ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-6214

Association of Single Nucleotide Polymorphisms with Infection Susceptibility in Patients with Severe Alcoholic Hepatitis

Adelina Horhat[1,2], Camelia Alexandra Coada[3], Mina Dana Ignat[1,4], Petra Fischer[4], Bogdan Procopet[1,4], Horia Stefanescu[1,4], Zeno Sparchez[1,4]

1) Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania;
2) Institut Mondor de Recherche Biomédicale, MINT-HEP Mondor Integrative Hepatology, Université Paris Est Créteil, Créteil, France;
3) Department of Morpho-functional sciences, University of Medicine and Pharmacy „Iuliu Hațieganu", Cluj-Napoca, Romania;
4) Liver Unit, Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania

Address for correspondence:
Camelia Alexandra Coada
Department of Morphofunctional Sciences,
Iuliu Haţieganu University of Medicine and Pharmacy,
Victor Babeş, No 8, 400012
Cluj-Napoca, Romania
coada_camelia_alexandra@elearn.umfcluj.ro

Received: 19.03.2025
Accepted: 14.05. 2025

ABSTRACT

Background & Aims: Severe alcoholic hepatitis is characterized by an increased risk of infections. Polymorphisms in immune response-related genes may influence susceptibility to infections in alcoholic hepatitis. This study aimed to investigate the association between two clusters of differentiation 14's (*CD14*) single nucleotide polymorphisms (SNPs), rs2569190 and rs5744455, and the occurrence of infections in severe alcoholic hepatitis, the response to corticotherapy and the mortality rates at one and three months.
Methods: Patients with severe alcoholic hepatitis were genotyped for *CD14* - rs2569190 and rs5744455 SNPs. Genotype and allele frequencies were compared between patients who presented infections and those who did not.
Results: A total of 97 patients with biopsy proven sAH were included in the study, out of which 47 (48.4 %) had an associated infection. rs5744455 SNP was significantly associated with the presence of infection. Patients carrying the rs5744455T variant allele had a lower incidence of infections compared to those with the wild-type allele (32% vs 68%; p=0.002). In contrast, the rs2569190 SNP revealed no significant differences, either in the single genotype analysis (p=0.608) or under a dominant model (p=0.318). Community-acquired infections were primarily urinary tract infections (21.65%), followed by pulmonary infections (4.12%), with *Escherichia coli* responsible for 41.67% of cases. Healthcare-associated infections were more varied, including urinary tract (7.22%), respiratory (6.19%), digestive (7.21%), cutaneous (3.09%), and blood infections (5.15%). *Klebsiella pneumoniae* was the most prevalent strain, accounting for 16.67% of these infections.
Conclusions: Our findings highlight a potential protective role of the *CD14* rs5744455T variant allele against infections in sAH, suggesting that genetic variability may influence infection susceptibility in this population.

Key words: alcoholic hepatitis – biomarkers – infections – single nucleotide polymorphisms – cluster of differentiation 14.

Abbreviations: AH: alcoholic hepatitis; AHHS: alcoholic hepatitis histological score; AST: aspartate transaminase; ArLD: alcohol related liver disease; ALT: alanine transaminase; CD: cluster of differentiation; CRP: C-reactive protein; DNA: deoxyribonucleic acid; EDTA: ethylenediaminetetraacetic acid; HVPG: hepatic venous pressure gradient; IL: interleukin; INR: international normalized ratio; IQR: interquartile range; LPS: lipopolysaccharides; MELD: model for end stage liver disease; NIAAA: National Institute on Alcohol Abuse and Alcoholism; PAMPS: pathogen-associated molecular pattern; sAH: severe alcoholic hepatitis; sAHi: infected sAH; sAHn: non-infected sAH; SNP: single nucleotide polymorphism; TLR4: Toll-like receptor 4; TNF α: tumor necrosis alpha.

INTRODUCTION

Severe alcoholic hepatitis (sAH) is marked by a high short-term mortality rate of 20–50% within 28 days [1, 2], partly due to the high incidence of infections [3]. The heightened susceptibility to infections is associated with immune system dysfunction, characterized by systemic inflammation and a reduced immune cell response to pathogens and their products [4, 5].

Immune dysfunction in monocytes in alcoholic hepatitis (AH) is characterized by impaired cytokine production, decreased polyfunctionality, transcriptional and epigenetic alterations leading to downregulation of critical innate immune and metabolic pathways [6].

The interaction between cluster of differentiation (CD) markers and interleukins (ILs) is crucial for immune regulation and inflammation. Elevated levels of pro-inflammatory ILs can exacerbate liver injury by promoting immune cell activation, which is reflected in the expression of CD markers, such as *CD14*, indicating a heightened inflammatory response in AH [7]. Toll-like receptor 4 (TLR4) is an essential pattern recognition receptor on immune cells and hepatocytes, recognizing pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), a component of Gram-negative bacterial membranes [8]. *CD14*, a co-receptor, enhances TLR4's ability to bind LPS, initiating an inflammatory response [9]. LPS binding activates promotes the expression of pro-inflammatory cytokines, particularly tumor necrosis factor alpha (TNFα). TNFα plays a key role in liver inflammation, hepatocyte apoptosis, and liver injury in AH [10, 11].

The *CD14* has a key role in facilitating LPS recognition and amplifying TLR4-mediated proinflammatory signaling hence contributing to altered innate immune sensitivity in the setting of alcohol-induced gut permeability and systemic inflammation. Genetic variants in the *CD14* gene, such as rs2569190 and rs5744455, have been associated with differential gene expression and circulating levels of soluble *CD14* (s*CD14*) thereby intensifying the inflammatory response or impairing resolution mechanisms, ultimately predisposing individuals to systemic infections. The pathogenic role of the *CD14* rs5744455 SNP in severe AH (sAH) likely stems from its influence on innate immune sensing and the host's inflammatory response to microbial products.

The innate immune receptor *CD14* plays a central role in mounting an effective immune response against pathogens. Genetic variants in the *CD14* gene, such as rs2569190 and rs5744455, have been associated with differential gene expression and circulating levels of s*CD14*, both of which influence susceptibility to infections [12].

Genetic variants in the *CD14* gene, such as rs2569190 and rs5744455 were selected for investigation based on their functional relevance in immune regulation and previous associations with infectious outcomes. In the context of alcoholic hepatitis variability in *CD14*-mediated immune responses may significantly modulate infection risk. Therefore, rs2569190 and rs5744455 represent biologically plausible genetic markers for assessing infection susceptibility in patients with AH.

The primary objective of this study was to evaluate the relationship between the *CD14* gene polymorphisms in patients with sAH and the risk of developing an infection. The secondary objectives were to evaluate the association between *CD14* polymorphisms and the response to corticosteroid therapy as well as the mortality rates at one, three and six months.

METHODS

Study Design and Patients' Selection

This was a prospective, observational study approved by the local Ethics Committee and conducted according to the Declaration of Helsinki and following the STARD criteria. Written informed consent was obtained from all the participants at the time of inclusion.

Consecutive patients with biopsy-proven sAH admitted to the Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania, between 2015 and 2017 were included. The NIAAA's criteria for sAH were used to screen patients: onset of jaundice within 60 days of heavy alcohol consumption (>40 or >60 g/day (females respectively males), total bilirubin > 3 mg/dL, aspartate aminotransferase (AST) up to 400 U/L, AST/alanine aminotransferase (ALT) ratio > 1.5 [13].

Liver biopsy was performed using the transjugular route and at the same time, hepatic venous pressure gradient (HVPG) was calculated as mentioned elsewhere [14].

Biopsies were formalin fixed in paraffin embedded with two histological sections with Hematoxylin & Eosin and Trichrome Masson and the histological features of AH were recorded: hepatocellular inflammation, polymorphonuclear infiltrate, steatosis, ballooning, bilirubinostasis, megamitochondria, and fibrosis. Alcoholic hepatitis histological score (AHHS) for prognostic stratification was calculated [15].

At inclusion, all patients had a complete laboratory work up as part of the standard care and were screened for bacterial infections by serum C-reactive protein (CRP), thoracic X-Ray, blood, and urine cultures.

Patients with sAH, defined as the Maddrey's discriminant function > 32 and/or model for end stage liver disease (MELD) > 21, and histological confirmation of sAH were treated with 40 mg of prednisone. A Lille score on day 7 was used to assess futility of corticotherapy [16]. If the patients had a favorable response (Lille Score 0.45), the prednisone was continued for 28 days.

Exclusion criteria were uncontrolled malignancy, human immunodeficiency virus infection, pregnancy, or age <18years.

Based on the presence of infection, patients in each group were divided into two subgroups: infected/non-infected sAH(sAHi/sAHn).

Patients were followed up at one and three months, and further standard bi-annual routine visits until liver transplantation or death.

Samples Collection and Analysis

Blood samples were collected from patients on EDTA vacutainers and stored at 4°C until further processing. The samples were processed at the Research Center for Functional Genomics, Biomedicine and Translational Medicine, Cluj-Napoca, Romania. Genomic DNA was extracted from whole blood using a commercially available kit from Qiagen (QIAamp DNA Blood Mini Kit) following manufacturer's instructions. Genotyping of the *CD14* rs5744455 SNP (also known as -651C>T) was done using a tetra primer ARMS-PCR, while the *CD14* rs2569190 SNP (also known as C260T) was genotyped using a PCR-RFLP protocol with *HaeIII* restriction enzyme digestion as previously described by Mertens et al. [17].

For routine serum tests and ELISA determinations we utilized Konelab 30i/60i device (Thermo Electron Corp, Finland).

Statistical Analysis

Statistical analysis was done using R version 4.3.2 (2023-10-31 ucrt)[18]. Continuous variables were reported with median and interquartile range (IQR) while categorical variables were reported with absolute numbers and frequencies. Significance between groups was tested using the Student t-test, Mann-Whitney test, Chi-Square or Fisher test as appropriate. Median follow-up time of the patient groups was calculated with the reverse Kaplan Meier method using the *prodlim* R package. Overall survival was calculated as the time between patient admission to the date of death or last follow-up. Statistical significance was tested using the log-rank test. Patients lost to follow-up or without the event at last follow-up were considered censored data. A p-value of less than 0.05 was considered significant.

RESULTS

Characteristics of the Study Groups

A total of 97 patients with biopsy proven sAH were included in the study, out of which 47 (48.4%) had an associated infection. Clinical and biological parameters are listed in Table I. Different severity scores and the response to corticotherapy are shown in Table II.

Regarding survival, the one- and three-month survival rate was significantly lower in sAHi (68% respectively 57%) compared to sAHn group (98% respectively 92%) (p<0.001). In the sAHi group, 72.3% had community acquired infections, and 61.7% healthcare associated infections. A detailed presentation of the infections is reported in Table III.

In the case of community-acquired infections, urinary tract infections (21.6 %) were the most common, followed by pulmonary infections (4.1 %). *Escherichia coli (E. coli)* was responsible for 41.6 % of cases.

In contrast, healthcare-associated infections showed a more diverse set of infection locations and bacterial strains. These included urinary tract (7.2 %), respiratory (6.2 %), digestive (7.2 %), skin (3 %), and blood cultures (5.1 %). *Klebsiella pneumoniae* was the most common strain, responsible for 16.6 % of infections. Other notable strains included Candida albicans (11.1 %), *Pseudomonas aeruginosa* (11.1 %), and *Staphylococcus epidermidis* (11.1 %).

With respect to the histological characteristics of sAH there are no statistically significant differences between sAHi/sAHn groups in terms of most histological features (fibrosis, bilirubinostasis, megamitochondria, steatosis, inflammation, and PMN infiltrate) (Table IV). The ballooning feature is almost universally present in both groups, with no difference in prevalence. Although no statistically significant differences were observed between the sAHi/sAHn groups, there was a noticeable trend of higher prevalence of panlobular fibrosis and canalicular/ductular bilirubinostasis in the infected group.

Association of *CD14* SNPs with the Presence of Infections in sAH

We examined the association of the two *CD14* SNPs with the occurrence of infections in patients with sAH. Analysis of the *CD14* rs5744455 SNP demonstrated a significant association in the single genotype analysis (p=0.008). This association was also seen when considering the carrier status of the variant allele and applying a dominant model. In detail, patients who were carriers of the rs5744455T variant allele presented less infections than those with the wild type of variant (32% vs 68%; p=0.002) (Table V).

In contrast, the analysis of the *CD14* rs2569190 SNP revealed no significant differences in allele frequency between the sAHi and sAHn groups, either in the single genotype analysis or under a dominant model (p=0.608 and p=0.318, respectively) (Table V).

Table I. Principal characteristics of the patients

Variable	Non-infected sAH N=50	Infected sAH N=47	p
Male, N (%)	43 (86)	36 (76.6)	0.234
Age (years), median (IQR)	52 (44; 60.75)	55 (46; 60.5)	0.825
Esophageal variceal bleed, N (%)	8 (17.02)	7 (15.56)	0.849
Ascites, N (%)	25 (51.02)	39 (84.78)	<0.001
Acute kidney injury, N (%)	2 (4.26)	5 (11.11)	0.215
Hepatic encephalopathy, N (%)	6 (12.77)	13 (28.89)	0.056
Community acquired infections, N (%)	NA	34 (72.34)	-
Healthcare-associated Infections, N (%)	NA	29 (61.7)	-
Total bilirubin (mg/dL), median (IQR)	5.2 (3.45;12.55)	7.7 (4.15; 16.2)	0.997
Serum Creatinine (mg/dL), median (IQR)	0.66 (0.55;0.81)	0.74 (0.63; 0.82)	0.334
Serum Albumin (g/dL), median (IQR)	3.05 (2.7;3.5)	2.75 (2.33; 3.1)	0.016
Prothrombin time (seconds), median (IQR)	25.45 (20.25; 32.62)	28.1 (24.55; 33.95)	0.019
INR, median (IQR)	1.67 (1.36; 2.14)	1.89 (1.64; 2.26)	0.015
Leucocytes (*10^3/uL), median (IQR)	7.87 (6.31; 11.63)	9.96 (7.02; 12.4)	0.323
Platelets (*10^3/uL), median (IQR)	114.5 (75.25; 179.25)	111 (83; 178.5)	0.498
TNF alpha (pg/mL), median (IQR)	51.9 (33.4; 95.1)	53.35 (36.9; 83.67)	0.539
hTLR4 (ng/mL), median (IQR)	0.07 (0.06; 0.4)	0.08 (0.07; 0.08)	0.843
hLPS (ng/mL), median (IQR)	14484.6 (5561.7; 22242.95)	17929.2 (12666.7; 30057.7)	0.628

sAH: severe alcoholic hepatitis; INR: international normalized ratio; TNF alpha: tumor necrosis alpha; hTLR4: human toll like receptor 4; hLPS: human lipopolysaccharide.

Table II. Liver severity scores and survival of the population

Variable		Non-infected sAH N=50	Infected sAH N=47	p
CHILD-PUGH Score, N (%)	A	6 (12.77)	1 (2.33)	0.019
	B	15 (31.91)	11 (25.58)	
	C	15 (31.91)	28 (65.12)	
MELD, median (IQR)		20 (17; 25)	22 (20; 27.5)	0.040
Maddrey score, median (IQR)		52 (25; 85.7)	69.5 (51.52; 96.92)	0.017
Lille score, median (IQR)		0.13 (0.09; 0.72)	0.32 (0.12; 0.54)	0.943
AHHS, median (IQR)		7.5 (7; 8)	8 (7.75; 8)	0.114
HVPG, median (IQR)		22 (18.5; 25)	20 (17; 23)	0.543
Response to corticotherapy, N (%)		15 (68.18)	22 (68.75)	0.965
One month survival rate, (%)		98	68	<0.001
Three-month survival rate, (%)		92	57	
Follow-up (months), median (IQR)		54.43 (42.40; 61.23)	60.83 (52.83; 69.10)	

AHHS: alcoholic hepatitis histologic score; HVPG: hepatic venous pressure gradient; MELD: model for end stage liver disease. For the rest of abbreviations see Table I.

Table III. Infection types and the etiological agents in patients with severe alcoholic hepatitis

Infection type		N (%)
Community acquired Infection		
Location	Urinary	21 (21.65)
	Pulmonary	4 (4.12)
	Blood cultures	1 (1.03)
	Skin	1 (1.03)
	Spontaneous Bacterial Peritonitis	1 (1.03)
Bacterial strain	*Escherichia coli*	5 (41.67)
	Enterococcus faecium	3 (25)
	Enterobacter cloacae	1 (8.33)
	Enterococcus faecalis	1 (8.33)
	Klebsiella pneumonie	1 (8.33)
	Proteus Mirabilis	1 (8.33)
Healthcare-associated Infection		
Location	Urinary	7 (7.22)
	Pulmonary	6 (6.19)
	Blood cultures	5 (5.15)
	Spontaneous Bacterial Peritonitis	1 (1.03)
	Digestive	7 (7.21)
	Skin	3 (3.09)
	Multiple	1 (1.03)
Bacterial strain	Candida albicans	2 (11.11)
	Candida krusei	1 (5.56)
	Clostridoides difficile	1 (5.56)
	Escherichia coli	1 (5.56)
	Enterococcus faecium	2 (11.11)
	Enterococcus faecalis	1 (5.56)
	Klebsiella pneumoniae	3 (16.67)
	Moraxella catarrhalis	1 (5.56)
	Pseudomonas aeruginosa	2 (11.11)
	Staphylococcus epidermidis	2 (11.11)

We then questioned whether the presence of these *CD14* genetic variants might influence the response to prednisone treatment. The analysis showed that none of the SNPs were significantly associated with the responses to prednisone therapy in these patients (Table VI).

SNPs Impact on Patient Mortality

Given the significant association with the patient's infection status, we next investigated whether the carrier status of the *CD14* rs5744455T SNP could also impact the survival outcomes of these patients. Thus, we performed a Kaplan-Meier analysis. The results indicated a trend towards an improved survival for carriers of the *CD14* rs5744455T variant. At one month, survival was 86% among T allele carriers, compared to 77% in patients with the CC genotype. At three months, survival rates declined for both groups. However, T allele carriers continued to show higher survival (77%) than those with the CC genotype (68%). This result, however, did not achieve statistical significance (p=0.340, log-rank test) (Fig. 1).

DISCUSSION

To the best of our knowledge this is the first study to investigate the association between two SNPs of the *CD14* gene (rs2569190 and rs5744455) and the occurrence of infections in patients with sAH. The results revealed different associations for each SNP, suggesting that the genetic variation in *CD14* might influence infection susceptibility in this population, but in a genotype-specific manner. The *CD14* rs5744455 SNP demonstrated a significant association with infection susceptibility in sAH. The single genotype analysis revealed a p-value of 0.008, indicating a clear difference between the groups, result further supported by the dominant model analysis (p=0.002).

Specifically, patients who were carriers of the rs5744455T variant allele had a lower frequency of infections compared to those with the wild-type variant. This suggests that the rs5744455T allele might have a protective effect against infections in sAH.

Table IV. Histological characteristics of the study groups

Variable		Non-infected sAH N=50	Infected sAH N=47	p-value
Fibrosis	Absent	2 (7.14)	1 (3.12)	0.39
	Zone 2/Zone 3	18 (64.29)	12 (37.5)	
	Panlobular	8 (28.57)	19 (59.38)	
Bilirubinostasis	No	7 (25)	4 (12.5)	0.324
	Hepatocellular	0 (0)	2 (6.25)	
	Canalicular or ductular	12 (42.86)	18 (56.25)	
	Canalicular or ductular plus hepatocellular	9 (32.14)	8 (25)	
Inflammation	Absent	1 (3.57)	1 (3.12)	0.262
	Mild	20 (71.43)	28 (87.5)	
	Severe	7 (25)	3 (9.38)	
PMN Infiltrate	No	1 (3.57)	1 (3.12)	0.069
	Mild	20 (71.43)	29 (90.62)	
	Severe	7 (25)	2 (6.25)	
Ballooning	Yes	26 (92.86)	30 (93.75)	1
Steatosis	Absent	1 (3.57)	3 (9.68)	0.575
	< 33%	8 (28.57)	8 (25.81)	
	33-66%	11 (39.29)	8 (25.81)	
	> 66%	8 (28.57)	12 (38.71)	
Megamitochondria	Yes	2 (7.14)	1 (3.12)	0.594

PMN: polymorphonuclear leucocytes.

Regarding the *CD14* rs2569190 SNP, there was no significant association with infection. The lack of association in our study may be attributable to various factors such as sample size, population heterogeneity, or other confounding genetic and environmental variables not accounted for in the analysis. Additionally, the functional impact of the rs2569190 SNP remains unclear, as its role in the regulation of *CD14* expression and subsequent immune response has yet to be fully elucidated.

There is an urgent need of finding biomarkers for prognosis and treatment response. The dynamic change in bilirubin over the first days of admission has been identified as an important prognostic indicator and a marker of response to corticosteroid treatment [19, 20]. On the contrary, older patients with hepatic encephalopathy, renal failure have a worse prognosis [21]. Also, the high incidence of infectious complications represents a critical determinant of poor clinical outcomes in patients with severe alcoholic hepatitis, contributing substantially to increased morbidity and mortality [22]. We evaluated whether the investigated SNPs were also associated with the susceptibility of developing infections. This study identifies a significant association between the rs5744455 SNP in the *CD14* gene and the susceptibility to infections in patients with sAH.

One study found that the -159 C>T promoter SNP led to increased levels of both soluble and membrane bound *CD14* which conferred a greater risk of alcoholic liver injury [23]. Similarly, it was shown that rs2569190T allele is associated with an increased risk of developing alcohol related liver disease (ArLD), further supporting the role of *CD14* genetic variants in ArLD [24]a process that is regulated by the *CD14* endotoxin receptor. Patients with ArLD had elevated levels of pro-inflammatory cytokines (LPS binding protein, TLR4, TNFα, IL1β, IFNγ, IL6) and lower levels of anti-inflammatory cytokines (IL10, IL4) compared to controls. The TT genotype in ArLD patients and controls showed higher pro-inflammatory and lower anti-inflammatory cytokines. Promoter activity in the region flanking the SNP suggests that the risk genotype influences *CD14* gene expression [24].

It is noteworthy that not all patients in our cohort had cirrhosis, which provides a unique opportunity to examine the influence of *CD14* polymorphisms across different stages of liver disease. While cirrhosis-associated immune dysfunction could modify the impact of genetic variations, our findings suggest that the SNP's effect on infection risk is likely independent of cirrhosis status, although the majority of our cohort was cirrhotic. In contrast, the advanced immune impairment observed in cirrhotic individuals may blunt or mask the protective effects of the T allele at rs5744455. This highlights the importance of considering the interaction between cirrhosis status and genetic variants when evaluating infection risk in sAH patients.

The rs5744455 SNP is located in the promoter region of the *CD14* gene and has been shown in previous studies to influence the expression of *CD14* [17].

As we clearly showed that there was a clear association between *CD14* rs5744455 SNP and infection susceptibility we went further and analyzed the *CD14* SNPs with the responses to prednisone therapy in these patients. This lack of association may be attributed to the small sample size of the cohort, which could limit the statistical power to detect potential relationships between the SNPs and corticotherapy outcomes.

Table V. Genotype distribution between the two groups of patients

SNP	Genotype	Non-infected sAH N=50	Infected sAH N=47	p-value
Overall				
CD14 rs2569190	TT	11 (22.92)	7 (14.89)	0.608
	CT	23 (47.92)	25 (53.19)	
	CC	14 (29.17)	15 (31.91)	
CD14 rs2569190	TT	11 (22.92)	7 (14.89)	0.318
	CT+CC	37 (77.08)	40 (85.11)	
CD14 rs5744455	CC	11 (31.43)	25 (67.57)	0.008
	CT	16 (45.71)	9 (24.32)	
	TT	8 (22.86)	3 (8.11)	
CD14 rs5744455	CC	11 (31.43)	25 (67.57)	0.002
	CT+TT	24 (68.57)	12 (32.43)	
At admission				
CD14 rs2569190	TT	12 (19.67)	6 (17.65)	0.729
	CT	29 (47.54)	19 (55.88)	
	CC	20 (32.79)	9 (26.47)	
CD14 rs2569190	TT	12 (19.67)	6 (17.65)	0.809
	CT+CC	49 (80.33)	28 (82.35)	
CD14 rs5744455	CC	19 (40.43)	17(68)	0.095
	CT	19 (40.43)	6 (24)	
	TT	9 (19.15)	2 (8)	
CD14 rs5744455	CC	19 (40.43)	17 (68)	0.026
	CT+TT	28 (59.57)	8 (32)	
During hospitalization				
CD14 rs2569190	TT	13 (19.7)	5 (17.24)	0.961
	CT	33 (50)	15 (51.72)	
	CC	20 (30.3)	9 (31.03)	
CD14 rs2569190	TT	13 (19.7)	5(17.24)	0.779
	CT+CC	53 (80.3)	24(82.76)	
CD14 rs5744455	CC	18 (38.3)	18 (72)	0.024
	CT	20 (42.55)	5 (20)	
	TT	9 (19.15)	2 (8)	
CD14 rs5744455	CC	18 (38.3)	18 (72)	0.006
	CT+TT	29 (61.7)	7 (28)	

CD14: cluster of differentiation 14; sAH: severe alcoholic hepatitis.

Table VI. Genotype distribution and the response to corticotherapy

SNP	Genotype	Prednisone response		
		Non responders	Responders	p-value
CD14 rs2569190	TT	3 (18.75)	7 (18.92)	1
	CT	8 (50)	19 (51.35)	
	CC	5 (31.25)	11 (29.73)	
CD14 rs2569190C carrier	TT	3 (18.75)	7 (18.92)	1
	CT+CC	13 (81.25)	30 (81.08)	
CD14 rs5744455	CC	6 (50)	14 (48.28)	1
	CT	5 (41.67)	12 (41.38)	
	TT	1 (8.33)	3 (10.34)	
CD14 rs5744455T carrier	CC	6 (50)	14 (48.28)	0.920
	CT+TT	6 (50)	15 (51.72)	

For abbreviations see Table V.

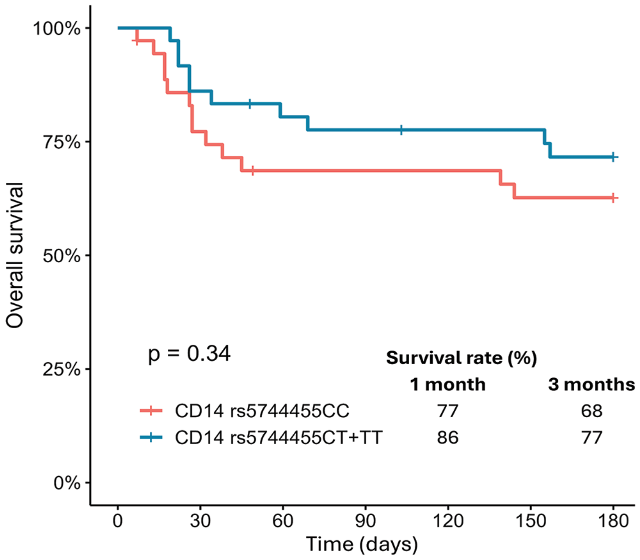

Fig. 1.

The observed trend toward improved survival for carriers of the *CD14* rs5744455T allele, while not statistically significant, suggests that this genetic variant may have a protective effect on survival in sAH patients. This finding aligns with the fact that the rs5744455T allele was associated with a lower frequency of infections in this cohort. Since infections are a major cause of morbidity and mortality in sAH, it is plausible that the reduced infection risk in rs5744455T carriers might contribute to better survival outcomes. However, the absence of statistical significance in the survival analysis (p=0.34) means that this observation should be viewed with caution.

The findings of this study suggest that genetic variation in the *CD14* gene, particularly at rs2569190 and rs5744455, may contribute to inter-individual differences in susceptibility to infections in patients with AH. Both SNPs are located in regulatory regions that influence *CD14* expression and s*CD14* levels, which are key modulators of the innate immune response. Specifically, the rs5744455 SNP in the promoter region may enhance *CD14* transcription, potentially leading to a heightened early immune response that facilitates pathogen clearance. Conversely, certain genotypes at rs2569190, located in the 5' untranslated region, have been associated with reduced *CD14* expression, potentially impairing microbial recognition and increasing the risk of systemic infection.

In AH, where gut barrier dysfunction and microbial translocation are hallmark features, these SNPs may play a pathogenic role by altering host capacity to detect and respond to translocated bacterial products. An insufficient *CD14*-mediated response could permit systemic spread of pathogens or their components, fueling chronic inflammation or secondary infections, both of which are critical determinants of prognosis in AH. On the other hand, excessive *CD14* activity may contribute to immune hyperactivation and tissue damage, suggesting that the functional consequences of these variants may be context dependent.

These observations underscore the potential of *CD14* polymorphisms as biomarkers of infection risk in AH and may inform personalized therapeutic strategies, such as targeted modulation of innate immune pathways.

While our study focused primarily on severe alcoholic hepatitis, it is important to consider whether the *CD14* SNPs (rs2569190 and rs5744455) also influence infection susceptibility in mild or moderate forms of AH. Given the role of *CD14* in immune recognition and infection response, it is plausible that these genetic variants could similarly affect the immune response in patients with less severe liver disease.

In mild and moderate AH, the immune system may still retain a certain degree of functionality, as liver dysfunction and inflammation are less advanced compared to severe AH or cirrhosis. Therefore, genetic factors such as the *CD14* SNPs could have a more prominent role in regulating immune responses to infections, especially in the absence of extensive immune suppression typically seen in more advanced stages of liver disease.

While the protective effect of the rs5744455T allele is interesting, further functional studies are needed to confirm how this variant influence immune function at a mechanistic level. Additionally, the clinical relevance and the potential for this variant to serve as a biomarker for infection risk in sAH patients' needs to be explored further.

Moreover, the findings of this study, although promising, are based on a relatively small cohort of patients, which limits the generalizability of the results. A larger sample size would increase the statistical power of the study and could help better define the role of these SNPs. Moreover, additional genetic factors that may interact with *CD14* variants, as well as other environmental factors should be explored in future studies to provide a more comprehensive understanding of the genetic underpinnings of infection risk in sAH.

CONCLUSIONS

The *CD14* rs2569190 SNP was not associated with infection occurrence in sAH patients, while the rs5744455 SNP showed a significant protective effect, with carriers of the T allele having a lower frequency of infections. This suggests that genetic variation in the *CD14* gene may influence the immune response and infection susceptibility in sAH patients. Future studies with larger cohorts and functional analyses are needed to confirm these findings and explore the potential clinical applications of these SNPs in predicting infection risk in severe alcoholic hepatitis.

Conflicts of interest: None to declare.

Authors' contributions: A.H., C.A.C. and P.F. conceived and designed the study. A.H., C.A.C., P.F., M.D.I. collected the data. C.A.C performed the analysis. H.S. and C.A.C provided the resources. A.H. and C.A.C drafted the manuscript. P.F. and M.D.I. revised the manuscript. H.S., Z.S. and B.P. supervised the study and critically revised the manuscript.

Acknowledgements: This study was financially supported by a grant from the Romanian Ministry of Education and Research (UEFISCDI): PN-II-RU-TE-2014-4. CAC was supported by a biennial AIRC fellowship for Italy code:23969. The authors would like to thank Dr.

Andreea Benea, and Dr. Stanca Pandrea for their professional support in serum sample preparation.

REFERENCES

1. Maddrey WC, Boitnott JK, Bedine MS, Weber FL Jr, Mezey E, White RI Jr. Corticosteroid therapy of alcoholic hepatitis. Gastroenterology 1978;75:193-199.
2. Bataller R, Arab JP, Shah VH. Alcohol-Associated Hepatitis. N Engl J Med 2022;387:2436-2448. doi:10.1056/NEJMra2207599
3. Louvet A, Wartel F, Castel H, et al. Infection in patients with severe alcoholic hepatitis treated with steroids: early response to therapy is the key factor. Gastroenterology 2009;137:541-548. doi:10.1053/j.gastro.2009.04.062
4. Clària J, Stauber RE, Coenraad MJ, et al. Systemic Inflammation in Decompensated Cirrhosis: Characterization and Role in Acute-on-chronic Liver Failure. Hepatology 2016;64:1249–1264. doi:10.1002/hep.28740
5. Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-Associated Immune Dysfunction: Distinctive Features and Clinical Relevance. J Hepatol 2014;61:1385–1396. doi:10.1016/j.jhep.2014.08.010
6. Weichselbaum L, Azouz A, Smolen KK, et al. Epigenetic Basis for monocyte dysfunction in patients with severe alcoholic hepatitis. J Hepatol 2020;73:303-314. doi:10.1016/j.jhep.2020.02.017
7. Kim A, Bellar A, McMullen MR, Li X, Nagy LE. Functionally Diverse Inflammatory Responses in Peripheral and Liver Monocytes in Alcohol-Associated Hepatitis. Hepatol Commun 2020;4:1459–1476. doi:10.1002/hep4.1563
8. Kawai T, Akira S. The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-like Receptors. Nat Immunol 2010;11:373–384. doi:10.1038/ni.1863
9. Sun J, Zhang J, Wang X, Ji F, Ronco C, Tian J, Yin Y. Gut-Liver Crosstalk in Sepsis-Induced Liver Injury. Crit Care 2020;24:614. doi:10.1186/s13054-020-03327-1
10. McClain CJ, Cohen DA. Increased Tumor Necrosis Factor Production by Monocytes in Alcoholic Hepatitis. Hepatology 1989;9:349-351. doi:10.1002/hep.1840090302
11. Aggarwal BB. Signalling Pathways of the TNF Superfamily: A Double-Edged Sword. *Nat.* Rev Immunol 2003;3:745–756. doi:10.1038/nri1184
12. Jiménez-Sousa MÁ, Liu P, Medrano LM, et al. Association of CD14 Rs2569190 Polymorphism with Mortality in Shock Septic Patients Who Underwent Major Cardiac or Abdominal Surgery: A Retrospective Study. Sci Rep 2018;8:2698. doi:10.1038/s41598-018-20766-7
13. Crabb DW, Bataller R, Chalasani NP, et al. Standard Definitions and Common Data Elements for Clinical Trials in Patients with Alcoholic Hepatitis: Recommendation from the NIAAA Alcoholic Hepatitis Consortia. Gastroenterology 2016;150:785–790. doi:10.1053/j.gastro.2016.02.042
14. Bosch J, Abraldes JG, Berzigotti A, García-Pagan JC. The Clinical Use of HVPG Measurements in Chronic Liver Disease. Nat Rev Gastroenterol Hepatol 2009;6:573–582. doi:10.1038/nrgastro.2009.149
15. Altamirano J, Miquel R, Katoonizadeh A, et al. A Histologic Scoring System for Prognosis of Patients with Alcoholic Hepatitis. Gastroenterology 2014111;146:1231-1239.e1-e6. doi:10.1053/j.gastro.2014.01.018
16. Louvet A, Naveau S, Abdelnour M, et al. The Lille Model: A New Tool for Therapeutic Strategy in Patients with Severe Alcoholic Hepatitis Treated with Steroids. Hepatology 2007;45:1348–1354. doi:10.1002/hep.21607
17. Mertens J, Bregadze R, Mansur A, et al. Functional Impact of Endotoxin Receptor CD14 Polymorphisms on Transcriptional Activity. J Mol Med 2009;87:815–824. doi:10.1007/s00109-009-0479-7
18. R Core Team (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.r-project.org
19. Lee M, Kim W, Cho Y, et al. Spontaneous Evolution in Bilirubin Levels Predicts Liver-Related Mortality in Patients with Alcoholic Hepatitis. PLoS ONE 2014;9:e100870. doi:10.1371/journal.pone.0100870
20. Parker R, Cabezas J, Altamirano J, et al. Trajectory of Serum Bilirubin Predicts Spontaneous Recovery in a Real-World Cohort of Patients With Alcoholic Hepatitis. Clin Gastroenterol Hepatol 2022;20:e289–e297. doi:10.1016/j.cgh.2021.01.042
21. Mathurin P, Duchatelle V, Ramond MJ, et al. Survival and Prognostic Factors in Patients with Severe Alcoholic Hepatitis Treated with Prednisolone. Gastroenterology 1996;110:1847–1853. doi:10.1053/gast.1996.v110.pm8964410
22. Karakike E. Infections in Severe Alcoholic Hepatitis. Ann Gastroenterol 2017;30:152-160. doi:10.20524/aog.2016.0101
23. Campos J, Gonzalez-Quintela A, Quinteiro C, et al. The −159 *C/T* Polymorphism in the Promoter Region of the *CD14* Gene Is Associated with Advanced Liver Disease and Higher Serum Levels of Ace-Phase Proteins in Heavy Drinkers. Alcohol Clin Exp Res 2005;29:1206–1213. doi:10.1097/01.ALC.0000171977.25531.7A
24. Järveläinen HA, Orpana A, Perola M, Savolainen VT, Karhunn PJ, Lindros KO. Promoter Polymorphism of the Cd14 Endotoxin Receptor Gene As A Risk Factor for Alcoholic Liver Disease. Hepatology 2001;33:1148–1153. doi:10.1053/jhep.2001.24236