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Impact of Sofosbuvir Plus Daclatasvir Therapy on the Frequency of CD200R$^+$ Dendritic Cells in Chronic Hepatitis C Virus Infection

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Abstract: Dendritic cells (DCs) play a crucial role in controlling viral infections. Little is known about the changes in frequencies of the DC subsets in patients with chronic hepatitis C (CHC), particularly in the era of interferon-free regimens. We aimed to evaluate the impact of sofosbuvir/daclatasvir on the frequency of different peripheral DC subsets, the expression of the inhibitory CD200R and its ligand CD200 on DC, and their relation to the treatment outcome. A total of 1000 patients with CHC were enrolled and treated with a fixed oral dose of 400 mg of sofosbuvir and 60 mg of daclatasvir for 12 weeks. A total of 940 patients achieved sustained virologic response (SVR), and only 60 patients were non-responders (NRs). The frequencies of the peripheral plasmacytoid (pDC) and myeloid (mDCs) subsets and their surface expressions of CD200R and CD200 molecules were analyzed using flow cytometry. This analysis included 60 non-responders (NR group), 60 randomly selected sustained virologic responders (SVR group) at baseline, and at the end of treatment, and 60 healthy controls. HCV infection was associated with a down-regulation in the frequency of mDC, compared to healthy controls. In addition, mDC in HCV-infected patients showed lower levels of CD200R. However, neither the pDC frequency nor their CD200R expression was significantly altered. Interestingly, by the end of therapy, the frequencies of circulating mDCs and CD200R$^+$mDC increased significantly in the SVR group and were even comparable to healthy controls. The levels of these cells were not normalized in the NR group. Percentages of mDCs and CD200R$^+$mDC subsets showed good prognostic accuracy for predicting virologic response to therapy. Our results showed that HCV infection was associated with modulation of the mDC frequency and their surface expression of CD200R. Successful daclatasvir and sofosbuvir combined therapy was associated with the normalization of the percentages of mDC and CD200R$^+$mDC.

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1. Introduction

Hepatitis C virus (HCV) infection remains a significant global health concern and contributes to chronic liver diseases, cirrhosis, and hepatocellular carcinoma (HCC). Despite the advent of direct-acting antivirals (DAAs), which have revolutionized HCV treatment by achieving a sustained virological response (SVR) in most patients, understanding the immune dysregulation associated with HCV remains critical for managing disease progression and co-infections. HCV is known to have a high propensity towards chronicity secondary to the poorly understood imbalance in the HCV-specific T cell response and/or the inefficient priming of antigen-presenting cells (APCs) that play a pivotal role in activating T cell responses [1–4].

Dendritic cells (DCs) are the most powerful professional APCs that play a central role in antiviral immune responses [5]. They lack the leukocyte lineage-specific markers CD3, CD14, CD16, CD19, CD20, and CD56 and predominantly express high levels of MHC class II (HLA-DR) [6,7]. Two distinct subsets of DCs are characterized in peripheral blood, namely the myeloid DCs (mDC), which are phenotypically characterized by being $CD11c^{+}$ $CD123^{-}$, and the plasmacytoid DCs (pDC), which are $CD11c^{-}CD123^{+}$ [8,9]. The main function of the mDC is in antigen processing and presentation and the subsequent T cell activation, while that of pDC is the regulation of the antiviral immune responses, and both produce high levels of type I interferon and cross-present viral antigens [5].

HCV infection is known to disrupt DC functionality, impairing their ability to activate T cells and produce antiviral cytokines, thereby contributing to viral persistence and immune evasion. However, there are conflicting results about the impact of chronic HCV infection on the DC frequency and functional capacity [10–19]. Some results highlighted the profound reduction in their numbers and function [17,18] and others did not [11,12,14,20–22].

CD200R is a member of the immunoglobulin super-family that is predominantly expressed on monocytes, macrophages, and dendritic cells [23]. CD200R delivers an inhibitory signal that dampens the immune response and decreases cytokine release [24].

Previous studies [25–27] have identified DCs as an accepted predictor of the response to interferon/ribavirin therapy. However, little is known about the changes in DC compartments in HCV-infected patients following treatment with the direct-acting antiviral agents in patients receiving the new interferon-free regimens, particularly in Egypt, where genotype 4 is predominant.

The impact of HCV on DC subsets and their regulatory pathways, particularly the CD200/CD200R axis, remains underexplored. Understanding these changes and their restoration following successful antiviral therapy can provide valuable insights into the immune reconstitution process and potential biomarkers of treatment response. Therefore, we aimed to evaluate the impact of the new interferon-free regimens on the frequency of different peripheral DC subsets. Also, we characterized the expression of the inhibitory CD200R on DCs and analyzed the relation between these cellular changes and treatment outcomes.

2. Materials and Methods

2.1. Ethics Statement

The study was conducted in accordance with ethical guidelines of the 1975 Helsinki Declaration and was approved by the local ethics committee of the Faculty of Medicine, Assiut University (IRB. 042024300524). All participants were adults, and all of them provided written informed consent before the collection of samples.

2.2. Patient Grouping and Selection Criteria

One thousand patients with chronic hepatitis C (CHC) were enrolled and treated with a 12-week course of oral antiviral therapy consisting of a combination of sofosbuvir (SOF; 400 mg) plus daclatasvir (DCV; 60 mg) (SOF/DCV) once daily. A total of 940 patients achieved a sustained virologic response (SVR), which is defined as undetectable HCV RNA at week 12 after the end of treatment. Patients were considered non-responders (NRs) if they had a detectable viremia at week 12 post-treatment [28]. At the end of the treatment, only 60 patients were NRs.

For each patient, two stored blood samples were analyzed; the first sample represented the baseline sample and was collected before starting the treatment regimen (week 0), and the second sample was collected at the 12th week post-treatment.

DC subsets were analyzed in the 60 NR patients and in 60 age- and sex-matched samples selected from the SVR group (randomly selected), one before treatment ($NR^{baseline}$ and $SVR^{baseline}$) and the other post-treatment (NR and SVR). A third group of healthy blood donors was used as a control group (n = 60).

2.3. Study Settings and Protocol

Enrolled patients were admitted to a tertiary care center for the management of viral hepatitis, Assiut University Hospitals, Assiut, Egypt between January 2018 and January 2019. Patients with detectable anti-HCV antibodies detected by ELISA and detectable HCV RNA detected by RT-PCR and who were eligible for HCV treatment with SOF/DCV were enrolled. All patients were aged older than 18 years and had only an HCV infection without any apparently concurrent medical illnesses.

Anti-HCV antibodies were detected by Ortho HCV Version 3.0 ELISA (Ortho Diagnostics Systems, Raritan, NJ, USA), with 99.95% specificity and 100% sensitivity. Detection of the plasma HCV viral load was performed using the Artus HCV-RG RT-PCR Kit (cat no. 4518265, Qiagen) using standardized quantitative real-time PCR according to the manufacturer's protocol, and amplification was performed by the ABI 7500 Fast Real-Time PCR Thermal cycler (Applied Biosystems, Foster City, CA, USA). The detection limit was $\leq$15 IU/mL, and the linear range was 15 IU/mL to 1×10^8 IU/mL.

Exclusion criteria: Patients were excluded if they were co-infected with the human immunodeficiency virus (HIV); co-infected with the hepatitis B virus (HBV); alcoholics; receiving hepatotoxic drugs, steroids, or other immune suppressive drugs; or those with other recognized causes of chronic liver disease.

2.4. Flow Cytometry Analysis

Plasmacytoid DC (pDC) and myeloid DC (mDC) in the peripheral blood of the selected patients were tested at baseline and 12 weeks after the cessation of treatment using flow cytometry. Briefly, peripheral blood mononuclear cells (PBMCs) were purified using Ficoll-Paque Plus gradient centrifugation (Sigma-Aldrich, St. Louis, MO, USA). Aliquots of purified PBMCs were cryopreserved and stored in liquid nitrogen on the day of testing. Cells were thawed, washed, and then surface-stained with a combination of FITC anti-lineage cocktail (CD3/14/16/19/20/56, clones UCHT1/HCD14/3G8/HIB19/2H7/HCD56), PE/Cy7 anti-HLA-DR (clone L243), PerCP/Cy5.5-labeled anti-CD11c (clone 3.9), Brilliant Violet 421 anti-human CD123 (clone 6H6), PE anti-CD200 (clone A18042B), and APC anti-CD200R (clone OX-108). Matching iso-type control antibodies were processed in a similar manner to determine cut-offs. All antibodies were purchased from BioLegend (USA). Cell acquisition and analysis were performed in the Flow Cytometry Unit, South Egypt Cancer Institute using the Canto II cell analyzer (BD Bioscience, CA, USA) and FlowJo V10 software (Tree Star Inc., Ashland, TN, USA).

Percentages of mDCs (Lin-, HLA-DR+, CD11c+, and CD123- cells) and pDCs (Lin-, HLA-DR+, CD11c-, and CD123+ cells) were assessed within PBMCs. Levels of CD200R and CD200 expressions were determined among both myeloid and plasmacytoid DCs. Gating strategies for identifying the DC subsets and their surface expression of CD200R are illustrated in Figure 1.

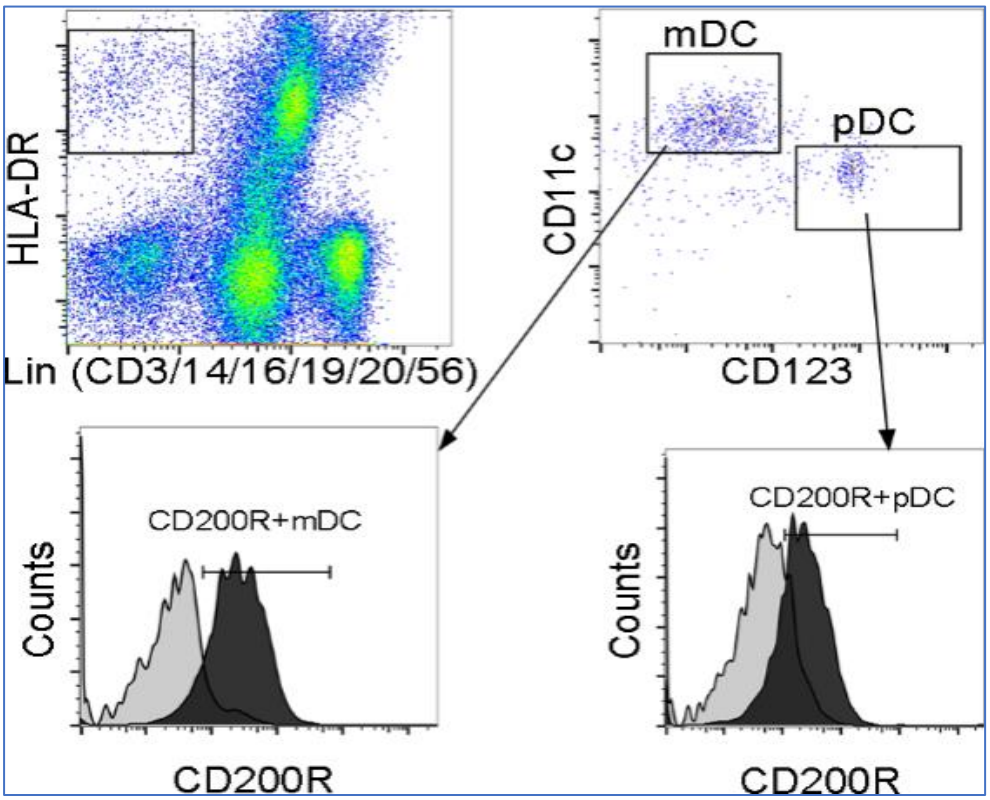

Figure 1. Gating strategy used to identify DC subsets and CD200R expression. PBMCs were stained with anti-lineage cocktail (CD3/14/16/19/20/56), anti-HLA-DR, labeled anti-CD11c, anti-human CD123, and APC anti-CD200R. DC subsets were identified by specific phenotype gating and analyzed for the expression of CD200R (black-filled histograms) against an isotype-matched control (gray-filled histograms).

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version 5. Percentages of cells were expressed as mean ± standard deviation (SD). Group comparisons were carried out using paired *t*-tests for normally distributed data. A p-value of <0.05 was considered statistically significant. Spearman correlation analysis was applied to evaluate associations between variables. Additionally, the areas under the receiver operating characteristic (ROC) curves were calculated to assess the performance of dendritic cell frequencies in predicting treatment outcomes. Sensitivity, specificity, and diagnostic accuracy, along with their 95% confidence intervals, were also estimated.

3. Results

The demographic and laboratory characteristics of the study groups are summarized in Table 1. No significant differences were observed between the $SVR^{baseline}$ and $NR^{baseline}$ groups in terms of age, sex distribution, or liver function parameters, including ALT, AST, albumin, and total bilirubin levels (all p-values > 0.05). Similarly, platelet counts were comparable between the two groups, indicating no overt baseline differences in hematological profiles. HCV RNA viral load was also similar between the $SVR^{baseline}$ and

$NR^{baseline}$ groups, with high levels of viremia detected in both groups before treatment ($p = 0.71$).

Table 1. Demographic and laboratory characteristics of the study patients at baseline and at the end of treatment.

Study Group	$SVR^{baseline}$ ($n = 60$)	$NR^{baseline}$ ($n = 60$)	SVR ($n = 60$)	NR ($n = 60$)	Healthy Controls ($n = 60$)	*p*-Value $SVR^{baseline}$ Vs $NR^{baseline}$
Sex (female/male)	14/46	12/48	14/46	12/48	18/42	0.86
Age (years)						
Mean ± SD	50 ± 10	52.0 ± 9	50 ± 10	52.0 ± 9	49 ± 10	0.37
Median (Range)	54 (30–65)	55 (40–70)	54 (30–65)	55 (40–70)	50 (30–60)	
ALT (IU/mL)						
Mean ± SD	60 ± 14	65 ± 20	32.5 ± 17.5	67.5 ± 22.5	19.5 ± 5.5	0.38
Median (Range)	50 (35–90)	60 (30–95)	33 (25–55)	58 (30–98)	20 (5–30)	
AST(IU/mL)						
Mean ± SD	81.1 ± 52.4	79.0 ± 43.4	25.9 ± 18.9	92.2 ± 23	16 ± 7.5	0.95
Median (Range)	80 (45–140)	86 (40–150)	33 (25–52)	85 (42–161)	16 (5–30)	
Albumin (g/dL)						
Mean ± SD	3.9 ± 0.8	3.8 ± 0.9	3.8 ± 1.1	3.6 ± 0.4	4.5 ± 0.7	0.46
Median (Range)	3.5 (3–5)	3.3 (2.5–4.5)	3.8 (3.5–4.6)	3.1 (3–4.2)	4.5 (4.3–5.4)	
Total bilirubin (mg/dL)						
Mean ± SD	1.9 ± 1.5	2.1 ± 1.3	1.2 ± 0.8	2.2 ± 1.4	0.5 ± 0.4	0.29
Median (Range)	1.6 (1.4–3.2)	1.7 (1.3–3.5)	1.1 (0.6–2.1)	1.5 (1.2–3.6)	0.4 (0.2–1.1)	
Platelets $10^3/mm^3$						
Mean ±SD	185.7 ± 50.6	177.5 ± 45.2	190.8 ± 55.7	170 ± 53.7	254.5 ± 71	0.35
Median (Range)	184 (110–339)	175 (105–330)	191 (140–350)	180 (120–310)	256 (150–455)	
HCV RNA load (copies/mL)						0.71
Mean ± SD	$(2 \pm 1.6) \times 10^6$	$(2 \pm 1.8) \times 10^6$	Below detection limit	$(2.3 \pm 2) \times 10^6$	NA	
Median (Range)	$1.8\ (0.5–6.8) \times 10^6$	$2\ (1–7) \times 10^6$		$2\ (1–8.1) \times 10^6$		

Data are expressed as mean ± standard deviation, followed by medium (range).

3.1. Changes in Frequency of mDC Between Groups

Analysis of DC frequencies showed that, at baseline, mDCs were significantly lower in CHC patients naive to therapy ($SVR^{baseline}$ and $NR^{baseline}$), compared to healthy controls. The mean frequencies of mDCs in the $SVR^{baseline}$ and $NR^{baseline}$ groups were 55% and 56.4%, respectively, versus 73.6% in healthy controls ($p = 0.0015$ and 0.0016, respectively; Figure 2A).

However, following treatment, the mDC frequency in the SVR group increased significantly from 55% at baseline to 69%, achieving levels comparable to healthy individuals ($p = 0.0001$ and <0.05 compared to $SVR^{baseline}$ and healthy controls, respectively). In contrast, mDC frequencies in NR patients remained low, demonstrating no significant recovery post-treatment.

These findings indicate that successful viral elimination with sustained virologic response (SVR) is associated with the restoration of mDC frequencies to normal levels, while treatment failure (NR) is characterized by a persistent reduction in mDCs. This highlights the potential of mDC frequency as a biomarker for therapeutic success in CHC patients.

3.2. Changes in Frequency of pDC Between Groups

At baseline, there were no significant differences in the frequencies of pDCs between the $SVR^{baseline}$ or $NR^{baseline}$ group and healthy controls ($p = 0.0759$ and $p = 0.0698$, respectively; Figure 2B). Similarly, after treatment, no significant changes in pDC frequencies were observed within the SVR group (18.5% to 17.5%; $p = 0.6041$) or the NR group (19.7% to 17.7%; $p = 0.2657$). These results suggest that pDC frequencies remain relatively stable and are not significantly influenced by HCV infection or its clearance following treatment,

highlighting the distinct response of pDCs compared to other dendritic cell subsets, such as mDCs.

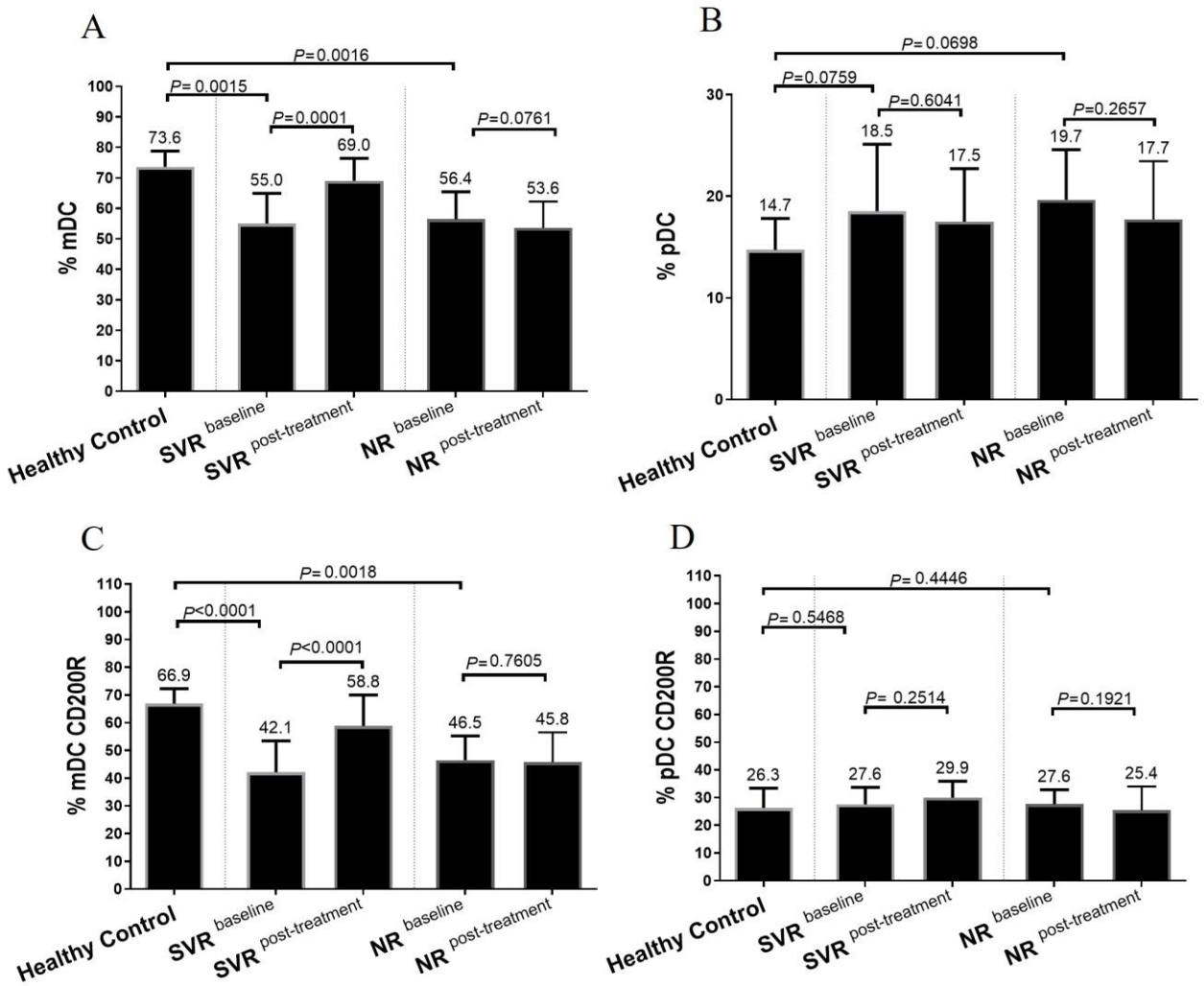

Figure 2. Changes in the frequency of mDC and pDC subsets and their CD200R expression across different study groups. (**A**) shows the percentage of myeloid dendritic cells (mDC), while (**B**) depicts the percentage of plasmacytoid dendritic cells (pDC). (**C**) presents the percentage of mDC expressing CD200R, and (**D**) illustrates the percentage of pDC expressing CD200R. **SVR**: patients who achieved sustained virological response; **NR**: Non-responders. Group comparisons were carried out using a paired *t*-test. Columns represent the mean, and error bars indicate the standard deviation.

3.3. Expression of the Inhibitory CD200R on DC

Evaluation of CD200R expression on dendritic cell subsets revealed that CD200R was more prominently expressed on the mDC subset of DC. $CD200R^{+}$mDC were significantly lower in treatment-naïve HCV-infected patients ($SVR^{baseline}$ and $NR^{baseline}$), compared to healthy controls. Mean $CD200R^{+}$mDC in $SVR^{baseline}$ and $NR^{baseline}$ were 42.1% and 46.5%, respectively, versus 66.9% in healthy controls ($p < 0.0001$ and $p = 0.0018$ respectively; Figure 2C).

Following treatment, patients who achieved SVR were associated with significantly elevated levels of $CD200R^{+}$mDC, compared with $SVR^{baseline}$ ($p < 0.0001$). The levels of CD200R+mDC increased to 58.8% in SVR individuals, reaching levels comparable to the healthy control group ($p > 0.05$ for comparison with healthy controls; Figure 2C), whereas no significant difference was observed between $NR^{baseline}$ and NR patients (mean = 46.5; $p = 0.76$).

On the other hand, frequencies of CD200R$^+$pDCs were not changed in either SVR or NR groups (Figure 2D). We further analyzed the possible modulation in the expression of CD200, the ligand for CD200R, on DC. However, no differences in the mean frequencies of CD200$^+$mDCs or CD200$^+$pDCs were observed among all study groups (p values > 0.05). These findings highlight a significant reduction in CD200R expression on mDCs in HCV-infected patients, which is restored with successful treatment. The persistent low levels in non-responders suggest a potential link between CD200R expression and treatment outcomes, supporting its role as a marker of immune regulation and therapeutic response in chronic HCV.

3.4. Receiver Operating Characteristic Curve Analysis for Predicting the Achievement of SVR

Receiver operating characteristic (ROC) curve analysis was carried out to determine the performance of the percentages of mDCs and mDCs expressing CD200R in predicting SVR in DCV plus SOF-treated CHC patients. Both the percentages of mDC and CD200R$^+$ mDC showed good accuracy in predicting the achievement of SVR ($p < 0.0001$ for both). The area under the ROC curve (AUC) for the percentages of mDC was 0.92 (95% CI = 0.79–0.98), and the best cut-off for predicting SVR was >59%, with a sensitivity and specificity of 90 and 80%, respectively. Meanwhile, the AUC for the percentages of CD200R$^+$ mDC was 0.8 (95% CI = 0.61–0.92), and the best cut-off for predicting SVR was >56%, respectively. No significant differences were observed between the two percentages in the accuracy of the prediction of SVR. On the other hand, the percentages of CD200$^+$ mDC did not show a good accuracy in predicting the achievement of SVR (Figure 3).

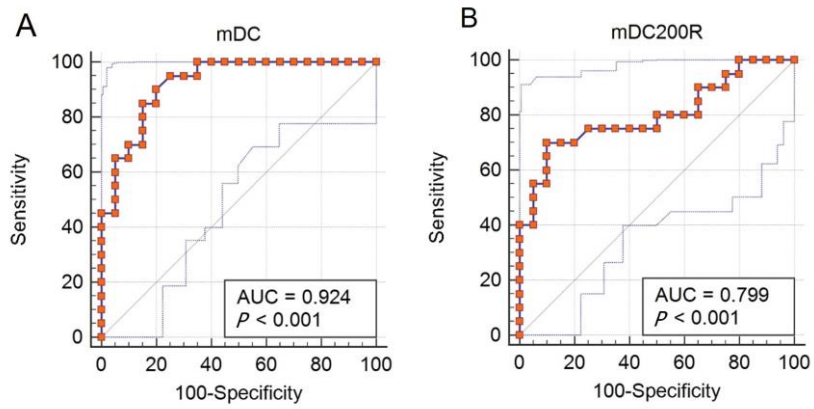

Figure 3. Receiver operating characteristic (ROC) curve analysis to determine the performance of the percentages of mDCs (**A**) and mDCs expressing CD200R (**B**) in predicting the SVR in DCV plus SOF-treated CHC patients.

4. Discussion

In Egypt, chronic HCV infection remains a significant health concern and may lead to cirrhosis and HCC. Although most patients achieve sustained virological response with DAAs, it is still crucial to understand the immune dysregulation associated with HCV and its variants to achieve ultimate control and to avoid recurrence, disease progression, and co-infections [29–32].

The findings of this study highlight the significant immunomodulatory effects of chronic HCV infection and its treatment with direct-acting antivirals (DAAs) on dendritic cell (DC) subsets and their expressions of CD200 and CD200R. Our results demonstrate that HCV infection is associated with a reduction in the frequency of myeloid DCs (mDCs) and the diminished expression of the inhibitory receptor CD200R on these cells, suggesting an impaired regulatory mechanism that may contribute to chronic inflammation and immune dysfunction. Interestingly, successful viral clearance following DAA therapy restored both

mDC frequency and CD200R expression, emphasizing the potential of these immunological markers as predictors of treatment response and immune restoration.

Daclatasvir and sofosbuvir, two newly discovered DAAs, are part of the preferred regimens in the WHO guidelines. They are much more effective, safer, and better-tolerated than the older therapies and can cure most persons with HCV [33,34]. Our study assesses the impact of a 12-week regimen of the DCV and SOF drugs on the frequencies of different peripheral DC subsets in CHC patients with genotype 4. Since liver biopsies were difficult to obtain, our analysis was restricted to the peripheral blood DCs that represent the most accessible source of DCs, rendering it an appropriate procedure for patient follow-up [3].

Our results, in agreement with some previous studies, showed that the frequencies of mDC were lower in CHC patients, compared with healthy controls [11,12,18,35,36]. In addition, Albert, Decalf [37] reported that pDC seems to be uninvolved in the immune pathogenesis of HCV infection. Some authors proposed that this DC deficiency is due to the HCV infection of DCs, making them more prone to apoptosis [3]. Another explanation for the reduction in mDC is possibly their mobilization into the inflamed liver or lymphoid tissues, causing their disappearance from the periphery, or maybe partially due to the reduction in DC progenitors [12].

Earlier studies have proved the increase in DC frequency following PEG-IFN ribavirin dual therapy [38,39]. Likewise, in our study, following 24 weeks of therapy of DCV plus SOF, the frequency of circulating mDCs increased significantly in patients who achieved SVR and was even comparable to normal subjects. On the contrary, the level of these cells was not normalized in the non-responders. This indicates that successful viral elimination is associated with the normalization of mDC levels.

The CD200:CD200R inhibitory signaling pathway plays a crucial role in controlling inflammation during viral infection. This inhibitory signaling may have a beneficial role in controlling unwanted immunopathology by protecting the host from the cytokine storm, which is a main cause of morbidity and mortality [40]. On the other hand, a growing number of viral pathogens have evolved means to manipulate this inhibitory pathway to down-regulate the host defenses, leading to viral evasion and persistence [41–43].

Different studies have assessed the role of CD200R in viral infections [40,44,45]. In some viral infections, the lack of CD200R signaling strongly enhances type I interferon production and viral clearance and improves the outcome of viral infections like mouse hepatitis coronavirus infection, while in others like the influenza A virus infection, the absence of CD200R signaling results in enhanced lung neutrophil influx and pathology [44]. Interestingly, some viruses express CD200 orthologues to induce the inhibitory CD200R signaling to dampen the immune response to favor viral growth and multiplication [43].

Our study is the first to investigate the frequencies of $CD200^+$ and $CD200R^+$ DC subsets during the course of HCV infection and their association with DAA treatment failure. Our results showed that $CD200R^+$mDC was lowered in CHC patients, increased significantly in patients with SVR, but remained low in NR patients. These findings were similar to a previous study that reported that the expression of virus-specific CD200R by $CD4^+$ T cells was significantly higher in HCV resolvers compared with chronically infected patients. These data collectively indicate that the up-regulation of this inhibitory receptor may be a critical step in the development of immune dysfunction during chronic HCV infections and that the response to the antiviral treatment could be attributable to this pathway [46].

The finding of a significant correlation between the HCV RNA load and the frequencies of both mDC and $CD200R^+$mDC supports previous reports that suggest that the DC defect seen in CHC patients is restricted to the period of active viral replication but can be resolved after viral clearance [27,47]. Moreover, the significant negative correlations

of CD200R^{+} mDC with ALT, AST, and the fibrosis grade support its prominent role in limiting inflammation, and thus, the decreased expression of CD200R is associated with the increased severity of hepatic fibrosis, which, as stated previously, is driven by inflammatory responses to injured tissue [48].

Taken together, the percentages of mDC and CD200R^{+}mDC could be used as good predictors of the achievement of SVR to DCV and SOF combined therapy. These observations not only deepen our understanding of the immune dysregulation associated with HCV but also underscore the value of monitoring DC phenotypes in evaluating therapeutic outcomes.

HCV infection significantly impacts both the frequency and function of T cells. Chronic HCV infection is associated with persistent antigen exposure, leading to the upregulation of inhibitory molecules such as programmed death-1 (PD-1) on CD8+ T cells, resulting in functional exhaustion and impaired antiviral responses. CD4+ T helper cells also show reduced proliferative capacity and cytokine production in chronic HCV infection. In addition, HCV-specific CD8+ memory T cell responses are often defective, contributing to the inability to clear the virus. Importantly, regulatory T cells (Tregs) are increased in HCV-infected patients, which suppresses antiviral immune responses and promote viral persistence [49–53]. HCV infection can also influence T cell responses in patients co-infected with other pathogens, such as Mycobacterium tuberculosis, potentially altering the immune response to these infections [1,54–56].

NK cells play a critical role in early antiviral defense, but their function is also compromised during HCV infection. Chronic HCV infection results in an increased frequency of CD56^{bright} NK cells (regulatory subset) and a decreased frequency of CD56^{dim} NK cells (cytotoxic subset). Also, HCV induces the downregulation of activating receptors, such as NKG2D and NKp46, leading to reduced NK cell-mediated cytotoxicity. Moreover, NK cells exhibit impaired production of interferon-gamma (IFN-γ), which is crucial for antiviral immunity, but may overproduce pro-inflammatory cytokines like TNF-α, contributing to liver damage [57,58]. Additionally, HCV infection affects B cell frequency and function, contributing to immune dysregulation. HCV-specific neutralizing antibody responses are often delayed or inadequate, facilitating viral persistence [59,60].

Our findings underscore the critical role of dendritic cell subsets and their immunoregulatory markers, such as CD200R, in the pathogenesis of HCV and highlight their potential as biomarkers for predicting therapeutic outcomes, paving the way for future studies to explore targeted immunomodulatory strategies in the management of chronic viral infections.

5. Conclusions

HCV infection was associated with a reduction in the mDC subset of DC and its CD200R expression. These immunological changes were restored following successful DCV and SOF combined therapy.

Author Contributions: H.F.H., M.A.E.-M., and O.E.-B. conceived and designed the experiments; H.F.H., O.E.-B., D.S., E.H.S., M.E.A., D.S.M., and M.A.E.-M. performed the experiments; M.A.M., H.I.S., and A.A.S.M. recruited patients, carried out the clinical investigations, and collected patients' clinical data; H.F.H., M.A.M., H.I.S., M.E.A., D.S.M., O.E.-B., and M.A.E.-M. prepared the original draft of the manuscript; and all authors participated in data analysis, writing, review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with ethical guidelines of the 1975 Helsinki Declaration and was approved by the local ethics committee of Faculty of Medicine, Assiut University (IRB. 042024300524). All participants were adults, and all of them provided written informed consent before the collection of samples.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients to publish this paper.

Data Availability Statement: The datasets used in this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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