interfering with antigen presentation and activation, LY6E may contribute to the persistence of HBV infection. Furthermore, LY6E may also be involved in the modulation of interferon signaling pathways. For example, LY6E may impact the activation of ISGs such as MxA, PKR, or OAS that directly inhibit HBV replication.

Research has demonstrated a significant correlation between LY6E and the aggressive characteristics of HCC [39], suggesting that LY6E could be a valuable predictive biomarker for the onset of de novo HCC [40]. Furthermore, previous studies have proposed that TRIM6 may function as an innovative prognostic marker in HCC [41], highlighting its ability to suppress HBV transcription [27]. In the present study, we found that LY6E mRNA levels at weeks 12 and 24 were independent predictors of treatment response through both univariate and multivariate analyses. Furthermore, TRIM6 mRNA levels were independently associated with treatment response at the same intervals. Interestingly, univariate and multivariate analyses revealed that HBsAg was significantly associated with both VR and SR at weeks 0, 12, and 24. Moreover, ROC curve analysis revealed that the predictive value of LY6E mRNA levels for VR and SR was greatest at week 24, but TRIM6 exhibited the best predictive value for VR and SR at week 12. These findings indicate that the mRNA expressions of LY6E and TRIM6 in PBMCs of HBeAg-negative CHB patients during the early treatment with Peg-IFN-α may correlate with therapeutic efficacy and prognosis.

To enhance the accuracy of predicting therapeutic effects, we developed a predictive model that incorporates multiple predictors, including TRIM6, LY6E and HBsAg. Currently, nomograms are widely utilized as prognostic tools in medical studies due to their high accuracy, which is crucial for clinical decision-making. Therefore, we established a nomogram model based on the predictors TRIM6, LY6E and HBsAg to forecast treatment response in HBeAg-negative CHB patients.

The nomogram presented in this study revealed that the therapeutic effect diminishes as the levels of LY6E and HBsAg expression increase. Conversely, higher expression levels of TRIM6 are correlated with a better therapeutic effect. Additionally, the combined analysis of LY6E, TRIM6, and HBsAg may improve the predictive accuracy of treatment outcomes. The impact of the predictors LY6E, TRIM6, and HBsAg on therapeutic efficacy was clearly reflected using this nomogram.

While HBsAg levels exhibited significant predictive ability in our study, we believe that LY6E and TRIM6 possess potential value for further research and broader application. The combination of these biomarkers may offer a more comprehensive approach to predicting treatment outcomes, particularly in cases where HBsAg alone may not fully reflect the underlying immune dynamics or viral persistence. Additionally, in clinical practice, LY6E and TRIM6 may enhance the predictive ability of HBsAg. For example, patients may exhibit immune escape mechanisms or immune tolerance, resulting in a relatively stable or gradual decline in HBsAg levels. In these cases, alterations in LY6E and TRIM6 may offer more precise information about the immune response. This information could facilitate more precise efficacy predictions for individual patients.

We acknowledge that our study has certain limitations. First, the duration of patient follow-up was brief, making it impossible to assess long-term responses after treatment was discontinued. Additionally, we were unable to include HBV genotypes or HBcrAg levels in the multivariate analysis. Previous studies have demonstrated that the predominant HBV genotypes in the Chinese population are genotypes B and C; genotype C is often associated with poorer treatment outcomes and increased drug resistance, whereas genotype B tends to correlate with more favorable responses to antiviral therapies [42]. HBcrAg can provide valuable information on disease activity and treatment response, and lower HBcrAg levels may correlate with a better response [43, 44]. The absence

of HBcrAg data prevents a comprehensive evaluation of how viral replication levels may affect treatment outcomes. Thus, it is essential to collect HBV genotype and HBcrAg data when evaluating the associations between LY6E/TRIM6 and clinical response in future studies. Furthermore, the sample size of patients was relatively small. Therefore, studies with larger samples are needed to confirm the present results.

Conclusion

LY6E and TRIM6 mRNA levels in the PBMCs of HBeAg-negative CHB patients during the initial stages of Peg-IFN-α treatment may be correlated with therapeutic efficacy and patient prognosis. The identification of LY6E and TRIM6 as important early biomarkers could improve the management of interferon therapy in patients infected with HBV.

Abbreviations

HBsAg	Hepatitis B surface antigen
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
WBC	White blood cells
Tbil	Total bilirubin
PLT	Platelet
VR	Virological response
NVR	Non-virological response
LY6E	Lymphocyte antigen 6 family member E
TRIM6	Tripartite motif-containing protein 6
ROC	Receiver operating characteristic
AUC	Area under ROC curve
CI	Confidence interval
Peg-IFN-α	Pegylated interferon alpha
NA	Nucleos(t)ide analogues
PBMCs	Peripheral blood mononuclear cells
CHB	Chronic hepatitis B

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

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Author contributions

Testing and data collection were performed by H.P; The data analysis and interpretation was performed by Y.S. and Y.T; Draft of the manuscript was written by Y.S. and H.P; N.Y. and R.W. was responsible for patients follow-up; B.Q. and F.Y. were responsible for editing, reviewing, and approving the final manuscript; All authors approved the final version of the manuscript for submission.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethical approval was received from Ethical Review Committee of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China(Reference number: 2023 – 311), and the registration process was successfully completed on the designated website (https://www.medicalresearch.org.cn/login). All patients submitted written informed consent for their participation in the study. The study was conducted in adherence to relevant laws and institutional protocols and in accordance with the ethical standards of the Declaration of Helsinki.

Consent for publication

All authors have approved the final version of the manuscript. Consent for the publication of data, images, or information was obtained from all participants (or their legal guardians) where applicable.

Competing interests

The authors declare no competing interests.

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