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ORIGINAL ARTICLE

Interleukin-10 promoter gene polymorphisms are associated with the first major depressive episode in chronic hepatitis C patients

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KEYWORDS
ATA haplotype;
Chronic hepatitis C;
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First major depressive episode;

Summary
Aims: To investigate the association of *IL*10 SNPs in chronic hepatitis C (CHC) patients with and without the first major depressive episode (MDE), as well as their association with plasma levels of target cytokines.
Methods: A hundred and thirty two CHC patients (32 with and 100 without first MDE) and 98 controls were prospectively enrolled in this cross-sectional study. MDE was diagnosed by a psychiatrist, using the Mini International Neuropsychiatric Interview Plus 5.0. *IL*10 polymorphisms

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*IL*10 gene polymorphisms; *IL*10 SNPs

(−1082 G/A, −819C/T and −592C/A *IL*10 SNPs) were evaluated by Taqman SNP genotyping assay. Plasma concentrations of IL-2, IL-6, IL-10, IFN-γ and TNF-α were determined using the Human Th1/Th2 Cytometric Bead Array kit. The associations were investigated by logistic models.
Results: The frequencies of the studied *IL*10 SNPs did not differ between the CHC patients and controls. The first MDE was positive and independently associated with the *IL*10-1082*A, *IL*10-819*T and *IL*10-592*A (ATA) low producer haplotype (OR = 1.50; 95% CI = 1.11—2.04; *P* = 0.009) and current alcohol misuse (OR = 4.29; 95% CI = 1.22—15.05; *P* = 0.02), and inversely associated with increasing age (OR = 0.94; 95% CI = 0.91—0.98; *P* = 0.006). In addition, plasma level of TNF-α was significantly higher in the carriers than in the non-carriers of the *IL*10 ATA haplotype in patients with the first MDE. The IL-10 and IL-2 plasma levels were significantly higher in the carriers than in non-carriers of the *IL*10 GCC high producer haplotype, demonstrating the functionality of the studied *IL*10 polymorphisms.
Conclusions: This is the first study to demonstrate that the *IL*10 low producer ATA haplotype is associated with the first MDE in patients with CHC.

SNPs	single nucleotide polymorphisms
*IL*10	interleukin 10 gene
HCV	hepatitis C virus
MDD	major depressive disorder
CHC	chronic hepatitis C
TNF-α	tumour necrosis factor alpha
IL-1β	interleukin-1 beta
DSM IV	diagnostic and statistical manual of mental disorders IV
ICD-10	international classification of diseases
HIV	human immunodeficiency virus
HBV	hepatitis B virus
ALT	alanine aminotransferase
AST	aspartate amino transferase
γ-GT	gamma-glutamyl transpeptidase
ALP	alkaline phosphatase
PCR	polymerase chain reactions
IL-6	interleukin-6
IL-10	interleukin-10
IFN-γ	interferon gamma

Introduction

The hepatitis C virus (HCV) infection is a worldwide public health burden that affects around 71 million people [1,2]. Hepatic-associated diseases, such as cirrhosis and hepatocellular carcinoma, are well-recognized complications of chronic hepatitis C (CHC) [3]. Furthermore, CHC has been considered a systemic disease and several extrahepatic conditions that may increase morbidity and mortality have been described, for instance: mixed cryoglobulinemia, lymphoproliferative disorders, renal and cardiovascular diseases, insulin resistance, type 2 diabetes, sicca syndrome, rheumatoid arthritis-like polyarthritis and autoantibody production [4]. Additionally, CHC is associated with depression and anxiety symptoms [5,6], which impair the patient's health-related quality of life [4,7,8].

Although depressive disorders are more frequently observed in CHC patients than in the general population [6], as verified in other chronic diseases [9], the pathogenesis of HCV-related psychiatric symptoms has not been completely clarified. Previous investigations have demonstrated that the virus is able to cross the blood-brain barrier [10—13]. Enhanced plasma levels of pro-inflammatory interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) have also been observed during ongoing depression in subjects chronically infected with HCV [14]. The role played by the host's immune response in the occurrence of psychiatric disorders in CHC should, thus, be considered. In fact, a putative role of other pro-inflammatory cytokines in the genesis of depression in HCV is highlighted by the observation that experimental administration of exogenous INF-α, in both humans and animals, provokes depressive symptoms and that HCV treatment with interferon alpha (IFN-α) is linked to the major depressive disorder (MDD) [15—17].

Given the potential relevance of cytokines in mediating depressive manifestations [14,18—23], the effect of single nucleotide polymorphisms (SNPs), located in the coding region of cytokine genes, on the pathogenesis of HCV-associated psychiatric symptoms deserves to be evaluated [24—26]. Although *IL*10 polymorphisms have been associated with the severity of CHC and with the response to treatment in HCV-infected patients [27—30], we are unaware of studies evaluating their association with depression among CHC patients.

The well-studied *IL*10 SNPs located in the promoter region at the positions −1082 A/G (rs1800896), −819C/T (rs1800871) and −592C/A (rs1800872) have been associated with different expression of IL-10 [31,32]. Therefore, considering that the *IL*10 haplotypes GCC, ACC and ATA are associated with high, intermediate and low production of IL-10, respectively [31,32], we investigated whether they are associated with the first major depressive episode (MDE) in the patients with CHC. Further, we investigated the influ-

ence of *IL*10 haplotypes in the plasma levels of target cytokines.

Patients and methods

Study population

A hundred and forty patients with confirmed CHC attending the Viral Hepatitis Outpatient Clinic, University Hospital, Belo Horizonte (state of Minas Gerais), Brazil, were prospectively included in the study. The control group consisted of 98 consecutive volunteer blood donors from the hemocenter of Felício Rocho Hospital (Hemoter — Clínica Romeu Ibrahim de Carvalho), Belo Horizonte, Brazil. All patients and controls signed the informed consent form. The study was designed and conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Federal University of Minas Gerais/UFMG (ETIC 0631.0.203.000-09).

The exclusion criteria were: pregnancy, breastfeeding, hepatic encephalopathy, HBV/HCV or HCV/HIV co-infection, current antiviral or anti-depressant treatment, use of non-steroidal anti-inflammatory drugs or corticosteroids, and the presence of advanced disease such as decompensated liver cirrhosis, chronic kidney disease, heart failure, chronic pulmonary disease and neoplasia, including hepatocellular carcinoma.

The diagnosis of cirrhosis was based on standard clinical, biochemical, radiological and histological parameters [33,34]. The severity of liver dysfunction was assessed by Child-Pugh-Turcotte Score [35]. Compensated cirrhosis was defined as the absence of variceal bleeding, ascites and oedema, jaundice or symptomatic encephalopathy on physical examination, and decompensated cirrhosis as the presence of any of these complications [36].

All patients and controls underwent a psychiatric evaluation at baseline, including the administration of the Brazilian validated version of the Mini International Neuropsychiatric Interview Plus (M.I.N.I. Plus) [37]. This instrument is a semi structured diagnostic interview comprising the primary Axis I disorders of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) and of the International Classification of Diseases (ICD-10), which was designed for clinical practice and research in psychiatric and primary care settings [38]. Eight patients, diagnosed with a previous episode of depression, were excluded from the analysis. One hundred and thirty-two patients remained in the study.

All participants were from a similar socioeconomic level, as assessed by a previously validated questionnaire [7], which was based on income and educational level. All subjects were natives of Minas Gerais, state with the following ethnic background: 56.0% of European ancestry, 32.0% of African ancestry and 12.0% of Amerindian ancestry, homogeneously present in each subject, irrespective of their phenotype [39].

Laboratory parameters

Blood samples were obtained from each subject after 12—14-hour overnight fasting for the HCV-status, cytokine genotyping, plasma cytokine and biochemical assessments. Alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), albumin, total bilirubin and prothrombin time were evaluated by routine laboratory methods.

Aliquots of leukocytes and plasma were stored at $-80\,^{\circ}C$ until analysis.

DNA extraction and *IL*10 genotyping

DNA was extracted from the leukocytes with the QIAmp DNA mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's recommendations. The SNPs *IL*10 −1082G/A (rs1800896), *IL*10 −819C/T (rs1800871) and −592C/A (rs1800872) were analysed by pre-designed Taqman SNP genotyping assays (Real Time PCR 7500 System Applied Biosystems, Foster City, CA), using minor groove binding probes labelled with FAM (6 carboxy-fluoresceine) or VIC fluorocloromes to detect the polymorphic or wild alleles, respectively. PCR amplification was performed according to the manufacturer's instruction in a volume of 2 mL containing 10 ng genomic DNA. Thermal cycling of optical plates was performed on Real Time PCR System 7500 (Applied Biosystems). The sequences of synthetic oligonucleotides used were previously described by Turner et al. (1997) [31]. The probes and reaction conditions are described in Table 1.

Determination of cytokine plasma concentrations

Plasma concentrations of IL-6, IL-10, IFN-γ and TNF-α were determined using the Human Th_1/Th_2 Cytometric Bead Array (CBA) kit (BD Biosciences Pharmingen, San Jose, CA) according to the manufacturer's instructions. The maximum and minimum limits for detection of the five cytokines were 1.0 pg/mL and 5000.0 pg/mL, respectively.

Statistical analysis

The Hardy—Weinberg equilibrium of alleles at individual *loci* was assessed by two-tailed Chi^2 test or Fisher's exact test. Haplotype frequencies for pairs of alleles and linkage disequilibrium between the loci were estimated by using the program EH (available from: http://ftp://linkage.rockefeller.edu/software/eh/). The association of each variable, including ATA haplotype, increasing age, current alcohol misuse and IFN-γ plasma levels, with the first major depressive episode (dependent variable) was tested in univariate analysis. All the variables with a *P*-value of 0.20 or less were included in the full model of logistic regression. Odds ratio (OR) and 95% confidence interval (CI) were used as an estimate of the risk. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the fit of the models. In addition to the visual examination of histograms and box plots, the Kolmogorov—Smirnov goodness-of-fit test was used to assess the normality of the data. Because the data were not normally distributed, the two-tailed Mann—Whitney *U*-test was used to detect differences between groups. Correlation was done by Spearman's correlation. Data were analysed with the Statistical Pack-

Table 1 Probes and conditions used in the PCR to genotype *IL*10 SNPs at positions −592 (rs1800872), −819 (rs1800871) and −1082 (rs1800896).

*IL*10 gene	Probes (5'−3')	PCR conditions
−592[a]	CTTTCCAGAGACTGGCTTCCTACAG[T/G]ACAGGCGGGGTCACAGGATGTGTTC	60 °C − 1 min; 95 °C − 10 min; 50 cycles (95 °C − 15 s, 60 °C − 90 s) and 60 °C − 1 min
−819[b]	AGTGAGCAAACTGAGGCACAGAGAT[A/G]TTACATCACCTGTACAAGGGTACAC	60 °C − 1 min; 95 °C − 10 min; 50 cycles (95 °C − 15 s, 60 °C − 90 s) and 60 °C − 1 min
−1082[c]	TCCTCTTACCTATCCCTACTTCCCC[T/C]TCCCAAAGAAGCCTTAGTAGTGTTG	60 °C − 1 min; 95 °C − 10 min; 50 cycles (95 °C − 15 sec., 60 °C − 90 s) and 60 °C − 1 min

*IL*10: *interleukin*-10 gene.
[a] rs1800872.
[b] rs1800871.
[c] rs1800896.

age for the Social Sciences version 17 (SPSS Inc., Chicago, IL, USA). *P*-values ≤ 0.05 were considered significant.

Results

Distribution of the *IL*10 genotypes and cytokine levels in CHC patients and controls (blood donors)

The frequencies of the *IL*10 SNPs did not differ between the CHC patients and the blood donors (Table 2). All polymorphisms were in Hardy-Weinberg equilibrium ($P > 0.20$ for all SNPs) in the control group. The median plasma levels of IL-6, IL-10 and IFN-γ, but neither IL-2 nor TNF-α, were significantly higher in the CHC patients compared with the control group (Fig. 1A). Since the expression of cytokines may vary with aging [40], and considering that in our population young and middle-aged subjects (19—40 years and 41—60 years) had similar patterns of plasma cytokines, we analysed a subgroup of patients ($n = 95$) and controls ($n = 97$) aged 60 years or younger. The difference remained significant for IL-6, IL-10 and IFN-γ (Fig. 1B).

We confirmed that the *IL*10 polymorphisms are functional by demonstrating that the median [interquartile range (IQR)] plasma levels of IL-10 [4.49 (4.03—5.29) vs. 4.10 (3.91—5.29) pg/mL; $P = 0.01$] and IL-2 [1.32 (1.17—1.72) vs. 1.22 (1.09—1.53) pg/mL; $P = 0.03$)] were significantly higher in the carriers than in non-carriers of the GCC haplotype, respectively. The plasma levels of IL-10 were significantly correlated with those of IL-2 ($r = 0.50$, $P < 0.001$).

Characteristics of the patients chronically infected with hepatitis C virus with and without the first major depressive episode

The first MDE was observed in 32 (24.2%) patients with CHC and in one (1.0%) control subject ($P < 0.001$).

The demographic, clinical, histological, biochemical and virological characteristics of the 132 CHC included patients with ($n = 32$) and without ($n = 100$) the first MDE are shown in Table 3.

Among the patients enrolled, 14 (10.6%) had received previous treatment with INF-α for HCV, but all had finished the treatment at least 24 months before the beginning of the study.

Distribution of *IL*10 genotypes and plasma levels of cytokines in CHC patients with and without the first major depression episode

Higher frequencies of the *IL*10−1082 AA genotype ($P = 0.02$), as well as the ATA haplotype ($P = 0.009$) were observed in the patients with compared to those without the first MDE (Table 4). There were no significant differences of median plasma levels of IL-6, IL-10, IFN-γ and TNF-α between the CHC patients with and without the first MDE (Table 4). Nevertheless, in the group of patients with the first MDE, the ATA carriers had significantly higher plasma levels of TNF-α than the non-ATA carriers (Table 5).

Factors associated with the first major depressive episode (MDE) in patients with chronic hepatitis C

In the univariate analysis, *IL*10 SNPs, age, current alcohol misuse and plasma concentration of IFN-γ were associated with the first MDE (Tables 3 and 4). In the multivariate analysis, the first MDE remained positively associated with *IL*10 ATA haplotype and with current alcohol misuse as well as inversely associated with older age (Table 6). The associations remained when patients who had previously received IFN-α therapy ($n = 14$) were excluded from the analysis.

HCV viral load and HCV genotype in CHC patients with and without the ATA haplotype

The HCV viral load was higher in the carriers than in the non-carriers of the ATA haplotype [5.84 (IQR: 5.50—6.27) vs. 5.50 (IQR: 4.94—5.96) log/IU/mL; $P = 0.02$), but no difference was observed in respect of the HCV genotype ($P = 0.46$).

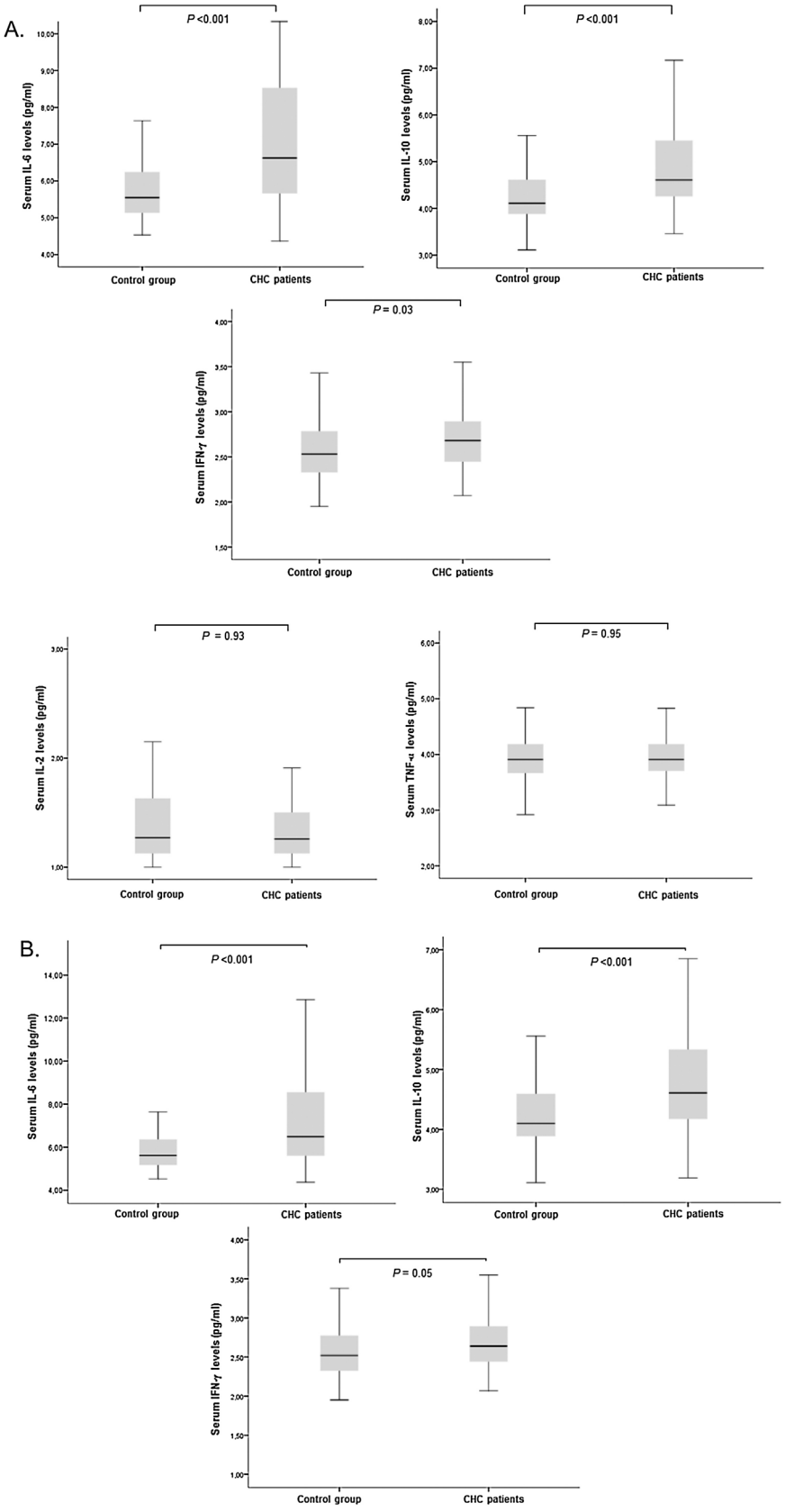

Figure 1 Box plots representing the plasma levels of (pg/ml) of IL-6, IL-10, IFN-γ, IL-2 and TNF-α in control group ($n = 98$) and in CHC patients ($n = 132$) Fig. 1A). Box plots representing the plasma levels of (pg/ml) of IL-6, IL-10 and IFN-γ in a subgroup of patients ($n = 95$) with CHC and controls ($n = 97$) aged 60 years or younger (Fig. 1B). The upper and lower limits of the boxes represent 75th and 25th percentiles, respectively; the horizontal bar across the box indicates the median and the end of the vertical lines indicates the minimum and maximum data values.

Table 2 Demographic characteristics and *IL*10 genotype distribution in patients with chronic hepatitis C ($n = 132$) and healthy controls ($n = 98$).

Variables	CHC *n* (%)	Control *n* (%)	*P*
Demographic			
Gender			0.32
Male	60 (45.5)	51 (52.0)	
Female	72 (54.5)	47 (48.0)	
Age (years)[a]	52.0 (45.0—60.3)	35.0 (27.0—43.3)	< 0.0001
*IL*10 genotypes			
−1082[b]			
G/G	22 (16.7)	16 (16.3)	
A/G	54 (40.9)	38 (38.8)	0.93
A/A	56 (42.4)	44 (44.9)	
−819[c] / −592[c]			
TT/AA	19 (14.4)	11 (11.2)	
CC/CC	54 (40.9)	41 (41.8)	0.78
CT/CA	59 (44.7)	46 (47.0)	
*IL*10 haplotypes[d]			
GCC (High producer)	48/98 (49.0)	29/61 (47.5)	0.86
ATA (Low producer)	50/98 (51.0)	32/61 (52.5)	

CHC: chronic hepatitis C; *n*: number of subjects; *IL*10: *interleukin*-10 gene.
[a] Median and interquartile range (IQR), 25th—75th percentile.
[b] Hardy-Weinberg equilibrium *P*-value = 0.22.
[c] Hardy-Weinberg equilibrium *P*-value = 0.34.
[d] Carriers of diplotypes GCC/ATA [cases ($n = 28$) and controls ($n = 25$)] and ACC/ACC [cases ($n = 6$) and controls ($n = 12$)], respectively, were removed from the analysis.

Discussion

Mood disorders have been reported to frequently occur in patients with chronic HCV infection [4—8] and the symptoms of depression have a significant negative impact in the patient's health-related quality of life [4,7,8]. In consonance with the literature, we found a high prevalence of the first MDE in our patients (24.2%). Although there is a sizeable body of scientific evidence linking depression and HCV infection, the biological mechanisms behind the co-occurrence of these conditions have not been completely clarified yet.

To the best of our knowledge, this is the first study to demonstrate that the *IL*10-1082*A, *IL*10-819*T and *IL*10-592*A (ATA) haplotype is associated with the first MDE in patients with CHC. Furthermore, the individuals carrying the *IL*10 GCC high producer haplotype had significantly higher plasma levels of IL-2 and IL-10. The IL-2 is a dual cytokine, showing both a redundant pro-inflammatory property and a non-redundant regulatory immune effect mediated by an enhancement of Treg lymphocytes, thus playing a down regulation by stimulating the secretion of IL-10 [39]. In fact, we also observed a strong correlation between IL-10 and IL-2 levels. Additionally, the functionality of the polymorphisms in our study was reinforced by the fact that among the CHC patients with the first MDE, those carrying the *IL*10 low producer ATA haplotype had significantly higher plasma levels of the pro-inflammatory TNF-α than the non-ATA carriers. Noteworthy and aligned with our findings, Loftis et al. (2008), evaluating CHC patients, demonstrated an association between the severity of depressive symptoms and increased expression of TNF-α [14]. Altogether, these findings point to a complex crosstalk between the immune cells and the central nervous system, involving potential common mediators, such as cytokines and their receptors. Moreover, the role played by HCV on mood disorders should not be disregarded, particularly the interaction orchestrated by the virus in the host's immune response [13,26—31] and in the brain [9—12].

In accordance with other studies [30,31], no significant difference was observed in the distribution of the three SNPs in the promoter region of the *IL*10 gene between the CHC patients and the controls. However, previous investigations have shown associations between the *IL*10 SNPs and clearance of HCV [30] and progression of the liver disease [29,30].

Given the potential relevance of cytokines in mediating depressive manifestations [14—23], there is currently little evidence of an effect of SNPs located in cytokine genes and/or endogenous cytokines on the pathogenesis of HCV-related psychiatric symptoms.

Although the development of direct antiviral agents (DAAs) has been causing immense modifications in the treatment of CHC, with sustained viral response rates that surpass 90.0% in real-life settings [41] these innovations have a high cost [42] and represent an obstacle for many health systems around the world. Nevertheless, Hengst et al. (2016) demonstrated that DAA-induced viral clearance does not completely restore the altered cytokine and chemokine milieu in CHC patients [43]. Furthermore, in a more recent investigation, the authors observed the persistence of neuropsychiatric impairment in HCV patients despite the clearance of the virus [44].

In the present study, we also observed that the first MDE was inversely associated with older age and positively associated with current alcohol misuse. Concerning the influence

Table 3 Demographic, clinical, histological, biochemical and virological data of the patients with chronic hepatitis C with ($n = 32$) and without ($n = 100$) the first major depressive episode.

Variables	First MDE		
	Present *n* (%)	Absent*n* (%)	*P*
Demographic			
Gender			
Male	14 (23.3)	46 (76.7)	0.83
Female	18 (25.0)	54 (75.0)	
Age (years)[a]	46.0 (43.0—52.0)	54.0 (47.5—62.0)	0.001
Psychiatric comorbidities			
Current alcohol misuse	7 (21.9)	6 (6.0)	0.009
Lifetime alcohol misuse	8 (25.0)	26 (26.0)	0.91
Current non-alcohol substance use	1 (3.1)	1 (1.0)	0.43
Lifetime non-alcohol substance use	4 (12.5)	3 (3.0)	0.06
Cirrhosis			
Absent	25 (70.8)	83 (76.9)	0.53
Present (compensated form)	7 (29.2)	17 (23.1)	
Histology[b]			
Grading[c]			
No/Mild activity (A0—A1)	9/15 (60.0)	36/73 (49.3)	0.45
Moderate/Severe activity (A2—A3)	6/15 (40.0)	37/73 (50.7)	
Staging[c]			
No/Mild fibrosis (F0—F1)	10/15 (66.7)	43/73 (58.9)	0.58
Moderate/Severe fibrosis (F2—F4)	5/15 (33.3)	30/73 (41.1)	
Biochemical data[a]			
Albumin (g/dL)	4.2 (3.9—4.4)	4.3 (4.0—4.5)	0.38
ALT(U/L)	56.0 (42.0—92.0)	63.0 (42.0—93.0)	0.73
ALP (U/L)	105.0 (82.6—166.0)	104.0 (79.6—167.8)	0.66
AST (U/L)	53.0 (38.0—106.0)	60.5 (40.3—86.0)	0.60
γ-GT (U/L)	74.0 (34.5—165.5)	75.0 (37.0—137.5)	0.92
Total bilirubin (mg/dL)	0.8 (0.7—1.1)	0.9 (0.5—1.4)	0.58
Prothrombin time	14.4 (13.0—18.0)	14.0 (12.9—15.3)	0.34
Virologicalparameters[d]			
Viral load HCV-RNA [Log10 (IU/mL)][a]	5.8 (5.40—6.31)	5.7 (5.2—6.2)	0.54
Genotype 1	19/25 (76.0)	70/81 (86.4)	0.35

MDE: major depressive episode; *n*: number of subjects; ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; γ-GGT: gamma-glutamyltranspeptidase; HCV: hepatitis C virus.

[a] Median and interquartile range (IQR), 25th—75th percentile.

[b] 88/132 (66.7%) patients with MDE ($n = 15$) and without MDE ($n = 73$).

[c] Staging and grading performed according to the METAVIR classification.

[d] The viral load quantification and the HCV genotyping were available for 106/132 (80.3%) patients.

of age at the onset of MDD, it is known that the prevalence of MDD follows a roughly linear increase through late middle age and a decrease in older adults [45,46]. Regarding alcohol misuse, several investigations have demonstrated the extent of the comorbidity between depression and alcohol use disorders [47—49].

The relationship linking HCV and depression is indubitably complex and multifaceted. In the present study, although an association between the HCV viral load and the first MDE was not observed, higher viremia was more often found in the patients carrying the *IL*10 ATA haplotype than in the non-carriers. Taken together, these results may suggest that the interplay between the host's immune response and the viral load could represent a favourable condition for the HCV's penetration and replication in the brain, where it might induce the release of pro-inflammatory mediators [50]. These assumptions are in line with previous investigations that demonstrated HCV tropism and replication in human cerebral microvascular endothelial cells [10,11]. In addition, increased levels of IL-1α, IL-1β, TNF-α, IL-12 and IL-18 in brain tissue autopsy from HCV-positive patients, compared to HCV-negative patients, have been reported [51].

It has to be emphasized that our results are strengthen by the fact that we studied only patients with chronic hepatitis C and compensated cirrhosis, avoiding potential confounders linked to advanced liver disease, which might interfere in the brain function. In addition, the depressive disorder was diagnosed by a psychiatrist, using a detailed psychiatric approach in combination with a semi-structured interview validated in Portuguese [37] and widely used by Brazilian researchers [52,53].

Table 4 Distribution of *IL*10 genotypes/haplotypes and plasma levels of cytokines in patients with chronic hepatitis C with ($n = 32$) and without ($n = 100$) the first major depressive episode.

Variables	First major depressive episode		
	Present $n = 32$	Absent $n = 100$	*P*
*IL*10 genotypes			
−1082			
GG	4 (12.5)	18 (18.0)	0.02
AG	8 (25.0)	46 (46.0)	
AA	20 (62.5)	36 (36.0)	
−819/−592			
TT/AA	7 (21.9)	12 (12.0)	0.09
CC/CC	8 (25.0)	46 (46.0)	
CT/CA	17 (53.1)	42 (42.0)	
*IL*10 Haplotypes[a]			0.009
GCC (High producer)	7/26 (27.0)	41/72 (57.0)	
ATA (Low producer)	19/26 (73.0)	31/72 (43.0)	
Plasma Cytokine levels (pg/mL)[b]			
IL-6	6.67 (5.70—8.82)	6.66 (5.67—8.64)	0.93
IL-10	4.78 (4.29—5.28)	4.61 (4.23—5.51)	0.57
IFN-γ	2.57 (2.31—2.84)	2.71 (2.46—2.93)	0.09
TNF-α	3.87 (3.55—4.18)	3.92 (3.72—4.18)	0.58

n: number of subjects; *IL*10: *interleukin*-10 gene.
[a] Carriers of GCC/ATA ($n = 28$) and ACC/ACC ($n = 6$) diplotypes were removed from the analysis.
[b] Median and interquartile range (IQR), 25th—75th percentile.

Table 5 Plasma levels of cytokines according to single-nucleotide polymorphisms in the promoter region at positions −1082, −819 and −592 of *IL10* gene in the patients with chronic hepatitis C with ($n = 32$) and without ($n = 100$) the first major depressive episode.

Patients with CHC	Cytokines (pg/mL)[a]	SNPs at *IL*10-1082, *IL*10-819 and *IL*10-592		
		ATA	non-ATA	*P*
Without the first MDE				
	IL-6	6.66 (5.62—9.86)	7.07 (5.76—8.41)	0.85
	IL-10	4.50 (4.03—5.39)	4.72 (4.49—5.79)	0.09
	IFN-γ	2.67 (2.41—2.80)	2.75 (2.46—3.13)	0.22
	TNF-α	3.98 (3.72—4.25)	3.84 (3.67—4.08)	0.21
With the first MDE				
	IL-6	6.64 (5.60—8.88)	6.73 (5.91—7.10)	0.96
	IL-1	4.86 (4.21—5.42)	4.78 (4.33—5.26)	0.85
	IFN-γ	2.60 (2.23—2.82)	2.55 (2.37—2.89)	0.73
	TNF-α	3.98 (3.69—4.24)	3.70 (3.41—3.80)	0.04

CHC: chronic hepatitis C; *IL*10-ATA: *interleukin*-10 gene ATA low producer haplotype; MDE: major depressive episode; IL: interleukin; IFN-γ: interferon gamma; TNF-α: tumour necrosis factor alpha.
[a] Median and interquartile range (IQR), 25th—75th percentile.

Table 6 Variables associated with the first major depressive episode in the patients with chronic hepatitis C.

Variables	Univariate analysis	Multivariate analysis		
	P	OR	95%CI	*P*
Increasing age	0.001	0.94	0.91—0.98	0.006
Current alcohol misuse	0.009	4.29	1.22—15.05	0.02
IFN-γ plasma level (pg/mL)	0.09	0.47	0.14—1.59	0.22
*IL*10-ATA haplotype	0.03	1.50	1.11—2.04	0.009

CI: confidential interval; OR: Odds Ratio; IFN-γ: interferon gamma; *IL*10-ATA: *interleukin*-10 gene ATA low producer haplotype.

The limitations of our study should also be considered. First, the subjects included were recruited from a referral centre and, consequently, may not be representative of all patients with CHC. Second, a smaller number of individuals formed the control group and finally, the cross-sectional nature of the investigation hindered the likelihood to recognize any cause-effect relationship between depression and *IL*10 SNPs in hepatitis C.

Conclusion

In summary, this is the first study to demonstrate that the *IL*10 low producer ATA haplotype is associated with the first MDE in patients with CHC. We also found increased plasma levels of TNF-α associated with the first MDE in the CHC patients. Our results indicate that cytokine dysregulation may have implications in the development of a depressive episode among subjects chronically infected by HCV. These findings reinforce the need for further studies focusing on the biological mechanisms of depression in CHC patients. The challenge remains for forthcoming research to identify potential inflammatory mediators involved in the crosstalk between HCV and the axis brain-liver. Moreover, better comprehension of these processes may positively influence the management strategies for decreasing the extra-hepatic manifestations and their negative impact on health-related quality of life in patients with CHC.

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Disclosure of interest

The authors declare that they have no competing interest.

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