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Sovaldi and killer cells in hepatitis C Virus infected patients in Egypt during infection with Covid 19

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ABSTRACT

In December 2019, Wuhan, China, reported a new case of coronavirus-induced pneumonia. The severe acute respiratory syndrome coronavirus was the name given to this virus upon its identification. The World Health Organization has designated coronavirus disease in 2019 as an abbreviation COVID-19.The treatment of chronic hepatitis C virus has revolutionized with the development of medications like Sovaldi that directly act on particular hepatitis C virus target structures. Almost all patients, regardless of their comorbidity, can now receive therapeutic treatment. One of the main cell types reacting to interferon is natural killer cells. Therefore, it seems sense to believe that natural killer cells will play a role in the body's reaction to interferon, an antiviral medication used to treat chronic hepatitis C infection. Natural killer cells recognize infected hepatoma cells with hepatitis C virus after interferon stimulation in a DNAX Accessory Molecule-1 dependent manner. The purpose of this study was to assess the relation between Covid-19, natural killer cells (CD16, CD56) and Sovaldi as anti-viral which used as a treatment of hepatitis C virus. Methods we analyzed data from 80 patients and 40 persons as a control recruited in hospital. We calculated the percentage of responders to treatment and effect of treatment. Results we found that the frequency of CD56 and CD16 in responders whom infected with covid 19 is high while in responders whom not infected with covid 19 and in non-responders was normal. Conclusions The study has demonstrated that Sovaldi improve the response of patients to covid 19 treatment.

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Introduction

Hepatitis C is an infectious disease that is caused by hepatitis C virus (HCV) and affects the liver (Hussein et al. 2025). The infection is always asymptomatic, but it causes inflammation of the liver (chronic hepatitis). This condition can progress to fibrosis, and cirrhosis. In some cases, the cirrhosis will change to liver failure or liver cancer. Hepatitis C virus (HCV) was a significant health problem. Until recently, Pegylated Interferon was used as a treatment for hepatitis C virus (HCV) infection. The treatment of chronic hepatitis C virus has been transformed by the development of medications like Sovaldi that directly act on specific hepatitis C virus target structures. Almost all patients, regardless of their comorbidity, can now receive successful treatment (Burchill et al., 2015). Interferon is a potent activator of Natural killer (NK) cells; therefore, it is not surprising

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that natural killer cell activation has been identified as a key factor associated with Sustained Virologic Response (SVR) to interferon-based therapies (Mondelli 2015). In contrast, Sovaldi would not be expected to have a direct effect on natural killer cell phenotype and function; however, rapid control of the virus could result in decreased endogenous interferon, which may result in decreased activation. Indeed, many studies on first-line Sovaldi therapy (Sovaldi / Ribavirin) suggest that natural killer cells may contribute to the clearance of the hepatitis C virus during Sovaldi therapy (Burchill et al., 2015). Early studies focused mainly on differences between responders and nonresponders or comparisons with external healthy control groups without liver disease. The elimination of hepatitis C virus (HCV) infection depends on the effectiveness, specificity, and rapidity of the innate and adaptive immune responses, as well as on the hepatitis C virus (HCV) replication rate (Brown & Neuman 2001). Hepatitis C virus (HCV) is treated with Sofosbuvir, which is marketed among other names as Sovaldi. (Sofosbuvir 2016). The Sovaldi drug is consumed orally and it is belongs to the nucleotide analog family, which inhibits the hepatitis C NS5B protein ("Sovaldi 400 mg film coated tablets" Summary of Product Characteristics 2016).

In the US, Sovaldi was licensed for medical use in 2013 after being found in 2007. ("Sovaldi- sofosbuvir tablet".2020) It is listed as an essential medicine on the World Health Organization's (WHO) list (World Health Organization 2021). Sovaldi is a substrate of P-glycoprotein, a transporter protein that returns medications and other materials from intestinal epithelial cells to the gut. Therefore, the intestinal P-glycoprotein-promoting drugs rifampicin may reduce the absorption of Sovaldi According to St. John's wort ("Sovaldi-sofosbuvir tablet". 2020). A management guideline for hepatitis C virus (HCV) was issued in 2016 by the American Association for the Study of Liver Diseases and infectious Diseases. According to this suggestion, Sovaldi is included in all first-line therapies for hepatitis C virus (HCV) genotypes 1, 2, 3, 4, 5, and 6, as well as some second-line treatments, when used in combination with other medications (Recommendations for Testing, Managing, & Treating Hepatitis C". 2016). For all genotypes, a combination of sovaldi and velpatasvir is advised, as it has a cure rate of at least 90 % and often nearly 100 %. Usually, the course of treatment is 12 weeks (EPCLUSA (sofosbuvir & velpatasvir) Prescribing information 2017). Depending on the patient's genotype, unique situation, and cost-effectiveness analysis, Sovaldi may also be taken in combination with other drugs and for a longer period throughout therapy. For instance, Sovaldi and ledipasvir, a viral NS5A inhibitor, can be used in combination to treat hepatitis C virus (HCV) infections caused by genotypes 1, 4, 5, and 6 (Harvoni-ledipasvir & sofosbuvir tablet 2022). Sovaldi can be used in combination with daclatasvir for the treatment of genotype 2 and 3 hepatitis C virus (HCV) infections in patients with cirrhosis or liver transplant recipients. Sometimes, weight-based ribavirin is included. When starting treatment for the hepatitis C virus (HCV), with Pegylated interferon and Sovaldi or without Sovaldi, is not advised (Recommendations for Testing, Managing & Treating Hepatitis C 2016).

With Sovaldi, the majority of patients can receive effective treatment for their hepatitis C virus without the need for pegylated interferon, an injectable medication with serious side effects that was a major ingredient in earlier drug combinations (Yau & Yoshida 2014). The persistence of hepatitis C virus (HCV) was due to infection at privileged (extrahepatic) sites, viral inhibition or mutation of antigen presentation, selective immune suppression, and negative regulation of hepatitis C virus (HCV) gene expression, immune type of T cells and the incomplete differentiation of memory T cells (Dustin & Rice 2007). In chronic hepatitis C virus (HCV) infection, specific CD 4+ or CD 8+ T cell responses play essential roles in the pathogenesis of liver damage. The cells of Natural killer (NK) are one of the primary cell populations responding to interferon. So, it makes sense to believe that interferon, an antiviral treatment for chronic hepatitis C virus (HCV), will affect natural killer cells (NK) (Mondelli 2015). Natural killer cells (NK) exhibit a DNAM-1-dependent pattern of recognition for hepatitis C virus-infected hepatoma cells upon Interferon stimulation. Furthermore, hepatitis C virus (HCV) replication is effectively decreased when interferon-stimulated natural killer cells interact with hepatitis C virus-infected hepatoma cells (Stegmann et al., 2012). The second phase drop in HCV-RNA levels with pegylated interferon therapy may be explained by these data (Stegmann et al., 2010). Reduced inhibitory NKG2A expression is correlated with interferon-based therapy success (Golden et al., 2011), higher natural and antibody-dependent Natural killer (NK) cytotoxicity (Oliviero et al., 2013), and normalization of natural killer (NK) cell levels as well as interferon production (Dessouki et al., 2010). Natural killer (NK) cells are replenished in the liver following a successful antiviral treatment.

Beginning in December 2019, the SARS Covid-19 epidemic spread over the world. By the end of January 2023, there had been over 600 million cases overall, and 6.8 million deaths had been reported (Saied et al. 2021, World Health Organization Dashboard 2022). Three of the seven coronaviruses that have epidemically outbreaks

in humans were SARS-Covid in 2002 (severe acute respiratory syndrome, or SARS), MERS-Covid-19 in 2012 (Middle East respiratory syndrome, or MERS), and currently SARS-CoV-2 (the current pandemic known as COVID-19), according to the Center for Disease Control and Prevention (CDC; https://www.cdc.Gov./coronavirus/types.html) (Wiersinga et al., 2020).

SARS-CoV-2 is a member of the Coronavirinae family and a genus of beta coronaviruses (Rehman et al., 2020). The viruses have a diameter of between 60 and 140 nm and are spherical or pleomorphic in form. With 27–32 kilobases (kb), coronaviruses have one of the biggest single-strand RNA genomes. On their surface, several coronaviruses encode for the proteins hemagglutinin esterase, 3a/b, and 4a/b (Mittal et al., 2020). The World Health Organization (WHO) classified the lineage B.1.1.529, also known as the Omicron variant, as a variant of concern (VOC) in November 2021. The spike protein of the Omicron variation has more than thirty different amino acid changes from the original forms. Numerous worrisome epidemiological characteristics describe it, including a decreased minimal infection dosage that leads to increased transmissibility, immune evasion that raises the risk of reinjection and breakthrough infections, and a compromised response to COVID-19 specific treatment (Tao et al., 2022). In replication, the difference between other variants of COVID-19 and the Omicron variant that the replication of Omicron is occurs mainly in the upper respiratory tract (e.g. pharynx), which can lead to higher transmission rates and milder disease (Shuai et al., 2022). Certain COVID-19 cases have been demonstrated to have increased ferritin, which may be associated with inferior clinical outcomes (Chen et al., 2020). As immunological biomarkers, ferritin and IL-6 are employed to diagnose COVID-19 patients. In COVID-19 cases, ferritin and C reactive protein (CRP) may be useful screening markers for the early identification of systemic inflammatory response syndrome (Melo et al., 2021).

The purpose of this study was to assess the relation between COVID-19, Natural killer cells (CD16, CD56) and effect of Sovaldi as a treatment of hepatitis C virus.

Material and Methods

General experimental procedure

This study contain 80 patients who were infected with HCV virus. Group I and III are infected with Covid-19, Group II and IV not infected with COVID-19. The infected patients with HCV virus received Sovaldi, Ribavirin, and they had a virological response determined for three months after completion of Sovaldi therapy. To diagnose the presence of hepatitis C we used an appropriate serology (HCV Ab) test. All cases received Sovaldi and Ribavirin therapy for three months. And group V 40 persons were healthy persons with matched age, sex and environmental status also included as controls.

This study was conducted on five different groups of participants. **Group I** included 20 patients who were positive for hepatitis C antibodies. After receiving treatment with Sovaldi and Ribavirin, their real-time PCR results for hepatitis C turned negative, which meant that they were considered responders to treatment. Despite this successful response, all of these patients were also infected with COVID-19.

Group II also consisted of 20 patients who were positive for hepatitis C antibodies. Like the first group, they too responded well to treatment with Sovaldi and Ribavirin, as their real-time PCR results for hepatitis C became negative, placing them in the responder category. However, unlike the first group, these patients were not infected with COVID-19.

Group III was made up of 20 patients who were positive for hepatitis C antibodies but had a different outcome after treatment. Their real-time PCR results remained positive even after therapy with Sovaldi and Ribavirin, meaning they were classified as non-responders. In addition to this, they were also infected with COVID-19.

Group IV included another 20 patients with hepatitis C antibody positivity who, like Group III, did not respond to treatment. Their PCR results for hepatitis C remained positive, so they were considered non-responders. However, in this group, the patients were not infected with COVID-19.

Finally, **Group V** was a control group of 40 healthy individuals. These participants were carefully selected to match the patients in terms of age, sex, and environmental background, ensuring a reliable basis for comparison with the other groups.All of cases were enrolled from ACMC, inpatient and outpatient department.

According to ICH good clinical practice, Declaration of Helsinki and World Health Organization guidelines, and the Research ethics committee of Faculty of medicine for Girls FMG-IRB, Egypt. And all experimental protocols were approved by the Research ethics committee of Faculty of medicine for Girls FMG-IRB, Egypt. And the sampling process of this study was carried out at ACMC (Arab contractor's medical center) and the college of medicine for girls FMG-IRB, Egypt. The college of medicine for girls FMG-IRB Ethical Committee (Approval code 2098/2023). Informed consent was obtained from all subjects according to the

ethical committee for human research in the Faculty of medicine for Girls (FMG-IRB), Egypt.

All patients in this study received combination therapy with Sofosbuvir (Sovaldi) and Ribavirin. Sofosbuvir was given as one tablet daily for three months, while Ribavirin was also administered as one tablet daily for the same period. At the end of the treatment duration, after three months, blood samples were collected from every patient for laboratory evaluation.

Both patients and controls included in the study were subjected to a full assessment, starting with demographic and clinical data collection. This included age, sex, place of residence, smoking status, presence of diabetes mellitus, and bilharzia antibody positivity.

Comprehensive laboratory investigations were carried out for all participants. Liver function tests included aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transaminase (GGT), total bilirubin, direct bilirubin, albumin (Alb), and C-reactive protein (CRP). These were analyzed using the Cobas C311 Roche Hitachi system (Japan). Tumor and inflammatory markers such as alpha-fetoprotein (AFP), interleukin-6 (IL-6), ferritin, procalcitonin (PCT), and COVID-19 IgM were assessed using the Cobas E411 Roche Hitachi system (Japan). Hematological and coagulation profiles were determined by complete blood count (CBC) using the Sysmex XN 500 system (Japan), international normalized ratio (INR) using the Stago ST4 system (USA), and D-dimer using the Cobas C311 Roche Hitachi system (Japan). In addition, immunological analysis was performed by detecting CD16 and CD56 natural killer cells through flow cytometry using the BD Biosciences Accuri C6.

The criteria for treatment response to Sofosbuvir therapy were based primarily on quantitative PCR results. A successful virological response was defined as a viral load of less than 10 copies after three months of treatment. Supportive criteria included improvements in liver function tests, normalization of complete blood count (CBC), and reductions in alpha-fetoprotein (AFP).

Sampling

Blood sample of (10ml) was drawn and divided into three aliquots, the first aliquot (4ml) was collected in two vaccutainre tubes with EDTA additive (anti-coagulant) , (2ml) in each tube, one for CBC and the other aliquot for NK cells (CD16 and CD56) and the second aliquot for INR and D Dimer. The third aliquot of sample was a serum sample which screened for liver functions, ferritin, AFP and other tests.

Data analysis

The data collected in this study were entered into a computer and analyzed using the Statistical Package for Social Science (SPSS), version 15.0.1 for Windows (SPSS Inc., Chicago, IL, 2001). The type of analysis chosen depended on the nature of the data obtained for each parameter. For descriptive statistics, the mean and standard deviation (± SD) were calculated to represent parametric data in a clear mathematical form. For non-mathematical or categorical information, frequencies and levels were presented to give a meaningful picture of the data distribution.

Analytical measurements were then performed to compare and explore relationships between different groups. The Student's t-test was applied to determine the statistical significance of differences between the means of two groups. For comparisons involving more than two groups, the ANOVA test was used, followed by a Post Hoc test to examine all possible pairwise group differences. To explore relationships between qualitative variables, the Chi-Square test was applied. In cases where the expected count was less than five in more than 20% of the cells, Fisher's exact test was used as a more reliable alternative.

The level of significance was determined using the p-value. A p-value greater than 0.05 was considered non-significant (NS), while a p-value less than 0.05 indicated statistical significance (S). When the p-value was less than 0.01, the result was regarded as highly significant (HS).

Results

A total of 120 people took part in this study. To better understand their health conditions and responses, they were divided into five groups. The groups were formed based on whether the participants had hepatitis C, how they responded to treatment with Sovaldi and Ribavirin, and whether or not they were also infected with COVID-19. Alongside these patients, a group of healthy individuals was also included to serve as a control, helping provide a clearer comparison with those affected by the illnesses.

The current study was conducted at Arab Contractors Medical Center (ACMC). And the number of cases whom included in this study was 80 patients. The age range was from 30-60 years. They were 49 (61.3 %) from urban and 31 (38.7 %) from rural areas, 26 (32.5 %) smokers and 54 (67.5 %) nonsmokers, 72 (90.0 %) male and 8 (10.0 %) female, 25 (31.3 %) diabetic patients and 55 (68.7 %) not diabetic (Table 1).

In responders the mean of the HCV by PCR result before treatment was high (187946 IU/ml) while after treatment it became very low (less than 10 IU/ml) (Table

Table 1 Description of demographic data among cases of this study

N = 80		Responders N = 40		Non Responders N = 40	
		N	%	N	%
Sex	Male	40	100 %	32	80.0 %
	Female	0	0 %	8	20.0 %
Age	Mean± SD	52.08 ± 6.13		50.52 ± 5.83	
	Range	(30 – 59) years		(39 – 60) years	
Bilharzia ab	Positive	4	10.0 %	8	20.0 %
	Negative	36	90.0%	32	80.0 %
Smoking	Smoker	10	25.0 %	16	40.0 %
	Non smoker	30	75.0 %	24	60.0 %
Diabetes	Diabetic	13	32.5 %	12	30.0 %
	Non diabetic	27	67.5 %	28	70.0 %
Residence	Urban	27	67.5 %	22	55.0 %
	Rural	13	32.5 %	18	45.0 %

Table 2. Comparison between responders and Non responders as regard load of viraemia (PCR) before and after treatment

PCR	Group				P	Sig
	Responders		Non responders			
	Mean	±SD	Mean	±SD		
Before *(10^3)	1879.46	449.34	833.41	673.81	.0001	HS
After	< 10.0	0.00	27.89* (10^3)	22.60	.0001	HS

2). It means that the difference between two groups (responders and non-responders) before and aftertreatment for hepatitis C virus was highly significant with respect to HCV by PCR result.

According to the findings, the frequency of natural killer cells, marked by CD16 and CD56, was noticeably higher in Group I compared to the other groups as seen in figures 1 and 2. In this group, CD16 reached a level of 14.9 and CD56 reached 13.6. By contrast, the levels of these natural killer cells remained within the normal range in all the other groups (Figs 3 and 4). These results are presented in Table (3), which highlights the differences in natural killer cell frequency across the studied groups

From table (4) we found that in group I the difference between groups before and after treatment from covid 19 was highly significant with covid 19-Igm, PCT, D Dimer, IL 6, CRP, WBCs and Lymphocyte, but with ferritin was not significant which means that the difference between responders in group I before and after treatment form covid 19 was highly significant with respect tocovid 19-Igm, PCT, D Dimer, IL 6, CRP, WBCs and Lymphocyte.

In table 5, we found that in group III the difference between groups before and after treatment from covid 19 was highly significant with covid 19-Igm, PCT, D Dimer, IL 6, CRP, WBCs and Lymphocyte, but with ferritin was not significant that means the difference between non responders in group III before and after treatment form covid 19 was highly significant with respect tocovid 19-Igm, PCT, D Dimer, IL 6, CRP, WBCs and Lymphocyte.

From table 6, we notice the frequency of Natural killer (CD 16) in responders whom infected with covid 19 was high (14.9) while responders whom mot infected with covid 19 is normal (8.9) and in non-responders who infected with covid 19 is normal (8.4) and in non-responders who not infected with covid 19 is normal (7.7). From all of these, it can be concluded that a higher frequency of Natural killer (CD 16) due to Sovaldi and Ribavirin, which improve their response to covid 19 therapy. It means that the difference between two groups (responders and non responders) before and after treatment form covid 19 was highly significant with respect to CD16. Also, in responders who infected with covid 19 the frequency of Natural killer (CD 56) was high (13.6) while in responders who not infected with

covid 19 is normal (8.9) and in non-responders is normal (8.4) and in non-responders who not infected with covid 19 is normal (7.7).

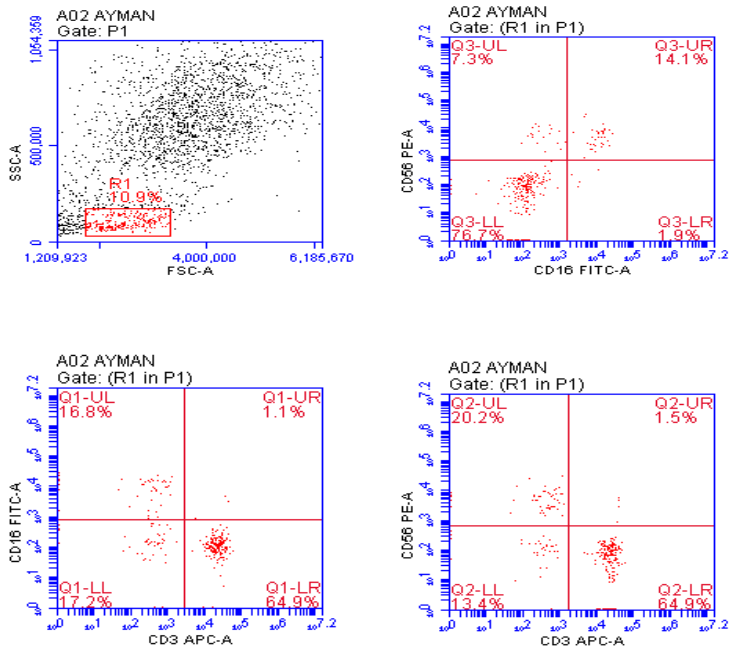

Fig 1. The frequency of CD16 and CD 56 in group I (responders for hepatitis C virus and positive covid 19)

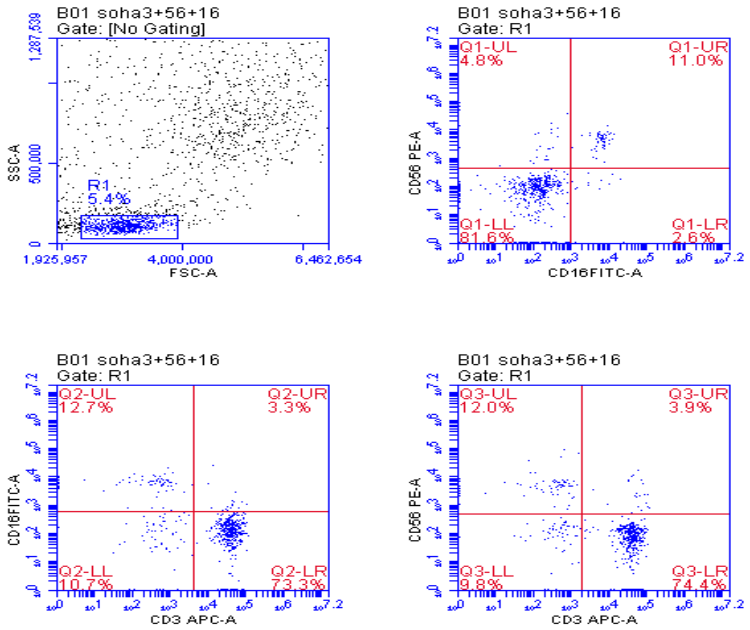

Fig 2. The frequency of CD16 and CD 56 in group II (responders for hepatitis C virus and negative covid 19).

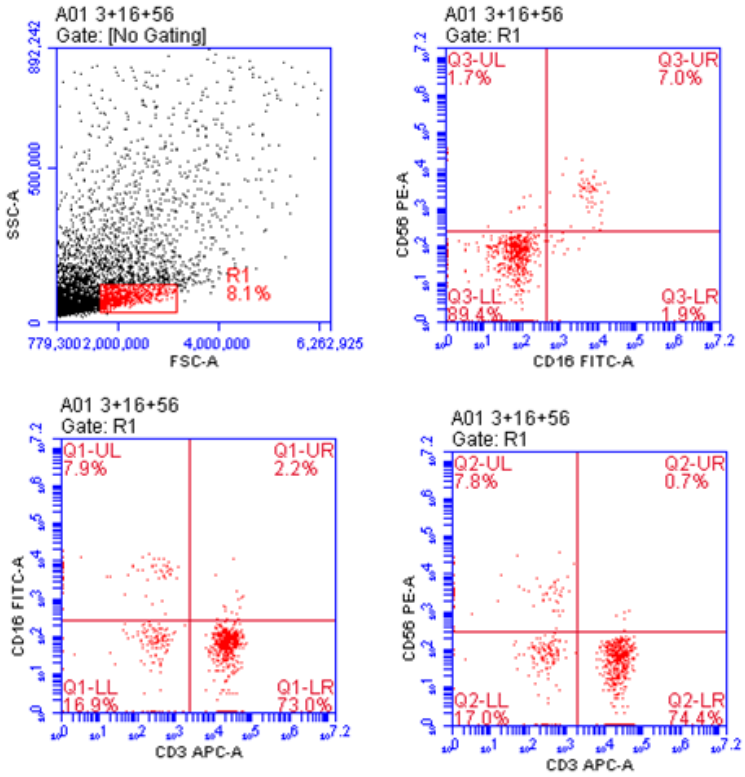

Fig 3. The frequency of CD16 and CD 56 in group III (non-responders for hepatitis C virus and positive covid 19).

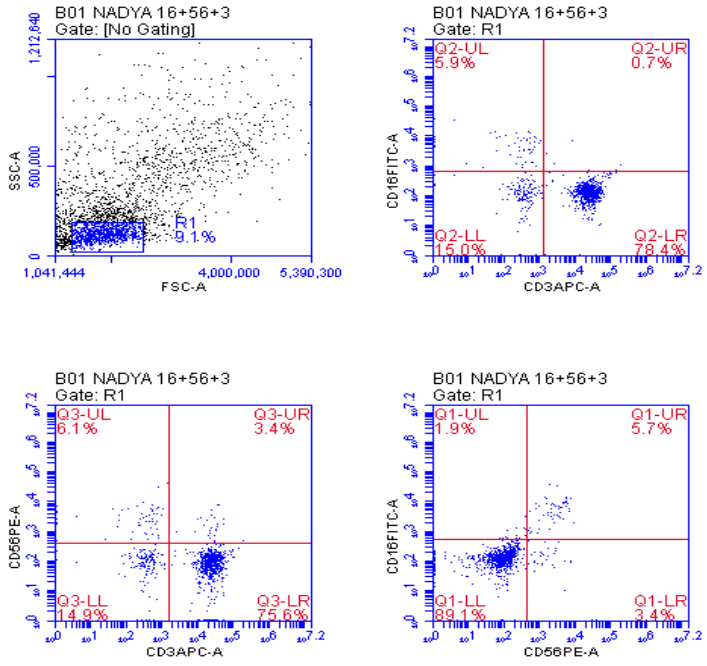

Fig 4. The frequency of CD16 and CD 56 in group IV (non-responders for hepatitis C virus and negative covid 19).

Table 3 Natural killers (Cd16, Cd56) frequency among groups

CD Type	Group									
	IA (Covid +ve)		II (Covid –ve)		III (Covid +ve)		IV (Covid -ve)		V (Control)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
CD 16	14.9	4.2	8.9	2.8	8.4	3.4	7.7	3.5	7.3	1.2
CD 56	13.6	5.8	8.1	2.8	6.7	3.7	6.9	3.7	8.2	0.9

Table 4 Comparison between group IA (infected with covid 19) before and after treatment from covid 19

Parameters	Group I Responders (covid + ve)				P	Sig
	Group I Responders (Before treatment)		Group I Responders (After treatment)			
	Mean	±SD	Mean	±SD		
WBCs	3.635	0.709	6.275	1.012	0.0001*	HS
Lymphocyte	0.816	0.1978	1.87	0.271	0.0001*	HS
Ferritin	416.8	513.487	547.65	354.90	0.1280*	NS
D Dimmer	4858.8	1758.8	276.6	86.94	0.0001*	HS
CRP	208.2	84.2	4.3045	1.624	0.0001*	HS
IL 6	147.3	33.1	4.8	1.291	0.0001*	HS
PCT	59.725	25.8420	0.7125	0.81319	0.0001*	HS
Covid 19-IgM	66.000	13.4017	5.140	1.6132	0.0001*	HS

Table 5 Comparison between group III (Infected with covid 19) before and after treatment from covid 19

Parameters	Group III Non Responders (covid +ve)				P	Sig
	Group III Non Responders (Before treatment)		Group III Non Responders (After treatment)			
	Mean	±SD	Mean	±SD		
WBCs	2.150	0.510	4.65	0.95	0.0001*	HS
Lymphocyte	0.616	0.21	1.28	0.32	0.0001*	HS
Ferritin	585.4	725.6	552.65	405.1	0.1280*	NS
D Dimmer	5642.8	1895.2	295.8	92.8	0.0001*	HS
CRP	246.5	59.9	5.1	2.1	0.0001*	HS
IL 6	174.3	14.6	5.6	1.57	0.0001*	HS
PCT	75.5	15.20	0.825	0.221	0.0001*	HS
Covid 19-IgM	72.000	11.17	6.2	1.25	0.0001*	HS

Table 6 Comparison between groups as regard CD 16 and CD 56

CD Type	Group										P	Sig
	I (Covid +ve)		II (Covid –ve)		III (Covid +ve)		IV (Covid -ve)		V (Control)			
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD		
CD 16	14.9	4.2	8.9	2.8	8.4	3.4	7.7	3.5	7.3	1.2	.0001	HS
CD 56	13.6	5.8	8.1	2.8	6.7	3.7	6.9	3.7	8.2	0.9		

This means that a higher frequency of Natural killer (CD 56) due to Sovaldi and Ribavirin which improve their response to covid 19 therapy.

It means that the difference between two groups (responders and non responders) before and after treatment form covid 19 was highly significant with respect to CD56. These results suggest the immune response plays an important role in determining the susceptibility to and the outcome of hepatitis C virus (HCV) infection. The possible association of hepatitis C virus (HCV) infection related parameters may contribute to the understanding of the pathogenesis of chronic hepatitis C, and hence its diagnosis, prevention and management.

Discussion

Earlier HCV work linked response to greater cytotoxic NK competence notably higher CD56^dim frequencies and perforin content while nonresponse has been associated with lower NK frequency and/or expansion of $CD56^{-}CD16^{+}$"dysfunctional" NK subsets. (Malengier-Devlies et al., 2022) This aligns with our normal levels in non-responders versus elevated NK in responders.

Across multiple cohorts, Interferon-free DAA therapy tends to attenuate/normalize NK activation during treatment, with functional/phenotypic "recovery" most evident during or after therapy rather than as a sustained increase (e.g., rapid early normalization followed by stabilization). In contrast, during p-Interferon/Ribavirin (IFN/RBV), early treatment is associated with NK activation and phenotypic shifts that track with Virologic response. (Ahlenstiel et al., 2011). This correspond with the pattern of our observation (higher CD16/CD56 among responders).

Several studies report that direct acting antiviral (DAAs) tend to normalize or attenuate NK cell activation rather than increase it during treatment, with phenotypic/function "recovery" more evident after therapy finishes. During peg-IFN/RBV therapy, early treatment phases are associated with NK activation and phenotypic shifts that correlate with Virologic response (Spaan et al., 2016) but we observe that a higher CD16/CD56 in responders echoes these interferon-era kinetics more than the "normalization" often seen with IFN-free DAA regimens.

Baseline NK features that favored response in earlier HCV studies included higher CD56^dim frequency and perforin, sometimes with lower CD16 expression; nonresponse has been linked to lower NK frequency or expansion of ($CD56^{-}CD16^{+}$) dysfunctional NK cells. While many DAA studies emphasize normalization, there are reports particularly in genotype-4 cohorts of increases in $CD16^{+}$ and $CD3^{-}CD56^{+}$ cells during SOF based regimens (e.g., SOF+DCV±RBV). (Childs et al. 2017) This aligns with Our "normal levels" in non-responders versus heightened NK in responders. We found that responders on Sofosbuvir& Ribavirin show elevated $CD16^{+}/CD56^{+}$ NK differs from the dominant DAA narrative of "attenuation/normalization," but is plausible given interferon-era parallels and some SOF-based reports especially in specific populations.

Most COVID-19 studies describe reduced absolute NK counts (both CD56^bright and CD56^dim) and shifts in subset composition, but our findings vary by time point and disease severity (e.g., decreased CD56^bright, shifts within CD56^dim). Some cohorts report early expansions or rebalancing within CD56^dim/CD16 subsets, while others note decreases in CD56^dim$CD16^{-}$ or emergence of unconventional subsets highlighting heterogeneity. (Maucourant et al., 2020). but our findings that are heterogeneous across disease severities and time points.

CD56^dim / CD16 dynamics: Data are mixed some cohorts show increases in CD56^dim early in disease or distinct rebalancing of activating/inhibitory receptors; others note decreases in CD56^dim$CD16^{-}$ after PCR positivity or expansions of unconventional subsets depending on severity and COVID-19 infection can acutely modulate NK cells, sometimes increasing CD56^dim or shifting CD16 expression, especially early potentially amplifying the NK changes you detected in responders (Maucourant et al., 2020). Our observation that concurrent COVID-19 is associated with higher CD16/CD56 frequencies in responders may reflect COVID-19 driven NK activation layered on top of HCV therapy effects. Given the heterogeneity in COVID-19 NK phenotypes, a time point- and severity-matched comparison is crucial.

Antiviral pressure can transiently activate or reshape NK compartments (stronger evidence with IFN/RBV; mixed with DAAs). Some SOF-based reports in genotype-4 populations show increases in $CD16^{+}/CD56^{+}$ populations during treatment consistent with your responders (Graydon et al., 2023).

Our data diverge from several DAA era studies that described attenuation/normalization of NK activation during IFN-free therapy, with recovery most evident post treatment; instead, we observed higher $CD16^{+}/CD56^{+}$ NK frequencies in responders, particularly when COVID-19 was present. This explained that acute SARS-CoV-2–driven NK activation superimposed on SOF/RBV modulated immunity yields a distinct phenotype in responders.

In our study for infected patients with hepatitis C virus we found that the frequency of Natural killer (CD 16) was high in responders whom was infected with covid 19 while in responders whom was not infected with covid 19 was normal. And so, in non-responders was normal. These means that a high frequency of Natural killer (CD 16) was observed due to Sovaldi and Ribavirin (anti-viral) therapy during infection with covid 19, And also, we found that the frequency of Natural killer (CD 56) was high in responders whom was infected with covid 19 while in responders whom was not infected with covid 19 was normal. And so, in non-responders was normal. These means that a high frequency of Natural killer (CD 56) was observed due to Sovaldi and Ribavirin (anti-viral) therapy during infection with covid 19, we found thatthe frequency of CD56 and CD16 in responders whom was infected with covid 19 was high while in responders whom was not infected with covid 19 and in non-responders was normal.These mean that a natural killer (CD16 and CD56) was a good point of comparison between groups.

Conclusions

We can conclude that the first group was response easily to treatment from covid-19. And this can be determined from the results of patients before treatment was high and after was normal. And it was a significant point for comparison. Likewise, in our study we found that in patients with hepatitis C virus home treated with Sovaldi and Ribavirin (anti-viral) therapy, the frequency of CD16 and CD56 was increased during infection with covid 19 and in not infected patients with covid 19 was normal.

So, it summarized that Sovaldi as a treatment of hepatitis c virus improves the response of patients to covid 19 therapy.

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Ethics approval and consent to participate

This study is performed according to ICH good clinical practice, Declaration of Helsinki and World Health Organization guidelines, and the Research ethics committee of Faculty of medicine for Girls FMG-IRB, Egypt. And all experimental protocols were approved by the Research ethics committee of Faculty of medicine for Girls FMG-IRB, Egypt. And the sampling process of this study was carried out at Arab contractor's medical center (ACMC) and the college of medicine for girls FMG-IRB, Egypt. The college of medicine for girls FMG-IRB Ethical Committee (Approval code 2098/2023).

Informed consent was obtained from all subjects according to the ethical committee for human research in the Faculty of medicine for Girls FMG-IRB, Egypt.Authors understand that their participation is voluntary.

Conflict of interests

All authors declare that they have no conflict of interests.

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References:

Ahlenstiel G, Edlich B, Hogdal LJ, Rotman Y, Noureddin M, Feld JJ, Holz LE, Titerence RH, Liang TJ, Rehermann B. (2011). Early changes in natural killer cell function indicate virologic response to interferon therapy for hepatitis C. Gastroenterology, 141(4):1231-9, 1239.e1-2. doi: 10.1053/j.gastro.2011.06.069.

Boussier J, Yatim N, Marchal A, Hadjadj J, Charbit B, El Sissy C, Carlier N, Pène F, Mouthon L, Tharaux PL, Bergeron A, Smadja DM, Rieux-Laucat F, Duffy D, Kernéis S, Frémeaux-Bacchi V, Terrier B. (2022). Severe COVID-19 is associated with hyperactivation of the alternative complement pathway. J Allergy Clin Immunol.;149(2):550-556.e2. doi: 10.1016/j.jaci.2021.11.004. Epub 2021 Nov 17. PMID: 34800432; PMCID: PMC8595971.

Brown P, Neuman M (2001): Immunopathogenesis of hepatitis C viral infection: Th1/Th2 responses and the role of cytokines. Clin Biochemistry; 34:167-71.

Burchill MA, Golden-Mason L, Wind-Rotolo M, Rosen HR. (2015). Memory re- differentiation and reduced lympho-cyte activation in chronic HCV-infected patients receiving direct-acting antivirals. J Viral Hepat 22: 983–991.doi:10.1111/jvh.12465.

Calvaruso V, Mazza M, Almasio PL (2011). Pegylated-interferon-α(2a) in clinical practice: how to manage patients suffering from side effects. Expert Opinion on Drug Safety 10 (3): 429–35. doi:10.1517/14740338.2011.559161

Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. (2020): "Clinical and immunological features of severe and moderate coronavirus disease 2019". The Journal of Clinical Investigation. 130 (5): 2620 2629. doi:10.1172/JCI137244. PMC 7190990. PMI D 32217835.

Childs K, Merritt E, Considine A, Sanchez-Fueyo A, Agarwal K, Martinez-Llordella M, Carey I. (2017). Immunological Predictors of Nonresponse to Directly Acting Antiviral Therapy in Patients With

Chronic Hepatitis C and Decompensated Cirrhosis. Open Forum Infect Dis. 3;4(2):ofx067.

Dessouki O, Kamiya Y, Nagahama H, Tanaka M, Suzu S, Sasaki Y, Okada S. (2010). Chronic hepatitis C viral infection reduces NK cell frequency and suppresses cytokine secretion: reversion by anti-viral treatment. BiochemBiophys Res Commun 393: 331–337. doi:10.1016/j.bbrc.2010.02.008.

Dustin B, Rice M. (2007): Flying under the radar: the inimunobiology of hepatitis C. Annu. Rev. Immunol.; 25:71-99.

EPCLUSA (sofosbuvir and velpatasvir) Prescribing information" 2017: (https://www.gilead.com/~/media/files/pdfs/medicines/liverdisease/epclusa/epclusa_pi.pdf?la=en) (PDF). Gilead Sciences, Inc. Archived (https://web.archive.org/web/20170630220003/http://gilead.com/~/media/files/pdfs/medicines/liver-disease/epclusa/epclusa_pi.pdf?la=en) (PDF) from the original on 30 June 2017. Retrieved 16 June 2017.

Golden-Mason L, Bambha KM, Cheng L, Howell CD, Taylor MW, Clark PJ, Afdhal N, Rosen HR, Virahep CSG. (2011). Natural killer inhibitory receptor expression associated with treatment failure and interleukin-28B genotype in patients with chronic hepatitis C. Hepatology 54: 1559–1569. doi:10.1002/hep.24556.

Graydon EK, Malloy AMW, Machmach K, Sun P, Paquin-Proulx D, Lizewski S, Lizewski R, Weir DL, Goforth CW, Anderson SK, Letizia AG, Mitre E. (2023). High baseline frequencies of natural killer cells are associated with asymptomatic SARS-CoV-2 infection. Curr Res Immunol. 15;4:100064. doi: 10.1016/j.crimmu.2023.100064.

Harvoni- ledipasvir and sofosbuvir tablet, film coated Harvoni- ledipasvir and sofosbuvir tablet, film coated Harvoni- ledipasvir and sofosbuvir pellet" 2022): (https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=f4ec77e4-bae8-4db0-b3d5-bde09c5fa075). DailyMed. Retrieved 26 January 2022.

Hussein A, Afifi M, Wardany A, Ramadan H, Gaber N. (2025). Expression of phosphatase and tensin homolog gene in hepatitis C virus induced hepatocellular carcinoma. Microbial Biosystems, 10(2), 97-105. doi: 10.21608/mb.2025.361569.1257

Malengier-Devlies B, Filtjens J, Ahmadzadeh K, Boeckx B, Vandenhaute J, De Visscher A, Bernaerts E, Mitera T, Jacobs C, Vanderbeke L, Van Mol P, Van Herck Y, Hermans G, Meersseman P, Wilmer A, Gouwy M, Garg AD, Humblet-Baron S, De Smet F, Martinod K, Wauters E, Proost P, Wouters C, Leclercq G, Lambrechts D, Wauters J, Matthys P. (2022). Severe COVID-19 patients display hyper-activated NK cells and NK cell-platelet aggregates. Front Immunol. 5;13:861251. doi: 10.3389/fimmu.2022.861251.

Maucourant C, Filipovic I, Ponzetta A, Aleman S, Cornillet M, Hertwig L, Strunz B, Lentini A, Reinius B, Brownlie D, Cuapio A, Ask EH, Hull RM, Haroun-Izquierdo A, Schaffer M, Klingström J, Folkesson E, Buggert M, Sandberg JK, Eriksson LI, Rooyackers O, Ljunggren HG, Malmberg KJ, Michaëlsson J, Marquardt N, Hammer Q, Strålin K, Björkström NK; Karolinska COVID-19 Study Group. (2020). Natural killer cell immunotypes related to COVID-19 disease severity. Sci Immunol. 21;5(50):eabd6832. doi: 10.1126/sciimmunol.abd6832.

Melo AKG, Milby KM, Caparroz ALMA, Pinto ACPN, Santos RRP, Rocha AP, Ferreira GA, Souza VA, Valadares LDA, Vieira RMRA, Pileggi GS, Trevisani VFM. (2021). Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: A living systematic review and meta-analysis. PLoS One. 29;16(6):e0253894. doi: 10.1371/journal.pone.0253894.

Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, Verma V. COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. (2020). PLoS Pathog. 21;16(8):e1008762. doi: 10.1371/journal.ppat.1008762.

Mondelli MU. (2015): Direct-acting antivirals cure innate immunity in chronic hepatitis C. Gastroenterology 149: 25–28. doi:10.1053/j.gastro.2015.05.026.

Oliviero B, Mele D, Degasperi E, Aghemo A, Cremonesi E, Rumi MG, Tinelli C, Varchetta S, Mantovani S, Colombo M, Mondelli MU. (2013). Natural killer cell dynamic profile is associated with treatment outcome in patients with chronic HCV infection. J Hepatol. ;59(1):38-44. doi: 10.1016/j.jhep.2013.03.003.

Recommendations for Testing, Managing, and Treating Hepatitis C (2016). (http://hcvguidelines.org/sites/default/files/HCV-Guidance_October_2016_a.pdf).

Rehman SU, Shafique L, Ihsan A, Liu Q. Evolutionary Trajectory for the Emergence of Novel

Coronavirus SARS-CoV-2 (2020). Pathogens. 23;9(3):240. doi: 10.3390/pathogens9030240.

Saied EM, El-Maradny YA, Osman AA, Darwish AMG, Abo Nahas HH, Niedbała G, Piekutowska M, Abdel-Rahman MA, Balbool BA, Abdel-Azeem AM. (2021): A Comprehensive Review about the Molecular Structure of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): Insights into Natural Products against COVID-19. Pharmaceutics.21;13(11):1759. doi: 10.3390/pharmaceutics13111759.

Shuai H, Chan JF, Hu B, Chai Y, Yuen TT, Yin F, Huang X, Yoon C, Hu JC, Liu H, Shi J, Liu Y, Zhu T, Zhang J, Hou Y, Wang Y, Lu L, Cai JP, Zhang AJ, Zhou J, Yuan S, Brindley MA, Zhang BZ, Huang JD, To KK, Yuen KY, Chu H. (2022). Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. Nature.;603(7902):693-699. doi: 10.1038/s41586-022-04442-5.

Spaan M, van Oord G, Kreefft K, Hou J, Hansen BE, Janssen HL, de Knegt RJ, Boonstra A. (2016) Immunological Analysis During Interferon-Free Therapy for Chronic Hepatitis C Virus Infection Reveals Modulation of the Natural Killer Cell Compartment. J Infect Dis.15;213(2):216-23. doi: 10.1093/infdis/jiv391. Epub 2015 Jul 28. PMID: 26223768.

Stegmann KA, Björkström NK, Veber H, Ciesek S, Riese P, Wiegand J, Hadem J, Suneetha PV, Jaroszewicz J, Wang C, et al. (2010). Interferon-α-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. Gastroenterology 138:1885-1897.e10.doi:10.1053/j.gastro.2010.01.051.

Stegmann KA, Björkström NK, Ciesek S, Lunemann S, Jaroszewicz J, Wiegand J, Malinski P, Dustin LB, Rice CM, Manns MP, Pietschmann T, Cornberg M, Ljunggren HG, Wedemeyer H. (2012). Interferon α-stimulated natural killer cells from patients with acute hepatitis C virus (HCV) infection recognize HCV-infected and uninfected hepatoma cells via DNAX accessory molecule-1. J Infect Dis. 1;205(9):1351-62. doi: 10.1093/infdis/jis210.

Sofosbuvir (2016). https://www.drugs.com/monograph/sofosbuvir.html). (2016): The American Society of Health-System Pharmacists. Archive (https://web.archive.org/web/20161201144328/https:// www.drugs.com/monograph/sofosbuvir.html) from the original on 1 December 2016.

Sovaldi 400 mg film coated tablets. (2016): Summary of Product Characteristics" (https://www.medicines.org.uk/emc/medicine/28539). UK Electronic Medicines Compendium. September 2016.

Sovaldi-sofosbuvir tablet (2020). Film coated Sovaldi-sofosbuvir pellet. (https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=80beab2c-396e-4a37-a4dc-40fdb62859cf). DailyMed. 27 September 2019. Retrieved 4 February 2020.

Sofosbuvir (Sovaldi) - Treatment - Hepatitis C Online"
(http://www.hepatitisc.uw.edu/page/treatment/drugs/sofosbuvir-drug). www.hepatitisc.uw.edu. Archived
(https://web.archive.org/web/20161223152415/http://www.hepatitisc.uw.edu/page/treatment/drugs/sofosbuvir-drug) from the original on 23 December 2016. Retrieved 8 January 2017.

Tao K, Tzou PL, Kosakovsky Pond SL, Ioannidis JPA, Shafer RW. (2022). Susceptibility of SARS-CoV-2 Omicron Variants to Therapeutic Monoclonal Antibodies: Systematic Review and Meta-analysis. Microbiol Spectr. 31;10(4):e0092622. doi: 10.1128/spectrum.00926-22. Epub 2022 Jun 14.

Wiersinga, W.J.; Rhodes, A.; Cheng, A.C.; Peacock, S.J.; Prescott, H.C. (2020). Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA 2020, 324, 782–793. Cells 2021, 10, 206 23 of 29.

World Health Organization (2019): World Health Organization model list of essential medicines: 21st list 2019. Geneva: World Health Organization. hdl:10665/325771 (https://hdl.handle.net/10665%2F325771). WHO/MVP/EMP/IAU/2019.06. License: CC BY-NC-SA 3.0 IGO.

World Health Organization (2021): World Health Organization model list of essential medicines: 22nd list (2021). Geneva: World Health Organization. hdl:10665/345533 (https://hdl.handle.net/10665%2F345533). HO/MHP/HPS/EML/2021.02.

World Health Organization Dashboard (2022): https:// covid 19. who. int/. Accessed 31.8.2022.

Yau AH, Yoshida EM. (2014). Hepatitis C drugs: the end of the pegylated interferon era and the emergence of all-oral interferon-free antiviral regimens: a concise review. Can J Gastroenterol Hepatol. 2014 Sep;28(8):445-51. doi: 10.1155/2014/549624.