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ORIGINAL ARTICLE

Predictive Value of Interleukin 6 and Interleukin 8 in Response to Treatment of Hepatitis C Virus

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SUMMARY

Background: **Chronic hepatitis C (CHC) infection is a major public health problem in many low- and middle-income countries. The study aimed to find out how interleukin IL-6 and IL-8 levels in the blood affect the virological response to direct-acting antivirals (DAAs) and to find useful clinical or immunological markers for the response to HCV treatment.**
Methods: **CHC patients from a real Egyptian population (n = 4,300), who were treated during the Egyptian national initiative to eliminate HCV at the Sherbin Central Hospital, Dakahlia Governorate, Ministry of Health, Egypt, were enrolled in our study. They were all patients who did not obtain a sustained virological response (SVR) (n = 75; non-responder; the response rate was 98.26%), and a total of 100 patients were randomly selected from patients who obtained SVR (responder) and were age- and gender-matched ($p > 0.05$) with non-responder patients. Serum levels of IL-6 and IL-8 were measured by commercial ELISA kits.**
Results: **Non-responder patients were associated with significantly high levels of ALT, AST, ALP, and total bilirubin. Non-responders had significantly ($p < 0.05$) higher baseline IL-6 (16.7 ± 4.92 pg/mL) and IL-8 (37.81 ± 10.55 pg/mL) levels compared to responders (12.68 ± 2.06, 29.06 ± 5.94 pg/mL, respectively). There was a substantial ($p < 0.05$) association between the combination of two cytokines and a high likelihood of treatment failure, as indicated by all parameters examined, with the highest correlation values seen.**
Conclusions**: The present study showed that increased IL-6 and IL-8 were associated with HCV treatment failure. Also, IL8 was associated with hepatic fibrosis.**
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KEYWORDS

Hepatitis C virus, interleukin 6, interleukin 8, direct-acting antivirals, treatment response

LIST OF ABBREVIATIONS

HCV - Hepatitis C virus
CHC - Chronic Hepatitis C
SVR - Sustained Virologic response
ALT - Alanine transaminase
AST - Aspartate aminotransferase
ALP - Alkaline phosphatase

AFP - Alpha-Fetoprotein
RT-PCR - Reverse transcription - polymerase chain reaction
IL-6 - Interleukin 6
FIB-4 - Fibrosis 4
IL-8 - Interleukin 8
DAAs - Direct-acting antivirals
NCCVH - Egyptian National Committee for Control of Viral Hepatitis
SOF - Sofosbuvir
DCV - Daclatasvir
OD - Optical density
ELISA - Enzyme-linked immunosorbent assay
SPSS - Statistical Package for Social Sciences
ROC - Receiver operating characteristics
AUC - Area under the curve

INTRODUCTION

Worldwide, an estimated 71 million persons were Hepatitis C virus (HCV)-infected. HCV is a major cause of death from infectious disease, and it is still the leading reason for liver transplantations in many areas around the world [1]. Therefore, an early recognition and effective management of the disease can modify its natural history [2]. In many patients, a successful antiviral treatment can prevent the short- and long-term complications of HCV infection [3].

Over the last few years, HCV treatment has rapidly changed with the development of new therapies and other advancements, and the chances of a cure are significantly increasing. Firstly, with the first-generation direct-acting antivirals (DAAs) boceprevir and telaprevir, individuals with HCV genotype 1 achieved sustained virologic response (SVR) rates of nearly 70% or greater, and the viral kinetics and hepatic fibrosis were the main predictors of response. With the more recent generations of pangenotypic antiviral therapies, there have been even higher SVR rates [2]. However, a small percentage of patients experience a relapse post treatment [4].

Little is known about predictors of failure to achieve SVR with DAAs. Although numerous clinical parameters predicted a poor response to pegylated IFN treatment (e.g. age, ethnicity, human immunodeficiency virus coinfection, insulin resistance, and interleukin [IL]-28b genotype), none of them are associated with a virological relapse after DAA based therapy [4,5].

IL-6 is a pivotal cytokine in inflammation-associated liver disease, and the current evidence suggests that it has both pro-inflammation and anti-inflammation effects, where IL-6 released from adiposity can promote inflammation and muscle-derived IL-6 can ameliorate inflammation. Furthermore, chronic exposure to high IL-6 levels can increase hepatic gluconeogenesis and impair lipid metabolism; however, IL-6 has hepatoprotective effects in acute liver injury. Also, a recent study suggested that IL-6 receptor (IL-6R) can reduce NAFLD-associated inflammation [6]. Therefore, the present study aimed to clarify whether IL 8 and IL 6 serum levels influence the virological response to DAAs and to identify useful clinical or immunological predictors for HCV treatment response.

MATERIALS AND METHODS

This real-world prospective study included patients with CHC infection who were recruited from and treated for HCV at the Sherbin Central Hospital, Sherbin, Dakahlia Governorate, Egypt, during Egypt's 100 Million Healthy Lives campaign.

Inclusion criteria were patients aged > 18 years with compensated hepatic disease, who were diagnosed with CHC infection by anti-HCV detection by using enzyme-linked immunosorbent assay and confirmed by real-time PCR. The exclusion criteria were determined according to the Egyptian national treatment program of Hepatitis C treatment protocol [7]. These included inadequately controlled diabetes mellitus, the inability to use effective contraception or pregnancy, extra-hepatic tumors except after 2 years of a disease-free interval, hepatocellular carcinoma except 6 months after intervention and with no evidence of activity by dynamic imaging, patients co-infected with HIV, INR > 1.7, serum bilirubin > 3 mg/dL, serum albumin < 2.8 g/dL, platelet count < 50,000/mm^3, and Child's C cirrhotic patients.

Accordingly, a total of 4,300 patients were scheduled to receive the new DAA therapy for the treatment of HCV infection, as recommended by the Egyptian National Committee for Control of Viral Hepatitis (NCCVH). They were prescribed sofosbuvir (SOF) and daclatasvir (DCV). For each patient, a PCR assay was done three times: 1) before treatment initiation to confirm the HCV diagnosis; 2) at week 12 after the treatment ended to estimate the end of treatment response (ETR); and 3) at week 24 (3 months) after treatment to assess SVR. According to treatment response, a total of 4,225 patients achieved SVR (a response rate of 98.25%), and 75 patients did not achieve SVR (non-responders). All non-responder patients were included in this study (n = 75), in addition to a total of 100 patients randomly selected from patients who obtained SVR (responders) and were age- and gender-matched with non-responder patients.

In fresh serum, laboratory investigations regarding the liver profile (including alanine and aspartate aminotransferase (ALT and AST), total bilirubin, albumin, alkaline phosphatase (ALP), and creatinine) were all performed by using commercial kits and an automated biochemistry analyzer (Response 920, DiaSys Diagnostic Systems, GmbH, Germany). Blood treated with EDTA-K3 was used for a complete blood count on the Sysmex automated analyzer (Sysmex, Japan). Citrated-anticoagulated blood samples were used to detect prothrombin time by using commercial kits (Spectrum, Egypt) and a semi-automatic coagulation analyzer (Thrombosta,

Behnk Elektronik, Germany). Serum AFP was measured by an immunoassay system (Siemens Healthcare Diagnostics, Germany).

RT-PCR for HCV diagnosis involved three main steps: viral RNA extraction, HCV-RNA conversion into complementary DNA (cDNA), and amplification and amplified product detection. According to the manufacturer's instructions, HCV-RNA extraction was performed by using the Artus® HCV RG RT-PCR kit (Qiagen GmbH, Germany). Viral load was determined by using the Rotor-Gene Q MDx (Qiagen, Germany) Light Cycler Real-Time PCR System, using Rotor-Gene 3,000 software version 6.23.

The sample size calculation

MedCalc (Belgium) software was used to calculate the sample size based on the previously reported predictive power of IL-6 (AUC = 0.839) in the prediction of HCV treatment failure [8]. A statistical power (1-β) level of 80% and a significance (α) level of 5% were used. The responders/non-responders' ratio was 1.17 [9], and the calculated total sample size for both groups was 20, so we think the sample size of our study (n = 175) was sufficient to perform statistical analysis.

Study outcomes

The study's primary outcome was the percentage of HCV-infected patients who achieved an SVR within 6 months of the end of the treatment. Secondary outcomes included baseline levels of two cytokines, IL-6 and IL-8, and their association (individually or in combination) with treatment response and/or failure.

Detection of interleukin 6 and 8

Commercial ELISA kits (Bioneovan, Beijing, China) were used based on sandwich-ELISA. Micro ELISA strip plate, provided in this kit, had been pre-coated with an antibody specific to human IL-6 or IL-8, appropriately. Standards or samples were added to the plate wells and combined with the specific antibody. Next, an antibody, that is conjugated to horseradish peroxidase and specific for IL-6 or IL-8, is put into each well. After washing, TMB substrate solution was added to each well. Only those wells that contain IL-6/IL-8 and HRP-conjugated IL-6/IL-8 antibodies will appear blue and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm, that is proportional to the concentration of IL-6 or IL-8. IL-6 or IL-8 levels in the samples were determined by comparing the OD values of samples to the standard curve.

Statistical methods

All statistical analyses were done by using SPSS version 20 and GraphPad Prism version 9.0. Numerical/continuous and categorical variables were presented as mean ± standard deviation (SD) and numbers, respectively. Data were appropriately compared with the Student´s *t*-test or the chi-squared test ($\chi 2$). The diagnostic performance of each cytokine for SVR prediction was expressed as sensitivity, specificity, positive predictive (PPV) and negative predictive values (NPV), and AUC (area under the receiver operating characteristic curve (ROC)). Statistical significance was set at $p < 0.05$.

RESULTS

Patients' baseline characteristics are summarized in Table 1. Patients who obtained SVR (responder) were age- and gender-matched ($p > 0.05$) with non-responder patients. Non-responder patients were associated with significant ($p < 0.05$) high levels of ALT, AST, ALP, and total bilirubin. Conversely, they had significantly ($p < 0.05$) decreased levels of albumin and platelet count. Moreover, non-responder patients were associated with significant ($p < 0.05$) high values of two common fibrosis markers (FIB-4 and APRI).

IL-6 and IL-8 levels in responder and non-responder patients

Non-responder patients were accompanied by a significant increase ($p < 0.05$) in baseline concentration of IL-6 (16.7 ± 4.92 pg/mL) and IL-8 (37.81 ± 10.55 pg/mL), when compared to responder patients (12.68 ± 2.06 and 29.06 ± 5.94 pg/mL, respectively). The ROC analysis revealed that both IL-6 (AUC of 0.835) and IL-8 (AUC of 0.808) have a good ability to separate non-responder patients with good sensitivity and specificity values (Table 2). To improve the accuracy of predicting treatment failure with HCV DAA regimens, we evaluated the combined use of IL-6 and IL-8 in treatment failure prediction. The risk was classified as follows: high-risk: both IL-6 (> 14 pg/mL) and IL-8 (> 31 pg/mL) were positive; moderate-risk: one of IL-6 or IL-8 was positive; low-risk: both IL-6 and IL-10 were negative (Figure 1). After this classification, we found that responder patients were associated with a low risk (69% were negative for both cytokines). At the same time, non-responder patients were associated with a high risk (72% were positive for both cytokines) (Figure 2). The best prediction ability was obtained for high-risk (Figure 3), compared to high and moderate combined (Figure 4**).** Elevated levels of IL-6 and IL-8 were significantly correlated with decreased levels of albumin ($r = -0.204$ ($p = 0.023$) and $r = -0.270$ ($p = 0.003$), respectively) and elevated fibrosis markers, including FIB-4 ($r = 0.22$ ($p = 0.020$) and $r = 0.32$ ($p = 0.001$), respectively] and APRI [$r = 0.23$ ($p = 0.021$) and $r = 0.30$ ($p = 0.012$), respectively] (Table 3). Interestingly, the high-risk, obtained from the combined use of the two cytokines, was significantly ($p < 0.05$) associated with all factors that were significantly associated with treatment failure (Table 4), with the highest correlation coefficients.

Table 1. Baseline patient characteristics.

Variables	Responder	Non-Responder	p-value
Gender (male/female)	40/60	30/45	0.252
Age (years)	49.5 ± 6.9	51.56 ± 7.0	0.069
ALT (U/L)	58.9 ± 15.9	70.1 ± 20.1	0.018
AST (U/L)	55.6 ± 15.1	69 ± 17.1	0.003
ALP (U/L)	96.1 ± 21	124.2 ± 33	0.019
Albumin (g/dL)	3.89 ± 0.56	3.38 ± 0.43	0.0001
Bilirubin (mg/dL)	0.97 ± 0.32	1.12 ± 0.26	0.010
Creatinine (mg/dL)	0.81 ± 0.16	0.87 ± 0.18	0.082
INR	1.14 ± 0.07	1.26 ± 0.14	0.071
α-fetoprotein (ng/mL)	3.53 ± 0.9	3.65 ± 1.1	0.772
Hemoglobin (g/dL)	13.08 ± 1.35	11.89 ± 2.58	0.073
Red blood cells (x 10^{12}/L)	4.99 ± 1.15	5.35 ± 1.86	0.084
White blood cells (x 10^9/L)	5.5 ± 1.36	4.9 ± 1.66	0.064
Platelets (x 10^9/L)	190.2 ± 36	156.7 ± 44	0.0001
FIB-4	1.95 ± 0.55	3.1 ± 1.0	0.0001
APRI	0.79 ± 0.21	1.19 ± 0.33	0.0001

Normally distributed variables were expressed as mean ± SD. Significant differences were determined by using the chi-squared test (χ^2) and Student´s *t*-test, appropriately. $p < 0.05$ was considered significant.
ALT - alanine aminotransferase, AST - aspartate aminotransferase, ALP - alkaline phosphatase, FIB-4 - fibrosis-4 score, APRI - AST-platelet ratio index.

Table 2. Accuracy of different cytokines for treatment failure prediction.

Categories	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
IL-6 ≥ 14 pg/mL	0.835 (0.78 - 0.90)	84	71	68.5	85.5	76.6
IL-8 ≥ 31 pg/mL	0.808 (0.74 - 0.88)	81.3	66	64.2	82.5	72.6

Cutoff values were obtained from ROC analysis. Non-responder patients were compared to responder patients.
PPV - positive predictive value, NPV - negative predictive value.

DISCUSSION

Egypt remains one of the ten countries with the greatest HCV burden in the world, posing a significant health and economic burden [9]. The traditional treatment options for HCV include peginterferon, ribavirin, and harvoni, which are associated with the reversion of liver fibrosis. In the past few years, a few novel HCV therapeutics, approved by the FDA, included Epclusa® (sofosbuvir) and OLYSIO® (simeprevir). Although DAAs report a high ratio of SVR, where HCV is no longer detected in blood plasma 24 weeks from the completion of anti-viral therapy, about 30% of the cases have been reported, where fibrosis has not improved post-SVR [10]. Cytokines are one of the immune system components that contribute to the host's response to invading infections. Infection, inflammation, and carcinogen-induced damage are all examples of cellular stressors that cause cytokines to be released. Several cytokines, especially those produced by CD4+ (cluster of differentiation 4) Th cells (T helper cells), are classified as Th1 or Th2 cytokines, and they mostly consist of interleukins (ILs). Th1 cytokines (e.g., IL-1, IL-2, IL-12p35, IL-12p40, IL-15, and non-ILs, such as tumor necrosis factor (TNF) and interferons (IFN)) are pro-inflammatory, whereas Th2 cytokines (e.g., IL-4, IL-8, IL-10, and IL-5) generate anti-inflammatory responses [11].
IL-6 is a pro-inflammatory and multifunctional cytokine that is located on the 7p21 chromosome. The hepatic response to infections and systemic inflammation is the

Table 3. Correlation between IL-6 and IL-8 factors associated with treatment failure.

	IL-6		IL-8	
Variable	***r* correlation coefficient**	**p-value**	***r* correlation coefficient**	**p-value**
ALT (U/L)	**0.11**	**0.216**	**0.15**	**0.092**
AST (U/L)	**0.11**	**0.192**	**0.14**	**0.135**
ALP (U/L)	**0.08**	**0.339**	**0.11**	**0.208**
Albumin (g/dL)	**-0.204**	**0.023** *	**-0.270**	**0.003** *
Bilirubin (mg/dL)	**0.11**	**0.217**	**0.16**	**0.088**
Platelets (x 10^9/L)	**-0.14**	**0.138**	**-0.17**	**0.073**
FIB-4	**0.22**	**0.020** *	**0.32**	**0.001** *
APRI	**0.23**	**0.021** *	**0.30**	**0.012** *

Pearson correlation coefficients were measured in linear relationships. p < 0.05 was considered significant.
ALT - alanine aminotransferase, AST - aspartate aminotransferase, ALP - alkaline phosphatase, FIB-4 - fibrosis-4 score, APRI - AST-platelet ratio index.

Table 4. Correlation between high-risk and factors associated with treatment failure.

Variable	*r* correlation coefficient	p-value
ALT (U/L)	**0.18**	**0.049** *
AST (U/L)	**0.23**	**0.009** *
ALP (U/L)	**0.19**	**0.040** *
Albumin (g/dL)	**-0.349**	**0.001** *
Bilirubin (mg/dL)	**0.19**	**0.031** *
Platelets (x 10^9/L)	**-0.19**	**0.050** *
FIB-4	**0.42**	**0.0001** *
APRI	**0.44**	**0.0001** *

Spearman correlation coefficients were measured in monotonic relationships. p < 0.05 was considered significant.
ALT - alanine aminotransferase, AST - aspartate aminotransferase, ALP - alkaline phosphatase, FIB-4 - fibrosis-4 score, APRI - AST-platelet ratio index.

key function of IL-6. Indeed, IL-6 produced by B cells, T cells, macrophages, and fibroblasts has a pleiotropic effect on inflammation, immune response, and hematopoiesis [12]. IL-6 was the most reported gene to be associated with HCV infection or HCC development in chronic HCV patients. The SNPs in the IL-6 gene have been reported to influence the histologic progression and clinical outcomes of HCV patients [12].

IL-8, also known as C-X-C motif ligand 8 (CXCL8), is a pro-inflammatory chemokine that acts as a chemoattractant of leukocytes. It is secreted by several cells, such as macrophages, neutrophils, and epithelial cells, to promote immune infiltration and angiogenesis and to mediate the activation and migration of peripheral blood neutrophils to tissues, thus increasing local inflammation [13].

Due to the quick stop of HCV replication and the subsequent clearance of viral antigens through DAAs, we wondered what the overall effects were on immune cells. So, the point of this study was to find out if IL 10 and IL 8 levels in the blood affect how the virus reacts to DAAs and to find useful clinical or immunological indicators for how well HCV treatment will work. Non-responder patients were associated with significant ($p < 0.05$) high levels of ALT, AST, ALP, and total bilirubin and with significantly ($p < 0.05$) decreased levels of albumin and platelet count. Moreover, non-responder patients were associated with significant ($p < 0.05$) high values of two common fibrosis markers (FIB-4 and APRI). Many factors have been demarcated to be of influence on the occurrence of the fibrosis regression process: age of the individual, genetic and epigenetic factors, rate of fibrosis progression (slow or rapid fibrosis), or disease-related factors like the etiology and staging of chronic liver disease [14-16]. A significant reduction of AST and ALT is a finding that is in agreement with

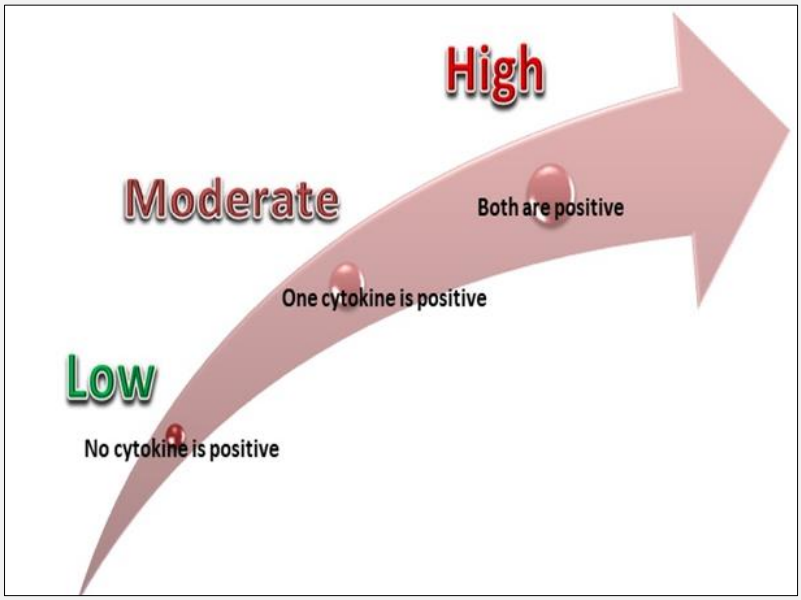

Figure 1. The classification of treatment failure risk according to IL-6 and IL-8 positivity.

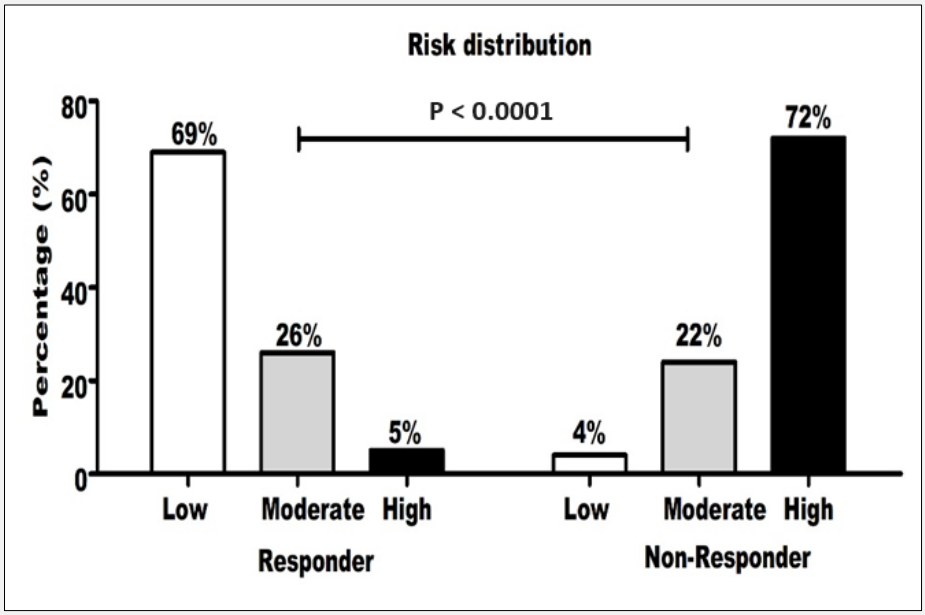

Figure 2. Risk distribution between responder and non-responder patients.

Differences were assessed by using the chi-squared test (χ^2). $p < 0.05$ was considered significant.

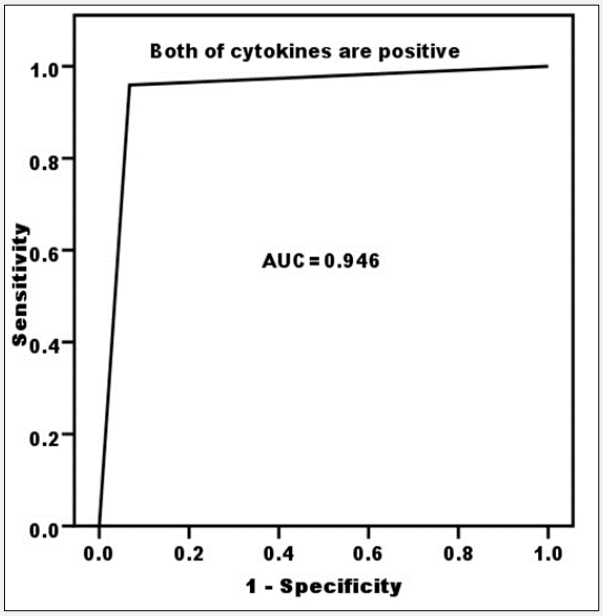

Figure 3. Area under the receiver operating characteristic curve (AUC) for separating non-responder from responder patients, when comparing high-risk to low-risk.

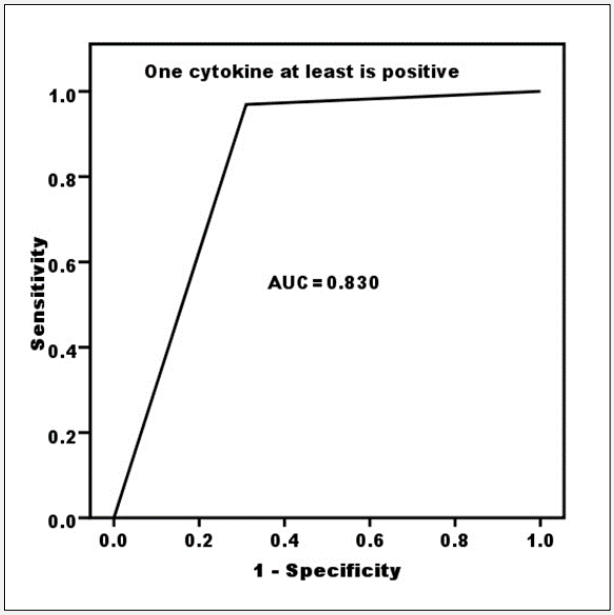

Figure 4. The area under the receiver operating characteristic curve (AUC) for separating non-responder from responder patients, when compared to high- and moderate-risk, and combined with low-risk.

those of other studies [17-20]. Also, a significant improvement in FIB-4 was observed in this study, as previously reported by Persico et al. and Tag-Adeen et al. [17,19]. The decline in FIB-4 values might primarily result from a reduction in necro-inflammation, as evidenced by decreased ALT levels. Hsu et al. assumed that the change in FIB-4 values might be due to the rapid decline in AST and ALT levels and, to a lesser extent, due to an increased platelet count [20].

The present study revealed that non-responder patients were accompanied by a significant increase ($p < 0.05$) in baseline concentration of IL-6 (16.7 ± 4.92 pg/mL) when compared to responder patients (12.68 ± 2.06 pg/mL). IL-6 gave an AUC of 0.835 for separating non-responder patients with good sensitivity and specificity values. This finding was in agreement with the results of Nattermann et al., who reported that a higher level of IL-6 is significantly associated with SVR, compared with a lower level [21]. Also, Mohamed et al. showed that the virological response during HCV therapy with SOF/SMV was associated with a significant decrease in IL-6 levels. High baseline IL-6 levels can be associated with poor clinical outcomes and impaired T-cell function in patients with unresectable HCC after Ate/Bev treatment [22]. In contrast, Cotler et al. reported that there was no significant difference in basal IL-6 levels between the groups of responders and non-responders to IFN therapy [23]. A possible explanation for this finding is that the IL-6 level could modulate the response to treatment by activating STAT3 by phosphorylation in hepatic stellate cells and by promoting their survival and proliferation. Furthermore, IFN-a activates STAT3, followed by the induction of a wide variety of antiviral and proapoptotic genes that may contribute to the antiviral and antitumor activities of IFN-a in human livers [24]. Myojin et al.´s study examined a small number of people treated with Ate/Bev as first- or second-line therapy and suggested that a relatively low IL-6 level (3.2 pg/mL) could be used as a prognostic factor [25]. Moreover, we found that elevated levels of IL-6 were significantly correlated with decreased levels of albumin and elevated fibrosis markers, including FIB and APRI. In line with our findings, Noh et al. found that serum TNF-α and IL-6 levels were inversely correlated with total protein and albumin levels in healthy subjects [26].

When we compared non-responder patients to responder patients, we saw that their baseline concentration of IL-8 was significantly higher ($p < 0.05$) than that of responder patients, which was 29.06 ± 5.94 pg/mL. IL-8 gave an AUC of 0.808 for separating non-responder patients with good sensitivity and specificity values. This may be explained by the fact that CXCL-8 is induced in response to the expression of the HCV NS5A protein and HCV replication. Moreover, CXCL-8 inhibits the antiviral actions of IFN-α, and recent studies indicate that CXCL-8 protein levels are associated with HCV replication [27].

This is in line with a previous study by Brochado-Kith et al. [28] that showed an improvement in these immune and liver disease biomarkers in plasma immune exhaustion (PD1), chemokines (CXCL10, CCL2, and CXCL-8), and cytokines (IL10), after achieving SVR with DAA therapy in HIV/HCV-coinfected individuals [27]. Koo et al. [29] showed that inhibition of CXCL-8 protein production inhibited chronic HCV replication in stable subgenomic replicon cells. CXCL-8 protein, but not mRNA, is correlated with HCV replication [30]. Also, previous studies showed that patients with chronic hepatitis C have elevations in serum levels of α-chemokine interleukin-8 (CXCL-8), and patients who are non-responsive to IFN therapy have high pretreatment levels of CXCL-8 [31,32]. When expressed in cell culture, the HCV NS5A protein induces CXCL-8, which is associated with the inhibition of the antiviral effects of IFN [32-34]. The present study revealed that IL-8 serum levels were closely associated with the progression of fibrosis or cirrhosis, as reflected by clinical scores (e.g., Child-Pugh, MELD) and laboratory tests indicating deteriorated liver function or progressed fibrosis. In one study of HCV-infected patients, serum IL-8 increased as the severity of chronic hepatitis C progressed to the development of hepatocarcinoma; therefore, it can be used as a prognostic factor for the development of this cancer [35].

Clément et al. showed that IL-8 secreted by injured hepatocytes was shown to activate collagen-producing hepatic stellate cells in vitro [36]. A study by Zimmermann et al. expands the profibrogenic potential of IL-8 by showing that the IL-8 signal is also amplified by 'non-classical', fibrosis-associated CD16+ monocytes and macrophages and that IL-8 is in chronically inflamed livers directly associated with the number of hepatic macrophages rather than hepatic neutrophils. Thus, our data support that IL-8 contributes to establishing a profibrogenic microenvironment in chronic liver diseases and proposes a novel role of IL-8 for the recruitment and activation of hepatic macrophages in human liver cirrhosis [37]. To the best of our knowledge, this is the first study to evaluate the combined use of IL-6 and IL-8 in treatment failure prediction. To improve the accuracy of predicting treatment failure with HCV DAA regimens, we evaluated the combined use of IL-6 and IL-8 in treatment failure prediction. The risk was classified as follows: high-risk: both IL-6 (> 14 pg/mL) and IL-8 (> 31 pg/mL) were positive; moderate-risk: one of IL-6 or IL-8 was positive; low-risk: both IL-6 and IL-10 were negative. A previous study identified the prognostic value of baseline IL-6 and IL-8 values in advanced HCC patients receiving sorafenib treatment [38]. Interestingly, the high-risk obtained from the combined use of the two cytokines was significantly ($p < 0.05$) associated with all factors that were significantly associated with treatment failure, with the highest correlation coefficients.

In many clinical settings, the currently available tools to predict SVR can help simplify the decision about discontinuing or starting treatment. But none of them can undertake an exact prediction. From this, the strength of

this study comes as this study evaluated, for the first time, the baseline levels of two important cytokines (IL-6 and IL-8) as predictors for HCV treatment failure. Also, although further studies are required, these cytokines may help in reducing the treatment length, as nowadays there is an increasing debate about this issue.

CONCLUSION

According to the study, patients with CHC had a lower response to treatment, when their levels of interleukin-6 and interleukin-8 were higher.

Ethics Approval:
The research protocol was approved by the Faculty of Medicine at Port Said University (serial number (92) BIO_003), under the ethical guidelines of the "Helsinki Declaration". All included cases gave their informed consent.

Declaration of Interest:
The authors declare that there are no financial or personal relationships that could have influenced the research.

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