Biomedicine & Pharmacotherapy 164 (2023) 114913

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Predictive plasma biomarkers of long-term increase in hepatic steatosis index after HCV eradication in HIV/HCV-coinfected patients

Rubén Martín-Escolano [a,1], Ana Virseda-Berdices [a,b,1], Juan Berenguer [b,c,d], Juan González-García [b,e,f], Oscar Brochado-Kith [a,b], Amanda Fernández-Rodríguez [a,b], Cristina Díez [b,c,d], Victor Hontañon [b,e,f], Salvador Resino [a,b,*,1], María Ángeles Jiménez-Sousa [a,b,*,1], the GeSIDA 10318 Study Group [2]

[a] Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología (CNM), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain
[b] Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[c] Unidad de Enfermedades Infecciosas/VIH; Hospital General Universitario "Gregorio Marañón", Madrid, Spain
[d] Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain
[e] Servicio de Medicina Interna-Unidad de VIH. Hospital Universitario La Paz. Madrid, Spain
[f] Instituto de Investigación Sanitaria La Paz (IdiPAZ). Madrid, Spain

ARTICLE INFO

Keywords:
DAAs therapy
HIV/HCV-coinfection
HIS
Plasma proteins
Predictive biomarkers
Steatosis

ABSTRACT

Hepatic steatosis is a common condition found in the liver of hepatitis C virus (HCV)-infected patients, contributing to more severe forms of liver disease. In addition, the human immunodeficiency virus (HIV) may accelerate this process. Alternatively, several immune checkpoint proteins have been reported to be upregulated and correlated with disease progression during HCV and HIV infections. In steatosis, a detrimental immune system activation has been established; however, the role of the immune checkpoints has not been addressed so far. Thus, this study aimed to evaluate the association between plasma immune checkpoint proteins at baseline (before antiviral therapy) with hepatic steatosis index (HSI) increase at the end of follow-up (~ five years after sustained virologic response (SVR)). We performed a multicenter retrospective study in 62 patients coinfected with HIV/HCV who started antiviral therapy. Immune checkpoint proteins were analyzed at baseline using a Luminex 200TM analyzer. The statistical association analysis was carried out using Generalized Linear Models (GLM) and Partial Least Squares Discriminant Analysis (PLS-DA). Fifty-three percent of the patients showed HSI increase from baseline to the end of follow-up. Higher immune checkpoint protein levels of BTLA, CD137 (4–1BB), CD80, GITR, LAG-3, and PD-L1 before HCV therapy were associated with a long-term increase in HSI after successful HCV therapy, suggesting a potential predictive role for early detection of progression towards steatosis in HIV/HCV-coinfected patients.

List of abbreviations: AIC, AKAike information criteria; ALT, alanine aminotransferase; AMR, arithmetic mean ratio; APCs, antigen-presenting cells; AST, aspartate aminotransferase; au, arbitrary units; BMI, body mass index; BTLA, B and T lymphocyte attenuator; ART, antiretroviral therapy; CD, cluster of differentiation; CI, confidence interval; DAAs, direct-acting antivirals; ESLD, end-stage liver disease; FDR, false discovery rate; FGL1, fibrinogen-like protein 1; FI, fluorescence intensity; Gal-3, galectin3; GITR, glucocorticoid-induced TNFR-related; GITRL, GITR ligand; GLM, Generalized Linear Models; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSI, Hepatic Steatosis Index; HVEM, herpesvirus entry mediator; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IQR, interquartile range; LAG-3, lymphocyte activation gene-3; LSECtin, liver and lymph node sinusoidal endothelial cell C-type lectin; LSM, liver stiffness measurement; MHC, major histocompatibility complex; NAFLD, non-alcoholic fatty liver disease; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; pegIFN, pegylated interferon; PLS-DA, Partial Least Squares Discriminant Analysis; SVR, sustained virologic response; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TNF, tumor necrosis factor; VIP, variable importance in the projection.

* Correspondence to: Centro Nacional de Microbiología, Instituto de Salud Carlos IIIIII (Campus Majadahonda), Carretera Majadahonda-Pozuelo, Km 2.2; 28220 Majadahonda, Madrid, Spain.
E-mail addresses: sresino@isciii.es (S. Resino), jimenezsousa@isciii.es (M.Á. Jiménez-Sousa).
[1] Authors contributed equally to this work
[2] See Appendix for the GeSIDA 10318 Study Group

https://doi.org/10.1016/j.biopha.2023.114913
Received 18 April 2023; Received in revised form 17 May 2023; Accepted 18 May 2023
Available online 20 May 2023

1. Introduction

Globally, an estimated 57 million people have hepatitis C virus (HCV) infection [1]. Without antiviral therapy, around 70% of HCV-infected people will develop chronic hepatitis C. Of these, 15–30% will develop cirrhosis over a 20–25 year period, resulting in a significant risk of end-stage liver disease (ESLD), hepatocellular carcinoma (HCC), and death [2]. Coinfection with human immunodeficiency virus (HIV) may accelerate this process, leading to more rapid HCV-associated liver disease progression than HCV-monoinfected patients [3,4]. Alternatively, hepatic steatosis is a common condition found in the liver of HCV-infected patients [5], contributing to more severe forms of liver disease [6,7]. In addition, people with HIV (PWH) gather a number of factors related to chronic inflammation that increase their risk of having steatosis, including HIV itself, viral coinfections, antiretroviral therapy (ART), or metabolic syndrome, among others [8]. The Hepatic Steatosis Index (HSI) is a simple index that can be very useful in screening hepatic steatosis [9,10]. This non-invasive index has been validated in PWH [11] and has been related to liver inflammation in this population [12].

Among anti-HCV treated PWH, HCV cure reduces liver and non-liver complications [13,14]. However, a significant risk of liver disease progression persists, particularly in HIV/HCV-coinfected patients [15,16]. In this sense, persistent molecular changes caused by chronic hepatitis C and associated with the risk of severe liver disease could explain that HCV cure only partially reduces this risk [17,18]. Metabolic dysfunction and nonalcoholic fatty liver disease (NAFLD), hallmarks of chronic hepatitis C, are related to liver inflammation, which may persist after direct-acting antiviral (DAA) treatment and further drive steatosis, NAFLD, and progression of liver disease after hepatitis C is cleared [19].

Several immune checkpoints have been reported to be upregulated during chronic hepatitis C [20,21]. Complete restoration may not be achieved after viral clearance, indicating that long-term antigenic stimulation drives an irreversible change in the immune system [22]. In HCV infection, immune checkpoints have been correlated with disease progression [23]. Signaling via these proteins can drive effector immune T cells into a state known as “exhaustion”, contributing to the reduction of effector function. Sustained expression of immune checkpoint molecules reduces the immune clearance of pathogens, favoring escape from immune control and disease progression [24]. Similarly, immune checkpoints are also observed to be upregulated in HIV infection on both $CD4^+$ and $CD8^+$ T cells, and correlated with disease progression as reflected in decreased T cell function, decreased $CD4^+$ T cell counts, increased viral RNA replication, and HIV reservoir enrichment [25]. In particular, in steatosis and progression to NAFLD, as well as in more severe liver disease, a detrimental role for $CD8^+$ and immune system activation has been established, so attention to immune checkpoint proteins has been given, especially to the PD-1/PD-L1 signaling pathway, which negatively regulates lymphocyte cytotoxic action [26]. However, there are no previous studies describing the role of immune checkpoints in the long-term disease evolution after successful HCV therapy in HIV/HCV-coinfected patients. In this regard, steatosis has been shown as a strong predictor of liver disease progression and severity, and patients achieving sustained virologic response (SVR) have demonstrated a different tendency towards steatosis [27–30]. Hence, identification of biomarkers to predict progression toward steatosis is highly desirable to identify patients who could benefit from closer monitoring.

1.1. Objective

This study evaluated the association between plasma immune checkpoint proteins at baseline (before antiviral therapy) with HSI increase at the end of follow-up (~ five years after SVR) in patients coinfected with HIV/HCV.

2. Material and methods

2.1. Study subjects

We performed a multicenter retrospective study in 62 HIV/HCV-coinfected patients on ART from 10 centers in Spain (see Appendix A). These patients had advanced fibrosis or cirrhosis and started interferon (IFN)-based therapy (peg-IFN-α/ribavirin or peg-IFN-α/ribavirin/DAAs) or IFN-free DAAs therapy between February 2012 and August 2016, achieving SVR (undetectable HCV-RNA load 12–24 weeks – depending on regimen – after the finalization of anti-HCV treatment). All patients had available clinical data and samples of frozen plasma at the start of HCV treatment (baseline). The end of follow-up (~ five years after SVR) was between January 2019 and May 2021. Patients with hepatitis B virus (HBV) coinfection, acute hepatitis C, or hepatic decompensation were excluded.

The study was approved by the Research Ethics Committee of the Institute of Health Carlos III (CEI PI 72_2021) and was conducted following the Declaration of Helsinki. All participants signed a written consent to participate in the study.

2.2. Clinical data and samples

Epidemiological, clinical, and virological characteristics were prospectively collected from patient’s medical records using an online form, which met all confidentiality requirements. This information was monitored.

Peripheral blood was collected in ethylenediaminetetraacetic acid tubes by venipuncture. On the same day of the extraction, samples were sent to the HIV HGM BioBank (http://hivhgmbiobank.com/?lang=en), where they were processed within 24 h post-extraction. Plasma was stored at − 80ºC until use.

2.3. Outcome variable

The HSI was calculated using the following formula: HSI = 8 x (alanine aminotransferase (ALT)/aspartate aminotransferase (AST) ratio)+ body mass index (BMI) (+2, if female; +2, if diabetes mellitus) [10]. The outcome variable was the change in HSI values from baseline to ~ five years after SVR (end of follow-up), coding this variable dichotomously: HSI increase ($\Delta HSI > 0$) versus HSI decrease ($\Delta HSI < 0$).

2.4. Multiplex immunoassays

Immuno-Oncology Checkpoint 14-Plex Human ProcartaPlex™ Panel 1 (Invitrogen™) was used to measure several plasma-soluble proteins using a Luminex 200™ analyzer (Luminex Corporation, Austin, TX, United States).

The plasma proteins measured were immune checkpoints that play a crucial role in the regulation of T cells, leading to either T cell exhaustion [B and T lymphocyte attenuator (BTLA), cluster of differentiation 80 (CD80), CD152(CTLA4), indoleamine 2,3-dioxygenase (IDO), lymphocyte activation gene-3 (LAG-3), programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3)] or stimulation [CD27, CD28, CD137(4–1BB), glucocorticoid-induced TNFR-related (GITR), and herpesvirus entry mediator (HVEM)]. The measured raw fluorescence intensity (FI) values (arbitrary units, a.u.) were used.

2.5. Statistical analysis

For the descriptive study, quantitative variables (clinical and epidemiological variables) were expressed as median (interquartile range, IQR), and categorical variables were shown as absolute count (percentage). Independent groups were compared using the Mann-

Whitney U and Chi-square tests for quantitative and categorical variables, respectively. The Wilcoxon signed range test was used to compare continuous dependent variables. Generalized Linear Models (GLM) with gamma distribution (log-link) were used to analyze the association between plasma immune checkpoint proteins at baseline and the change in HSI values (HSI increase vs. HSI decrease). This test provides the arithmetic mean ratio (AMR), the 95% of confidence interval (95% CI), and its level of significance. Also, GLM models were adjusted for the main available epidemiological and clinical characteristics (age, gender, HSI at baseline, liver stiffness measurement (LSM), HCV treatment (IFN-based therapy or DAAs), and time from baseline to follow-up time). These covariates were previously selected by a stepwise method (forward), where at each step, the covariates were considered to enter according to the lowest AKAike information criteria (AIC) for that specific model. BMI and AST were not considered as covariates because they were used to calculate the HSI. All p-values were corrected for multiple testing by using the Benjamini and Hochberg procedure. The level of significance was defined as p-value < 0.05 (two-tailed) and q-value < 0.1. The statistical analysis was done with R statistical package (R version 4.2.0. R Foundation for Statistical Computing, Vienna, Austria).

Finally, a supervised multivariate analysis using a Partial Least Squares Discriminant Analysis (PLS-DA) was performed with all significant plasma immune checkpoint proteins resulting from adjusted GLM models and the main epidemiological and clinical characteristics mentioned above. All variables were normalized by auto-scaled (mean-centered and then divided by the standard deviation of the variable). Permutation was carried out by separation distance (B/W) with a permutation number of 1000 to confirm the model's validity. The PLS-DA provides the variable importance in the projection (VIP) for each feature. The VIP score was used to classify and identify the most relevant variables, considering as relevant those VIP ≥ 1. The PLS-DA analysis was carried out with MetaboAnalyst 5.0 software (http://www.metaboanalyst.ca/).

3. Results

3.1. Patient characteristics

Characteristics of 62 HIV/HCV-coinfected patients are shown in Table 1. Overall, 48 (77.4%) were male, 40 (64.5%) were current smokers, and 25 (40.3%) and 49 (79.0%) had a prior history of alcohol intake and injection drug use, respectively. The median age was 50, and BMI was 24.5 kg/m^2. Regarding virological aspects, 70.7% were infected with HCV genotype 1, and the CD4$^+$ T cell count was 503 cells/mm^3.

We found almost half of the patients had an HSI decrease, while the other half had an HSI increase at the end of follow-up (~ five years after SVR), finding similar characteristics between both groups of patients, except for LSM (p-value = 0.014; Table 1). HSI changes from baseline to the end of follow-up were statistically significant for both groups (p-values < 0.001; Fig. 1). Besides, significant differences in HSI values between groups were found at the end of the follow-up (p-value = 0.011; Fig. 1).

3.2. Association analysis

Unadjusted GLM models showed significant direct associations (q-value < 0.1) between plasma levels of BTLA, CD137(4–1BB), CD152 (CTLA4), CD28, CD80, GITR, LAG-3, PD-1, PD-L1, and PD-L2 at baseline and HSI increase at the end of follow-up (~ five years after SVR) (Supplementary Table 1). In adjusted GLM models, we only found relevant direct associations (q-value < 0.1) for six immune checkpoint proteins (Fig. 2): BTLA (aAMR = 1.75; q-value= 0.022), CD137(4–1BB) (aAMR = 1.69; q-value= 0.032), CD80 (aAMR = 1.81; q-value= 0.057), GITR (aAMR = 2.30; q-value= 0.032), LAG-3 (aAMR = 1.32; q-value= 0.081), and PD-L1 (aAMR = 1.34; q-value= 0.022).

Table 1
Clinical, epidemiological, and virological characteristics of HIV/HCV-coinfected patients according to values of hepatic steatosis index (HSI).

	All patients	Patients with HSI increase	Patients with HSI decrease	*p*
No.	62	33 (53.2%)	29 (46.8%)	
Age (years)	50 (47–53)	50 (46–54)	51 (47–53)	0.989
Gender (male)	48 (77.4%)	24 (72.7%)	24 (82.8%)	0.523
BMI (kg/m^2)	24.5 (21.9–28.8)	24.6 (22.7–29.3)	23.6 (21.4–26.6)	0.125
Smoker				0.281
Never	4 (6.5%)	3 (9.1%)	1 (3.4%)	
Previous (>6 months)	18 (29.0%)	7 (21.2%)	11 (37.9%)	
Current	40 (64.5%)	23 (69.7%)	17 (58.6%)	
Alcohol intake (>50 g/day)				0.890
Never	34 (54.8%)	18 (54.5%)	16 (55.2%)	
Previous (>6 months)	25 (40.3%)	13 (39.4%)	12 (41.4%)	
Current	3 (4.8%)	2 (6.1%)	1 (3.4%)	
Intravenous drug user				0.375
Never	13 (21.0%)	5 (15.4%)	8 (27.6%)	
Previous (>6 months)	49 (79.0%)	28 (84.8%)	21 (72.4%)	
Current	0 (0%)			
Previous HCV therapy	38 (61.3%)	21 (63.6%)	17 (58.6%)	0.886
Diabetes mellitus	5 (8.1%)	2 (6.1%)	3 (10.3%)	0.880
Liver markers				
HSI	33.9 (29.1–37.2)	33.2 (28.8–37.2)	34.5 (31.0–37.2)	0.128
LSM (kPa)	17.9 (13.3–27.5)	24.8 (14.5–35.0)	14.3 (11.9–26.0)	0.014
AST (IU/L)	69.0 (45.5–103.8)	77.0 (57.0–123.0)	57.0 (40.0–87.0)	0.092
ALT(IU/L)	72.0 (42.3–99.8)	69.0 (47.0–103.0)	62.0 (45.0–111.0)	0.933
Diabetes mellitus	5 (8.1%)	2 (6.1%)	3 (10.3%)	0.880
HCV markers				
HCV genotype (n = 58)				0.302
1	41 (70.7%)	23 (71.9%)	18 (69.2%)	
3	11 (19.0%)	7 (21.9%)	4 (15.4%)	
4	5 (8.6%)	1 (3.1%)	4 (15.4%)	
Others	1 (1.7%)	1 (3.1%)	0 (0.0%)	
Log_{10} HCV-RNA (IU/mL) (n = 61)	6.2 (5.8–6.7)	6.1 (5.8–6.5)	6.3 (6.0–6.7)	0.225
HCV-RNA > 850.000 IU/mL (n = 61)	44 (71.0%)	22 (66.7%)	22 (75.9%)	0.606
HCV therapy				0.102
IFN-based	44 (71.0%)	20 (60.6%)	24 (82.8%)	
DAAs	18 (29.0%)	13 (39.4%)	5 (17.2%)	
HIV markers				
Previous AIDS	2 (3.2%)	0 (0.0%)	2 (6.9%)	0.416
Nadir CD4 + /mm^3 (n = 61)	162 (83–245)	130 (83–245)	174 (88–240)	0.879
Nadir < 200 CD4 + /mm^3 (n = 61)	39 (63.9%)	21 (63.6%)	18 (64.3%)	0.999
Baseline CD4 + T-cells/mm^3	503 (303–723)	492 (280–762)	515 (361–706)	0.999
Baseline < 500 CD4 + /mm^3	31 (50.0%)	17 (51.5%)	14 (48.3%)	0.999
HIV antiretroviral therapy				
NRTI + NNRTI	18 (29.0%)	12 (36.4)	6 (20.7%)	0.443
NRTI + II	24 (38.7%)	12 (36.4)	12 (41.4%)	
NRTI + PI	11 (17.7%)	4 (12.1)	7 (24.1%)	
Others	9 (14.5%)	5 (15.2)	4 (13.8%)	

Statistics: The values are expressed as the absolute number (percentage) and median (interquartile range). P-values were calculated by the Chi-square test and the Mann-Whitney U test.
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HSI,

hepatic steatosis index; BMI, body mass index; HCV, hepatitis C virus; LSM, liver stiffness measurement; kPa, kilopascal; IU, international units; pegIFN, pegylated interferon; DAAs, direct-acting antivirals; HCV-RNA, viral load of hepatitis C; AIDS, acquired immune deficiency syndrome; NRTI, nucleoside analogue HIV reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue HIV reverse transcriptase inhibitor; II, HIV integrase inhibitor; PI, HIV protease inhibitor.

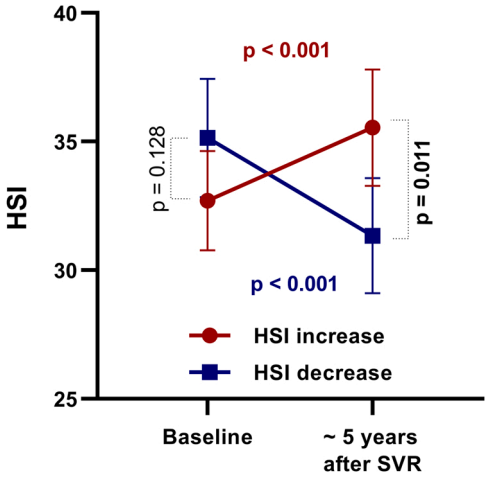

Fig. 1. Evolution of the raw data of hepatic steatosis index (HSI) from baseline to the end of follow-up (~ five years after SVR) in HIV/HCV-coinfected patients, stratifying by HSI evolution throughout the follow-up (HSI ≤0 vs. HSI >0). Statistics: Data represent the crude means and 95% confidence interval for each group of patients. P-values were calculated by the Mann-Whitney test for transversal analysis and the Wilcoxon test for longitudinal analysis between paired samples. Abbreviations: HSI, hepatic steatosis index; SVR, sustained virological response.

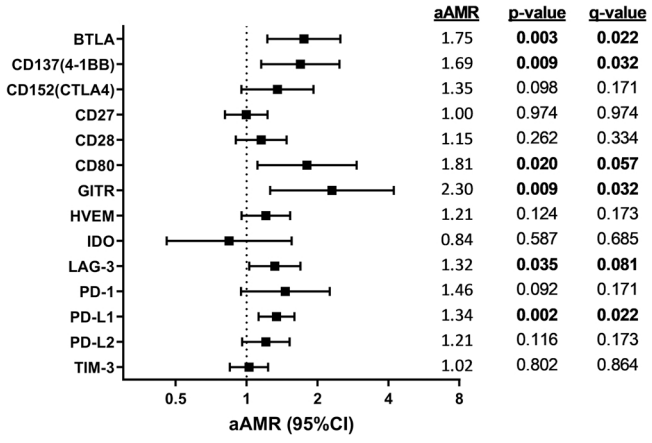

Fig. 2. Association of plasma immune checkpoint proteins at baseline with increased hepatic steatosis index (HSI>0) at the end of follow-up (~five years after SVR) in HIV/HCV-coinfected patients. Statistics: Data were calculated by Generalized Linear Models (GLM) with a gamma distribution (log-link) adjusted by age, gender, HSI at baseline, liver stiffness measurement, HCV therapy (IFN-based therapy or DAAs), and time from baseline to follow-up time, previously selected by a stepwise method (see Results Section). The q-values represent p-values corrected for multiple testing using the False Discovery Rate (FDR). Significant differences are shown in bold. Abbreviations: AMR, arithmetic mean ratio; aAMR, adjusted AMR; 95%CI, 95% of confidence interval; p, level of significance; BTLA, B, and T lymphocyte attenuator; CD, cluster of differentiation; GITR, glucocorticoid-induced TNFR-related; HVEM, herpesvirus entry mediator; IDO, indoleamine 2,3-dioxygenase; LAG-3, lymphocyte activation gene-3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

3.3. Supervised discriminant analysis

A PLS-DA including relevant immune checkpoint proteins resulting from adjusted GLM models (q-value <0.1) and epidemiological and clinical characteristics at baseline was performed to predict the change in HSI values during the follow-up. PLS-DA was validated by permutation (p-value = 0.014). All the significant immune checkpoint proteins showed a VIP ≥ 1, with values higher than those obtained by epidemiological and clinical characteristics, especially PD-L1 (Fig. 3).

4. Discussion

The information available in the literature about the association between plasma immune checkpoint proteins and steatosis is limited. This study describes for the first time the association between plasma levels of six immune checkpoint proteins (BTLA, CD137(4–1BB), CD80, GITR, LAG-3, and PD-L1) before HCV therapy and the long-term HSI increase (~ five years after SVR) in HIV/HCV-coinfected patients. Besides, the PLS-DA corroborated these findings since all significant immune checkpoint proteins showed a VIP > 1, especially PD-L1, suggesting a critical role of these biomarkers in the immunopathology of hepatic steatosis. Therefore, since hepatic steatosis progresses in about half of the treated patients, these plasma immune checkpoint proteins at baseline could serve as biomarkers of steatosis progression despite clearance of HCV.

It is widely known that HCV replication is closely related to the increase in lipid biosynthesis and a decrease in its degradation, favoring the accumulation of intracellular lipids (steatosis) [31]. Thus, chronic hepatitis C promotes metabolic dysfunction and NAFLD. However, it is still unclear how HCV elimination affects the course of these metabolic alterations. Some studies have shown a reduction in hepatic steatosis in HCV-infected patients who achieved SVR [27,28]. Other authors showed a decrease in steatosis only in patients infected with HCV genotype 3, in both HCV monoinfected and HIV/HCV-coinfected patients [32,33]. However, other reports have shown a tendency towards

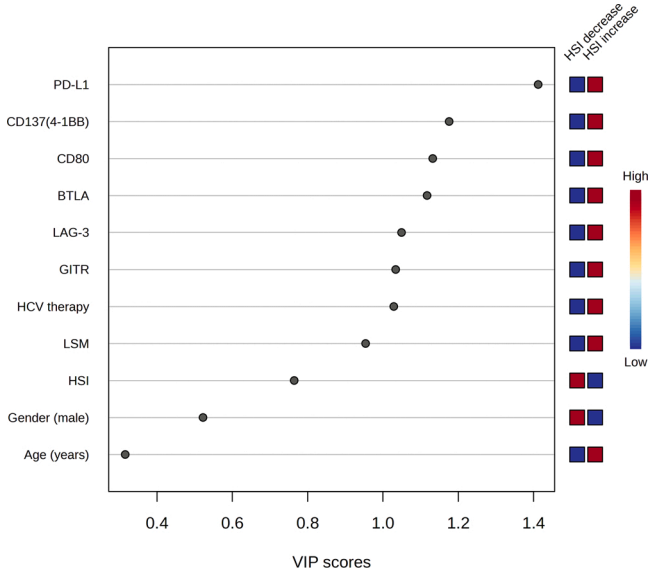

Fig. 3. Multivariate analysis by Partial least squares discriminant analysis (PLS-DA) for HSI increase. Variable importance in projection (VIP) of the main clinical variables and significant checkpoint biomarkers for predicting increase in HSI in HIV/HCV-coinfected patients. The VIP score measures the variable's importance and allows them to be ranked according to their importance. Abbreviations: BTLA, B, and T lymphocyte attenuator; CD, cluster of differentiation; GITR, glucocorticoid-induced TNFR-related; LAG-3, lymphocyte activation gene-3; PD-L1, programmed death-ligand 1; HCV, hepatitis C virus; LSM, liver stiffness measurement; HSI, hepatic steatosis index.

increased steatosis after SVR [29,30,34,35]. This is a critical issue since steatosis predicts poor outcomes in patients who achieve SVR [36,37]. Therefore, identifying biomarkers that predict progression toward steatosis could permit identifying patients who could benefit from closer monitoring after HCV eradication.

Chronic HIV and HCV infection promote T cell exhaustion, characterized by impaired immune function and increased expression of immune checkpoint proteins, which may decrease after viral control or elimination [22,38]. However, many studies show that only partial restoration of immune function is achieved after HCV clearance with DAAs, indicating some irreversible changes due to excessive long-term antigenic stimulation [22]. Likewise, immune checkpoint proteins are upregulated in HIV infection on T cells [39–41], which decline after ART, but remain elevated compared to healthy people [41]. Our findings suggest that the combined effect of excessive long-term antigenic stimulation by HCV and HIV infection could have promoted the overexpression of immune checkpoint proteins (BTLA, CD137(4–1BB), CD80, GITR, LAG-3, and PD-L1), in an attempt to normalize immune function.

PD-L1 is mainly expressed on immune cells upon stimulation with proinflammatory cytokines or bacterial products [42]. Thus, HCV induces strong PD-L1 upregulation in immune cells [22,43]. PD-L1 has also been detected in the liver of patients with hepatitis C, which directly correlates with the degree of liver inflammation, and impairs antiviral host immunity [42]. Moreover, PD-L1 is involved in obesity pathogenesis since its expression in adipose tissues is positively associated with visceral fat accumulation [44]. Patients with NAFLD show increased levels of PD-L1 [26]. Soluble PD-L1 is generated by proteolytic cleavage of membrane-bound PD-L1 [45], finding PD-L1 overexpressed in the plasma of patients with chronic hepatitis C [46]. The biological activity of soluble PD-L1 remains unclear [45]. Still, regardless of its mechanism of action, elevated soluble PD-L1 seems to be a marker of an immune system pathway that tries to control its inhibition/activation.

BTLA binds to HVEM to provide inhibitory signals in activated B and T cells, decreasing cell activation, cytokine production, and proliferation [47]. BTLA is overexpressed on T-cells after activation [48,49]. The activity of soluble BTLA remains unclear, but it may be a means of controlling T-cell inhibition. Soluble BTLA is significantly elevated in the plasma of septic patients with a high risk of disease progression and death [50], so it could be a marker of an activated immune system pathway.

Patients with chronic HCV and HIV infection show a higher expression of LAG-3 on T-cells and NK cells [51]. LAG-3 is expressed in activated immune cells and interacts with its canonical ligand, major histocompatibility complex (MHC) class II expressed on the surface of antigen-presenting cells (APCs), negatively modulating T cell function [52]. However, other binding partners such as liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin; also known as CLEC4G), galectin3 (Gal-3), and fibrinogen-like protein 1 (FGL1) have been proposed [52], molecules expressed in the liver and related to developing steatosis and metabolic disorders [53–55]. Therefore, the involvement of soluble LAG-3 in the pathophysiology of steatosis cannot be ruled out.

CD80, expressed mainly on APCs, binds CD28 (stimulatory signal) and CTLA-4 (inhibitory signal) on T cells modulating the immune response. CD80 is relevant in regulating obesity-related inflammatory reactions in adipose tissue and liver and, therefore, participates in the development and progression of steatosis and NAFLD [56]. Soluble CD80, generated by alternative splicing, engages in a complex regulatory network of the immune system since it shows stimulatory and inhibitory effects similar to those of membrane CD80 [57].

CD137 (4–1BB) is expressed on the activated leukocytes' surface and CD137 ligand (CD137L) on antigen-presenting cells (APCs). Interaction between CD137 and CD137L activates APCs and leukocytes, enhancing immune response. Soluble CD137 inhibits the interaction between CD137 and CD137L, downregulates CD137L on APCs, and suppresses T-cell activation [58]. In HCV-infected patients, serum CD137 was increased in cirrhotic patients, was associated with inflammation, and positively correlated with serum tumor necrosis factor (TNF) after SVR [59], possibly as an attempt to control the activation of the immune system. Moreover, high soluble CD137 levels are associated with inflammatory and metabolic parameters and are increased in the subcutaneous adipose tissues of obese patients [60], which could be related to the development of steatosis and NAFLD.

Another agonist molecule for the immune system is GITR, expressed on various types of immune cells, which binds to its ligand (GITRL) expressed on APCs, promoting effector T-cell function and inhibiting Treg function [61]. Besides, soluble GITR can also upregulate the proinflammatory response.

4.1. Study limitations

Our study is limited by the small size that could have restricted the detection of positive associations with other immune checkpoint proteins. Another limitation is the retrospective study design that may have introduced biases, such as different HCV therapy for treating patients (IFN and DAA-based treatment). However, we controlled for these variables by including them as covariates in the GLM model. Finally, liver biopsy, the gold standard for the diagnosis of NAFLD was not available since this technique has been avoided in clinical practice for a long time. Instead, the non-invasive HSI was used, which has shown excellent diagnostic performance in previous studies.

5. Conclusions

Higher plasma levels of different immune checkpoint proteins before HCV therapy were associated with a long-term increase in HSI after successful HCV therapy, suggesting a possible role in the pathophysiology of steatosis in patients coinfected with HIV/HCV. Further studies are needed to evaluate the utility of these plasma protein profiles for identifying HIV/HCV-coinfected patients who need closer monitoring after HCV eradication.

Ethics approval

The study was approved by the Research Ethics Committee of the Institute of Health Carlos III (CEI PI 72_2021), and was conducted following the Declaration of Helsinki. All participants signed a written consent to participate in the study.

Funding

This study was supported by the Instituto de Salud Carlos III (ISCIII) [grant numbers CP17CIII/00007, PI18CIII/00028 and PI21CIII/00033 to MAJS, PI17/00657 and PI20/00474 to JB, PI17/00903 and PI20/00507 to JGG, PI18CIII/00020 to AFR, and PI17CIII/00003 and PI20CIII/00004 to SR] and the Ministerio de Ciencia e Innovación (AEI, PID2021-126781OB-I00 to AFR). The study was also funded by the CIBER -Consorcio Centro de Investigación Biomédica en Red- (CB 2021), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación and Unión Europea – NextGenerationEU (CB21/13/00044). M.A.J.-S. is Miguel Servet researcher supported and funded by ISCIII (grant numbers CP17CIII/00007). R.M.-E. is Juan de la Cierva researcher supported and funded by MICINN of Spain (FJC2020-042865-I).

CRediT authorship contribution statement

Funding acquisition: MAJS and SR. Study concept and design: MAJS and SR. Patients' selection and clinical data acquisition: JB, JGG, CD, VH. Sample preparation and immunoassays: AVB and RME. Statistical analysis and interpretation of data: AVB, RME, and OBK. Writing – original draft preparation: AVB and RME. Writing – Review & Editing:

MAJS, SR, and AFR. Supervision and visualization: MAJS and SR.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study would not have been possible without the collaboration of all the patients, medical and nursery staff, and data managers who participated in the project. We want to acknowledge the patients in this study for their participation and the HIV BioBank integrated into the Spanish AIDS Research Network and collaborating Centers (http://hivhgmbiobank.com/donor-area/hospitals-and-centres-transferring-samples/?lang=en) for the generous gifts of clinical samples used in this work. The HIV BioBank, integrated into the Spanish AIDS Research Network, is partially funded by the RD16/0025/0019 project as part of the Plan Nacional R + D + I and cofinanced by ISCIII- Subdirección General de Evaluación and el Fondo Europeo de Desarrollo Regional (FEDER).

This study would not have been possible without the collaboration of all the patients, medical and nursery staff, and data managers who participated in the project.

Authors' information

Not applicable.

Appendix A. Members of the GeSIDA 10318 Study Group

Hospital General Universitario Gregorio Marañón, Madrid: A Carrero, P Miralles, JC López, F Parras, B Padilla, T Aldamiz-Echevarría, F Tejerina, C Díez, L Pérez-Latorre, C Fanciulli, I Gutiérrez, M Ramírez, S Carretero, JM Bellón, J Bermejo, and J Berenguer. Hospital Universitario La Paz, Madrid: V Hontañón, JR Arribas, mL Montes, I Bernardino, JF Pascual, F Zamora, JM Peña, F Arnalich, M Díaz, J González-García. Hospital Universitari Vall d'Hebron, Barcelona: E Van den Eynde, M Pérez, E Ribera, M Crespo. Hospital Universitario Príncipe de Asturias, Alcalá de Henares: A Arranz, E Casas, J de Miguel, S Schroeder, J Sanz. Hospital Donostia, San Sebastián: MJ Bustinduy, JA Iribarren, F Rodríguez-Arrondo, MA Von-Wichmann. Hospital Universitario de La Princesa, Madrid: J Sanz, I Santos. Hospital Clínico San Carlos, Madrid: J Vergas, MJ Téllez. Hospital Clínico Universitario, Valencia: A Ferrer, MJ Galindo. Hospital Universitario Ramón y Cajal, Madrid: JL Casado, F Dronda, A Moreno, MJ Pérez-Elías, MA Sanfrutos, S Moreno, C Quereda. Hospital General Universitario, Valencia: L Ortiz, E Ortega. Fundación SEIMC-GESIDA, Madrid: M Yllescas, P Crespo, E Aznar, H Esteban.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114913.

References

[1] Polaris Observatory HCVC, : Global change in hepatitis C virus prevalence and cascade of care between 2015 and 2020: a modelling study, Lancet Gastroenterol. Hepatol. 7 (5) (2022) 396–415.
[2] C.W. Spearman, G.M. Dusheiko, M. Hellard, M. Sonderup, Hepatitis C, Lancet 394 (10207) (2019) 1451–1466.
[3] J. Macias, J. Berenguer, M.A. Japon, J.A. Giron-Gonzalez, A. Rivero, L.F. Lopez-Cortes, A. Moreno, M. Marquez, J.A. Iribarren, E. Ortega, et al., Hepatic steatosis and steatohepatitis in human immunodeficiency virus/hepatitis C virus-coinfected patients, Hepatology 56 (4) (2012) 1261–1270.
[4] A. Vallet-Pichard, S. Pol, Natural history and predictors of severity of chronic hepatitis C virus (HCV) and human immunodeficiency virus (HIV) co-infection, J. Hepatol. 44 (1 Suppl) (2006) S28–34.
[5] J. Modaresi Esfeh, K. Ansari-Gilani, Steatosis and hepatitis C, Gastroenterol. Rep. 4 (1) (2016) 24–29.
[6] D. Attia, S. Abdel Alem, W. El-Akel, W. Abdel-Razek, M. Eslam, Y. Fouad, I. Waked, Prevalence and clinical characteristics of patients with metabolic dysfunction-associated fatty liver disease with hepatitis C virus infection-a population-based study, Aliment Pharm. Ther. 56 (11–12) (2022) 1581–1590.
[7] M.N. Kim, K. Han, J. Yoo, S.G. Hwang, S.H. Ahn, Increased risk of hepatocellular carcinoma and mortality in chronic viral hepatitis with concurrent fatty liver, Aliment Pharm. Ther. 55 (1) (2022) 97–107.
[8] C.E. Coronel-Castillo, X. Qi, J. Contreras-Carmona, O.L. Ramirez-Perez, N. Mendez-Sanchez, Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in HIV infection: a metabolic approach of an infectious disease, Expert Rev. Gastroenterol. Hepatol. 13 (6) (2019) 531–540.
[9] A. Okada, G. Yamada, T. Kimura, Y. Hagiwara, S. Yamaguchi, K.I. Kurakawa, M. Nangaku, T. Yamauchi, Y. Matsuyama, T. Kadowaki, Diagnostic ability using fatty liver and metabolic markers for metabolic-associated fatty liver disease stratified by metabolic/glycemic abnormalities, J. Diabetes Invest. 14 (3) (2023) 463–478.
[10] J.H. Lee, D. Kim, H.J. Kim, C.H. Lee, J.I. Yang, W. Kim, Y.J. Kim, J.H. Yoon, S. H. Cho, M.W. Sung, et al., Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease, Dig. Liver Dis. 42 (7) (2010) 503–508.
[11] C. Yanavich, A.G. Pacheco, S.W. Cardoso, E.P. Nunes, U. Chaves, G. Freitas, R. Santos, M. Morata, V.G. Veloso, B. Grinsztejn, et al., Diagnostic value of serological biomarkers for detection of non-alcoholic fatty liver disease (NAFLD) and/or advanced liver fibrosis in people living with HIV, HIV Med 22 (6) (2021) 445–456.
[12] K.W. Chew, K. Wu, K. Tassiopoulos, F.J. Palella, S. Naggie, N.S. Utay, E.T. Overton, M. Sulkowski, Liver Inflammation is common and linked to metabolic derangements in persons with treated human immunodeficiency Virus (HIV), Clin. Infect. Dis. 76 (3) (2023) e571–e579.
[13] J. Berenguer, E. Rodriguez-Castellano, A. Carrero, M.A. Von Wichmann, M. Montero, M.J. Galindo, J. Mallolas, M. Crespo, M.J. Tellez, C. Quereda, et al., Eradication of hepatitis C virus and non-liver-related non-acquired immune deficiency syndrome-related events in human immunodeficiency virus/hepatitis C virus coinfection, Hepatology 66 (2) (2017) 344–356.
[14] J. Berenguer, J. Alvarez-Pellicer, P.M. Martin, J. Lopez-Aldeguer, M.A. Von-Wichmann, C. Quereda, J. Mallolas, J. Sanz, C. Tural, J.M. Bellon, et al., Sustained virological response to interferon plus ribavirin reduces liver-related complications and mortality in patients coinfected with human immunodeficiency virus and hepatitis C virus, Hepatology 50 (2) (2009) 407–413.
[15] C. Diez, J. Berenguer, L. Ibanez-Samaniego, E. Llop, L. Perez-Latorre, M. V. Catalina, V. Hontanon, M.A. Jimenez-Sousa, T. Aldamiz-Echevarria, J. Martinez, et al., Persistence of clinically significant portal hypertension after eradication of hepatitis C virus in patients with advanced cirrhosis, Clin. Infect. Dis. 71 (10) (2020) 2726–2729.
[16] M. Santos, A. Corma-Gomez, M. Fernandez-Fuertes, A. Gonzalez-Serna, P. Rincon, L.M. Real, J.A. Pineda, J. Macias, Burden of significant liver damage in people living with HIV after microelimination of the hepatitis C virus, J. Infect. 86 (1) (2023) 41–46.
[17] N. Hamdane, F. Juhling, E. Crouchet, H. El Saghire, C. Thumann, M.A. Oudot, S. Bandiera, A. Saviano, C. Ponsolles, A.A. Roca Suarez, et al., HCV-induced epigenetic changes associated with liver cancer risk persist after sustained virologic response, Gastroenterology 156 (8) (2019) 2313–2329, e2317.
[18] S. Perez, A. Kaspi, T. Domovitz, A. Davidovich, A. Lavi-Itzkovitz, T. Meirson, J. Alison Holmes, C.Y. Dai, C.F. Huang, R.T. Chung, et al., Hepatitis C virus leaves an epigenetic signature post cure of infection by direct-acting antivirals, PLoS Genet 15 (6) (2019), e1008181.
[19] Y. Fouad, J.V. Lazarus, F. Negro, M. Peck-Radosavljevic, S.K. Sarin, P. Ferenci, G. Esmat, H. Ghazinian, A. Nakajima, M. Silva, et al., MAFLD considerations as a part of the global hepatitis C elimination effort: an international perspective, Aliment Pharm. Ther. 53 (10) (2021) 1080–1089.
[20] N.H. Shoukry, C.M. Walker, T cell responses during HBV and HCV infections: similar but not quite the same? Curr. Opin. Virol. 51 (2021) 80–86.
[21] M.N. Wykes, S.R. Lewin, Immune checkpoint blockade in infectious diseases, Nat. Rev. Immunol. 18 (2) (2018) 91–104.
[22] S. Osuch, K.J. Metzner, K. Caraballo Cortes, Reversal of T cell exhaustion in chronic HCV infection, Viruses 12 (2020) 8.
[23] L. Chen, X. Yu, C. Lv, Y. Dai, T. Wang, S. Zheng, Y. Qin, X. Zhou, Y. Wang, H. Pei, et al., Increase in serum soluble tim-3 level is related to the progression of diseases after hepatitis virus infection, Front Med. 9 (2022), 880909.
[24] E.J. Wherry, T cell exhaustion, Nat. Immunol. 12 (6) (2011) 492–499.
[25] Y. Sun, J. Xue, Expression profile and biological role of immune checkpoints in disease progression of HIV/SIV infection, Viruses 14 (2022) 3.
[26] R. Lombardi, R. Piciotti, P. Dongiovanni, M. Meroni, S. Fargion, A.L. Fracanzani, PD-1/PD-L1 immuno-mediated therapy in NAFLD: advantages and obstacles in the treatment of advanced disease, Int J. Mol. Sci. 23 (2022) 5.
[27] T. Tada, T. Kumada, H. Toyoda, Y. Sone, K. Takeshima, S. Ogawa, T. Goto, A. Wakahata, M. Nakashima, M. Nakamuta, et al., Viral eradication reduces both liver stiffness and steatosis in patients with chronic hepatitis C virus infection who received direct-acting anti-viral therapy, Aliment Pharm. Ther. 47 (7) (2018) 1012–1022.
[28] N. Kobayashi, H. Iijima, T. Tada, T. Kumada, M. Yoshida, T. Aoki, T. Nishimura, C. Nakano, R. Takata, K. Yoh, et al., Changes in liver stiffness and steatosis among patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response, Eur. J. Gastroenterol. Hepatol. 30 (5) (2018) 546–551.
[29] G. Rout, B. Nayak, A.H. Patel, D. Gunjan, V. Singh, S. Kedia, Shalimar, Therapy with oral directly acting agents in hepatitis C infection is associated with reduction

in fibrosis and increase in hepatic steatosis on transient elastography, J. Clin. Exp. Hepatol. 9 (2) (2019) 207–214.
[30] A. Cespiati, S. Petta, R. Lombardi, V. Di Marco, V. Calvaruso, C. Bertelli, G. Pisano, E. Fatta, G. Sigon, F. Iuculano, et al., Metabolic comorbidities and male sex influence steatosis in chronic hepatitis C after viral eradication by direct-acting antiviral therapy (DAAs): Evaluation by the controlled attenuation parameter (CAP), Dig. Liver Dis. 53 (10) (2021) 1301–1307.
[31] W. Elgretli, T. Chen, N. Kronfli, G. Sebastiani, Hepatitis C virus-lipid interplay: pathogenesis and clinical impact, Biomedicines 11 (2023) 2.
[32] M. Rodriguez-Torres, S. Govindarajan, R. Sola, N. Clumeck, E. Lissen, M. Pessoa, P. Buggisch, J. Main, J. Depamphilis, D.T. Dieterich, Hepatic steatosis in HIV/HCV co-infected patients: correlates, efficacy and outcomes of anti-HCV therapy: a paired liver biopsy study, J. Hepatol. 48 (5) (2008) 756–764.
[33] H.M. Patton, K. Patel, C. Behling, D. Bylund, L.M. Blatt, M. Vallee, S. Heaton, A. Conrad, P.J. Pockros, J.G. McHutchison, The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients, J. Hepatol. 40 (3) (2004) 484–490.
[34] N. Chuaypen, S. Siripongsakun, P. Hiranrat, N. Tanpowpong, A. Avihingsanon, P. Tangkijvanich, Improvement of liver fibrosis, but not steatosis, after HCV eradication as assessment by MR-based imaging: Role of metabolic derangement and host genetic variants, PLoS One 17 (6) (2022), e0269641.
[35] A. Trifan, E. Stratina, A. Rotaru, R. Stafie, S. Zenovia, R. Nastasa, L. Huiban, C. Sfarti, C. Cojocariu, T. Cuciureanu, et al., Changes in liver steatosis using controlled attenuation parameter among patients with chronic hepatitis C infection treated with direct-acting antivirals therapy who achieved sustained virological response, Diagnostics 12 (2022) 3.
[36] N. Peleg, A. Issachar, O. Sneh Arbib, M. Cohen-Naftaly, Y. Harif, E. Oxtrud, M. Braun, M. Leshno, A. Barsheshet, A. Shlomai, Liver steatosis is a major predictor of poor outcomes in chronic hepatitis C patients with sustained virological response, J. Viral Hepat. 26 (11) (2019) 1257–1265.
[37] J.N. Benhammou, A.M. Moon, J.R. Pisegna, F. Su, P. Vutien, C.A. Moylan, G. N. Ioannou, Nonalcoholic fatty liver Disease risk factors affect liver-related outcomes after direct-acting antiviral treatment for hepatitis C, Dig. Dis. Sci. 66 (7) (2021) 2394–2406.
[38] Y.T. Chan, H.C. Cheong, T.F. Tang, R. Rajasuriar, K.K. Cheng, C.Y. Looi, W. F. Wong, A. Kamarulzaman, Immune Checkpoint molecules and glucose metabolism in HIV-induced T cell exhaustion, Biomedicines 10 (2022) 11.
[39] L. Trautmann, L. Janbazian, N. Chomont, E.A. Said, S. Gimmig, B. Bessette, M. R. Boulassel, E. Delwart, H. Sepulveda, R.S. Balderas, et al., Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction, Nat. Med 12 (10) (2006) 1198–1202.
[40] D.E. Kaufmann, D.G. Kavanagh, F. Pereyra, J.J. Zaunders, E.W. Mackey, T. Miura, S. Palmer, M. Brockman, A. Rathod, A. Piechocka-Trocha, et al., Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction, Nat. Immunol. 8 (11) (2007) 1246–1254.
[41] G.M. Chew, T. Fujita, G.M. Webb, B.J. Burwitz, H.L. Wu, J.S. Reed, K.B. Hammond, K.L. Clayton, N. Ishii, M. Abdel-Mohsen, et al., TIGIT marks exhausted t cells, correlates with disease progression, and serves as a target for immune restoration in HIV and SIV infection, PLoS Pathog. 12 (1) (2016), e1005349.
[42] S.J. Park, Y.S. Hahn, Hepatocytes infected with hepatitis C virus change immunological features in the liver microenvironment, Clin. Mol. Hepatol. 29 (1) (2023) 65–76.
[43] G. Schonrich, M.J. Raftery, The PD-1/PD-L1 axis and virus infections: a delicate balance, Front Cell Infect. Microbiol 9 (2019) 207.
[44] M. Yang, S. Liu, C. Zhang, The related metabolic diseases and treatments of obesity, Healthcare 10 (2022) 9.
[45] M. Niu, Y. Liu, M. Yi, D. Jiao, K. Wu, Biological characteristics and clinical significance of soluble PD-1/PD-L1 and Exosomal PD-L1 in Cancer, Front Immunol. 13 (2022), 827921.
[46] S. Yamagiwa, T. Ishikawa, N. Waguri, S. Sugitani, K. Kamimura, A. Tsuchiya, M. Takamura, H. Kawai, S. Terai, Increase of soluble programmed cell death ligand 1 in patients with chronic hepatitis C, Int J. Med Sci. 14 (5) (2017) 403–411.
[47] Z. Ning, K. Liu, H. Xiong, Roles of BTLA in immunity and immune disorders, Front Immunol. 12 (2021), 654960.
[48] M. Barathan, R. Mohamed, J. Vadivelu, L.Y. Chang, R. Vignesh, J. Krishnan, P. Sigamani, A. Saeidi, M.R. Ram, V. Velu, et al., CD8+ T cells of chronic HCV-infected patients express multiple negative immune checkpoints following stimulation with HCV peptides, Cell Immunol. 313 (2017) 1–9.
[49] C. Ackermann, M. Smits, R. Woost, J.M. Eberhard, S. Peine, S. Kummer, M. Marget, T. Kuntzen, W.W. Kwok, A.W. Lohse, et al., HCV-specific CD4+ T cells of patients with acute and chronic HCV infection display high expression of TIGIT and other co-inhibitory molecules, Sci. Rep. 9 (1) (2019) 10624.
[50] A. Lange, J. Sunden-Cullberg, A. Magnuson, O. Hultgren, Soluble B and T lymphocyte attenuator correlates to disease severity in sepsis and high levels are associated with an increased risk of mortality, PLoS One 12 (1) (2017), e0169176.
[51] M.A. Cox, R. Nechanitzky, T.W. Mak, Check point inhibitors as therapies for infectious diseases, Curr. Opin. Immunol. 48 (2017) 61–67.
[52] S.E.A. Burnell, L. Capitani, B.J. MacLachlan, G.H. Mason, A.M. Gallimore, A. Godkin, Seven mysteries of LAG-3: a multi-faceted immune receptor of increasing complexity, Immunother. Adv. 2 (ltab025) (2022) 1.
[53] E. Pandey, A.S. Nour, E.N. Harris, Prominent receptors of liver sinusoidal endothelial cells in liver homeostasis and disease, Front Physiol. 11 (2020) 873.
[54] D. Ezhilarasan, Unraveling the pathophysiologic role of galectin-3 in chronically injured liver, J. Cell Physiol. (2023).
[55] X.H. Liu, L.W. Qi, R.N. Alolga, Q. Liu, Implication of the hepatokine, fibrinogen-like protein 1 in liver diseases, metabolic disorders and cancer: The need to harness its full potential, Int J. Biol. Sci. 18 (1) (2022) 292–300.
[56] A. Chatzigeorgiou, K.J. Chung, R. Garcia-Martin, V.I. Alexaki, A. Klotzsche-von Ameln, J. Phieler, D. Sprott, W. Kanczkowski, T. Tzanavari, M. Bdeir, et al., Dual role of B7 costimulation in obesity-related nonalcoholic steatohepatitis and metabolic dysregulation, Hepatology 60 (4) (2014) 1196–1210.
[57] M. Khan, S. Arooj, H. Wang, Soluble B7-CD28 family inhibitory immune checkpoint proteins and anti-cancer immunotherapy, Front Immunol. 12 (2021), 651634.
[58] M. Rojas, L.S. Heuer, W. Zhang, Y.G. Chen, W.M. Ridgway, The long and winding road: From mouse linkage studies to a novel human therapeutic pathway in type 1 diabetes, Front Immunol. 13 (2022), 918837.
[59] K. Weigand, G. Peschel, J. Grimm, K. Luu, D. Schacherer, R. Wiest, M. Muller, H. Schwarz, C. Buechler, Soluble CD137 is a novel serum marker of liver cirrhosis in patients with hepatitis C and alcohol-associated disease etiology, Eur. J. Immunol. 52 (4) (2022) 633–645.
[60] T.H. Tu, C.S. Kim, J.H. Kang, I.S. Nam-Goong, C.W. Nam, E.S. Kim, Y.I. Kim, J. I. Choi, T. Kawada, T. Goto, et al., Levels of 4-1BB transcripts and soluble 4-1BB protein are elevated in the adipose tissue of human obese subjects and are associated with inflammatory and metabolic parameters, Int J. Obes. 38 (8) (2014) 1075–1082.
[61] J. Tian, B. Zhang, K. Rui, S. Wang, The role of GITR/GITRL interaction in autoimmune diseases, Front Immunol. 11 (2020), 588682.