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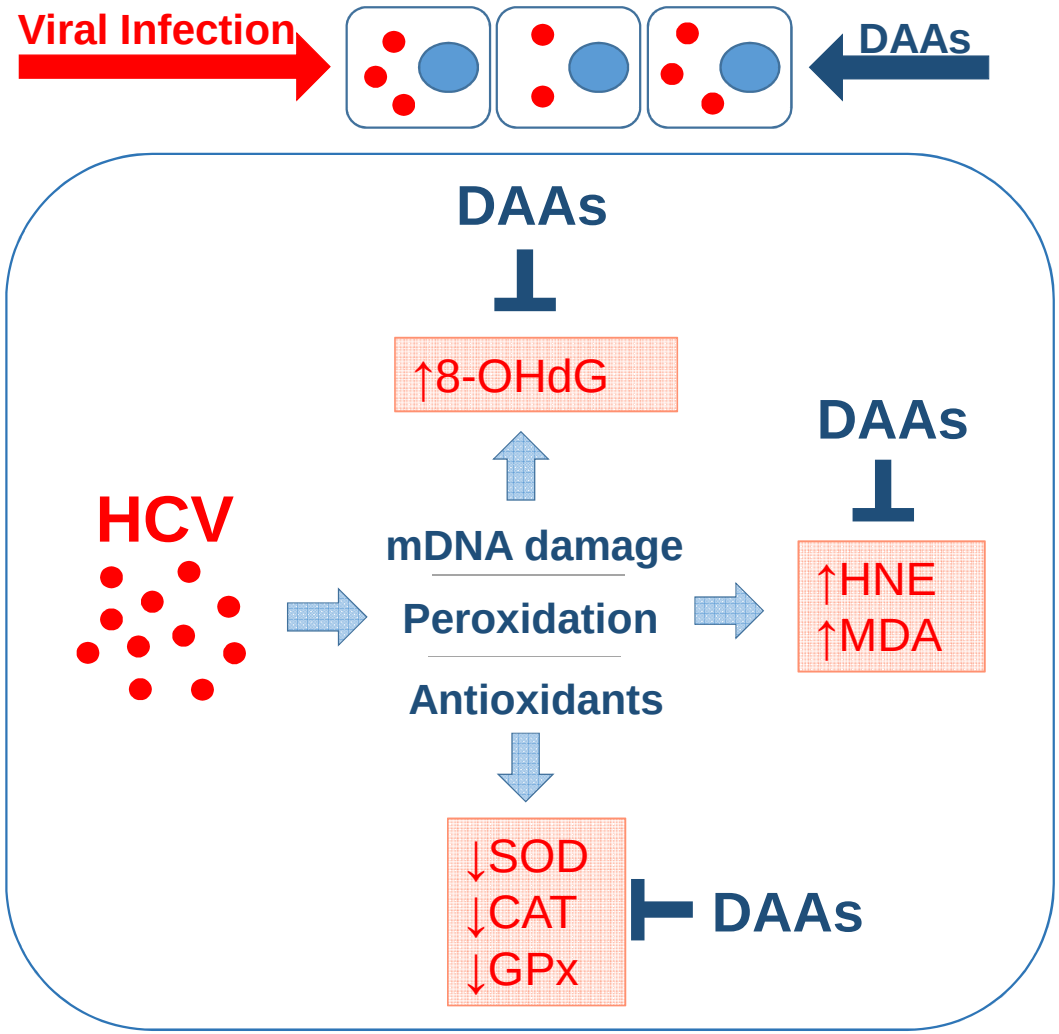
Viral Infection
DAAs
DAAs
↑8-OHdG
DAAs
HCV
mDNA damage
Peroxidation
Antioxidants
↑HNE
↑MDA
↓SOD
↓CAT
↓GPx
DAAs

DIRECT-ACTING ANTIVIRALS RESTORE SYSTEMIC REDOX HOMEOSTASIS IN CHRONIC HCV PATIENTS

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Abstract

Chronic hepatitis C therapy has completely changed in the last years due to the availability of direct-acting antivirals (DAAs). Removing the virus may be not enough since chronic infection deeply modifies immune system and cellular metabolism along decades of inflammation. Oxidative stress plays a significant role in maintaining systemic inflammation during chronic HCV infection. Other than removing the virus, effective therapy could counteract oxidative stress. This study investigated the impact of DAA treatment on circulating markers of oxidative stress and antioxidant defence in a cohort of patients affected by chronic hepatitis C. To this, an observational study on 196 patients who started therapy with DAA for HCV-related hepatitis was performed. Patients were assessed at baseline, 4 weeks after the initiation of therapy (4wks), at the end of treatment (EoT), and 12 weeks after the EoT (SVR12). Circulating oxidative stress was determined by measuring serum hydroxynonenal (HNE)- and malondialdehyde (MDA)-protein adducts, and 8-hydroxydeoxyguanosine (8-OHdG). Antioxidant status was evaluated by measuring the enzymatic activity and mRNA expression of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in peripheral blood mononuclear cells. We observed a reduction of serum 8-OHdG at 4wks, while the circulating level of both HNE- and MDA-protein adducts diminished at EoT; all these markers persisted low at SVR12. On the other side, we reported an increase in the enzymatic activity of all the antioxidant enzymes in PBMC at EoT and SVR12. Taking into account circulating 8-OHdG and antioxidant enzyme activities, patients with high fibrosis stage were those that had the most benefit from DAA therapy. To conclude, this study indicates that treatment with DAAs improves the circulating redox status of patients affected by chronic hepatitis C. This positive impact of DAA therapy may be related to its effectiveness on cutting down viremia and pro-inflammatory markers.

Keywords: Direct-acting antivirals; redox balance; HCV infection

Introduction

Chronic hepatitis C virus (HCV) infection affects 1% of the population and is a main cause of cirrhosis and hepatocellular carcinoma (HCC) with approximately 400 000 annual deaths worldwide (1). Effective treatment of chronic HCV infection, defined by a sustained virologic response (SVR), is determinant to manage this considerable public health problem. In the last years, the clinical management of patients has greatly improved because of the availability of direct-acting antivirals (DAAs), which are safe and effective at eradicating HCV infection (2).

Indeed, patients who achieve an SVR after DAA treatment show less cirrhosis-related complications, as well as reduced risk for mortality and hepatocellular carcinoma (3, 4). At the same time, clearance of the infection does not reduce risks to develop cirrhosis and cancer to the initial level, observed in the uninfected population (5). One of the factors that contributes to liver injury is the alteration of the redox balance of the host cell, by increasing production of reactive oxygen species (ROS) and alteration in the expression of scavenging enzymes (5).

Oxidative stress, defined as the production of reactive species which overwhelms the antioxidant defences, is a process deeply involved in the progression of liver damage during chronic hepatitis C (6). Indeed, patients affected by chronic hepatitis C present with altered pro- and antioxidant markers in both body fluids and liver specimens (7-11).

Excessive oxidative markers may be the result of chronic inflammation, iron overload, but also HCV encoded proteins (12).The non-structural protein 5A affects the intracellular Ca^{2+} signalling, which in turn triggers production of reactive species by mitochondria (13), and the HCV core protein may cause oxidative damage exerting a direct effect on mitochondria (14). In addition, chronic HCV infection is associated with decreased circulating antioxidant defences, such as reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (9, 15). Recent evidence also suggests that the disruption of redox balance observed in infected patients is irrespective of HCV genotype (16). However, much less is known about redox biology of viral infections since studies so far have been focused on the mechanism by which HCV augment ROS

production and limits antioxidant defense. Several studies have suggested that HCV replication and production of infectious virions are suppressed by lipid peroxides whose production is augmented by the infection (17).

By removing the noxious stimulus of the virus and inflammation, rapid and complete response observed early after starting DAA therapy might normalize redox homeostasis in chronically infected HCV patients. The response to a traditional antiviral regimen (pegylated interferon α-2b and oral ribavirin) is accompanied by an improvement in circulating redox balance, while failure is characterized by persistent oxidative stress (18).

With an efficacy near to 100%, DAAs may be beneficial to the systemic redox balance and play a role in limiting liver damage and starting fibrosis regression. The present study was designed to investigate the impact of DAA treatment on circulating markers of oxidative damage and antioxidant defence in a cohort of patients affected by chronic hepatitis C.

Patients and Methods

Study population and design

We conducted an observational study on 196 HCV+ and HIV- patients who started therapy with DAA as standard-of-care treatment for HCV-related hepatitis. All patients were referred to the C.U.R.E. (Centro per la Ricerca e la Cura delle Epatopatie) at the University of Foggia, Italy, between March 2015 and December 2018. Patients with concurrent active cancer and other chronic diseases or taking antioxidant supplementation were excluded. Fifty-seven percent of patients were females and the median age was 63 years (range 35-90 yrs). Plasma was collected from patients at multiple time points, including at baseline on the day of starting therapy (T0), after 4 weeks of drug administration (4wks), on the day of the final treatment (EoT), and at 12 weeks post-treatment (sustained virological response, SVR12). Patients with all the samples collected at baseline, during (4wks) and at the end of treatment (EoT), and after the treatment (SVR12) were considered for the final analysis. Liver fibrosis was assessed by transient elastography, performed with the Fibroscan® (Echosense, Paris, France) medical device, using the M or the XL probe after overnight fasting

following standard requirements of the Echosense (19). Stiffness cut-off values reported by Arena et al were used for staging liver fibrosis during transient elastography (20). 7.8 KPa, 10.8 KPa and 14.8 KPa were used as cut-off points for mild fibrosis, significant fibrosis and cirrhosis, respectively. The Authors' institutional review board approved the clinical investigation, which was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all patients to participate in the study. Hepatitis C treatment antiviral therapy and treatment duration (12 or 24 weeks) were indicated for each patient according to the viral genotype/subtype and the severity of liver disease, according to European Medicine Agency and National Drug Agency (AIFA) reimbursement restriction.

Patients received one of the following regimens:

1. sofosbuvir + simeprevir;
2. sofosbuvir/ledipasvir;
3. sofosbuvir + daclatasvir;
4. ombitasvir/paritaprevir/ritonavir ± dasabuvir (3D);
5. glecaprevir/pibrentasvir

Real-time polymerase chain reaction (PCR) was used for HCV-RNA quantification (Abbott RealT*ime* HCV Amplification Reagent Kit on Abbott Automatic m2000 system), with a lower detection of 12 IU/mL. Patients were followed up monthly with clinical and laboratory evaluations during antiviral therapy. Virologic response was assessed at week 4, at the end of treatment, and at 12 weeks after the end of treatment to determine the SVR. A rapid virologic response (RVR) was defined as undetectable HCV-RNA after 4 weeks of therapy. SVR12 was defined as undetectable HCV-RNA 12 weeks after the treatment completion. In patients without a complete 12-week follow-up, the final virologic and biochemical status was reported. Failure of DAAs and any adverse events were also recorded.

Laboratory measurements

Blood samples were obtained from an antecubital vein between 8:00 and 9:00 AM, after an overnight fast, and immediately processed. Laboratory measurements included the detection of HCV-RNA and the analysis of serum cytokines.

Cytokine measurement was performed using Biochip Array Technology (BAT) by Randox Laboratories. The array was used to measure serum levels of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNFα). The assay was performed using Randox apparatus and manufacturer's recommendations. Samples were tested in duplicate, and expressed as mean of two measurements.

Peripheral blood mononuclear cells (PBMC) were immediately isolated by a rapid Ficoll–Hystopaque centrifugation for 30 min at 900×g (21). Serum was separated within 24 h of sample collection. Eppendorf test tubes filled with separated serum samples were stored at −80°C until further analysis.

Serum fluorescent adducts formed between peroxidation-derived aldehydes (HNE and MDA) and proteins were measured by spectrofluorimetry as previously reported (27). The concentration of 8-hydroxydeoxyguanosine (8-OHdG) in serum was analyzed using highly sensitive 8-OHdG ELISA monitoring kit (Jaica, Fukuroi, Japan), according to the manufacturer's instructions (22).

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were analysed in PBMC by spectrophotometry method following the respective Cayman Assay kits procedures: SOD (n° 706002), CAT (n° 707002), GPx (n° 703102).

RNA isolation and quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

Total cellular RNA was isolated from PBMC samples using the RNAeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. Samples were quantified by absorption spectrophotometry, and RNA integrity was confirmed using nondenaturing agarose gel electrophoresis. cDNA was obtained using a random hexamer primer and a SuperScript III Reverse Transcriptase kit as described by the manufacturer (Invitrogen, Frederick, MD, USA). A PCR

master mix containing the specific primers (superoxide dismutase 1 (SOD1): forward, TGT GGG GAA GCA TTA AAG G; reverse, CCG TGT TTT CTG GAT AGA GG; catalase (CAT): forward, GCC ATT GCC ACA GGA AAG TA; reverse, CCA ACT GGG ATG AGA GGG TA; glutathione peroxidase (GSH-Px): forward, GGA GAC CTC ACC CTG TAC C; reverse, GTC ATT CAC CAT GTC CAC C; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, CAA GGC TGA GAA CGG GAA; reverse: 59-GCA TCG CCC CAC TTG ATT TT-39) was added, along with AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). Real-time quantification of mRNA was performed with a SYBR Green I assay and evaluated using an iCycler detection system (Bio-Rad Laboratories). The threshold cycle (CT) was determined, and the relative genes expression was subsequently calculated as follows: fold change=$2^{-\Delta(\Delta CT)}$, where $\Delta_{CT}=CT_{target}-CT_{housekeeping}$ and $\Delta(\Delta CT)=\Delta CT_{treated}-\Delta CT_{control}$.

Statistical analysis

Data were expressed as count and percentages for qualitative values, and as mean ± standard deviation of the mean (SDM) for quantitative variables. Gaussian distribution of the samples was evaluated by Kolgomorov–Smirnov test. Temporal trends of laboratory markers were studied using the one-way analysis of variance (ANOVA). Each analysis was conducted for the whole study population. Further analyses were performed comparing patients with no or mild fibrosis (F0/F1) vs significant fibrosis (F3/F4), according to the METAVIR scoring system (23), genotype 1 vs genotype non-1, sofosbuvir-based vs sofosbuvir-free treatment, RVR vs non-RVR. The significance of differences between different groups at each time studied was assessed by the two-way analysis of variance (ANOVA), to test the main effects of time and fibrosis/genotype/treatment/response as between-subject factor; the interaction time × fibrosis/genotype/treatment/response was studied, and a Sidak test was applied as post hoc test for multiple comparisons. A linear regression model was used to analyze the association between changes in serum HCV-RNA or TNF-α and circulating markers of oxidative stress (HNE- and MDA-protein adducts, 8-OHdG). All tests were 2-sided, and

P values <0.05 were considered statistically significant. Statistical analysis was performed with the Statistical Package for Social Sciences version 23.0 (SPSS, Inc., Chicago, IL) and the package Graph-Pad Prism 6.0 for Windows (GraphPad Software, Inc., San Diego, CA).

Results

Baseline patient characteristics

Baseline characteristics of patients are shown in Table 1. Nearly half of study population (45%) had baseline ALT levels ≤ 1.5 x ULN. The median baseline log10 HCV RNA level was 5.58 IU/mL (range 2.3–6.9 IU/mL) and genotype 1 was the most frequently detected (61%). 96 patients (49%) were treated with sofosbuvir-based regimens while 100 patients (51%) were treated with sofosbuvir-free regimen. All study participants reached SVR at the end of the treatment and 6 relapses were observed after treatment. A rapid virologic response (RVR) was observed in 61 (31%) patients.

HCV viremia during antiviral treatment and after the end of treatment is shown in Figure 1.

Effect of antiviral treatment on circulating redox balance

To assess the impact of DAA treatment on systemic redox balance, we measured circulating markers of oxidative stress (such as serum proteins oxidatively modified by lipoperoxidative reactions, as well as oxidatively damaged DNA), and the serum antioxidant capacity. We observed a significant decrease of both HNE- and MDA-protein adducts in the serum of patients at the end of treatment, which persisted 12 weeks after treatment discontinuation (Figure 2A-B). Of interest, serum levels of 8-OHdG were reduced at 4 weeks after initiation of therapy and persisted low at the end of treatment and at SVR12 (Figure 2C). On the other side, we did not notice any significant change in the serum trolox equivalent antioxidant capacity at any time of the study. Taken together, these results suggest that the improvement in circulating redox balance observed in HCV patients treated with DAA is associated with a reduction in the oxidative injury to proteins and DNA, even though we did not describe an increased systemic reactive species scavenging ability.

Since diabetes may associate to increased oxidative stress, a subgroup analysis including only diabetic patients was performed; trend and magnitude of changes were similar to the overall population (data not shown).

Effect of antiviral treatment on circulating inflammation cytokines

Cytokine profile during and after treatment is reported in Figure 3. IL-6 and IL-8 did not change across time points (Fig 3A and 3B) as compared to baseline whereas IL-10 and TNF-α normalized at the end of treatment onwards (Fig 3C and 3D). At the same time IL-10 significantly decreased from 1.53 pg/ml to 0.2 pg/ml ($p<0.05$) and TNF-α from 4.24 pg/mL to 2.82 pg/mL ($p<0.01$) and this was confirmed at SVR (IL-10 0.1 pg/ml ad TNF-α 0.8 pg/ml, respectively). The same findings were confirmed in a subgroup analysis by treatment regimen used.

DAA treatment restores redox balance by improving antioxidant enzymes activity

To better analyze the impact of DAA treatment on the circulating redox balance, and to clarify the possible mechanism of reduced oxidative damage, the enzymatic activity of several antioxidant enzymes (such as SOD, CAT, and GPx) was performed in PBMC extracted from patients at each time point. Of note, the SOD activity was reduced at 4 weeks after initiation of therapy, but it increased with respect to baseline at the end of treatment and persisted higher at SVR12 (Figure 4A). As compared to baseline, we observed an increase in both the CAT and the GPx activity at 4 weeks after initiation of therapy, which continued to rise until the end of the study period (Figure 4B-C).

We also evaluated the mRNA expression of antioxidant genes in PBMC. We noticed that the SOD gene expression followed a similar trend to its enzymatic activity during and after the DAA treatment (Figure 3D). On the contrary, gene expression of CAT and GPx was increased after 4 weeks of DAA therapy and at the EoT with respect to baseline, but it decreased to baseline level at SVR 12 (Figure 4E-F).

Association between HCV genotype, cirrhosis and treatment regimen on circulating redox markers

We then compared the impact of DAA treatment on markers of redox balance and antioxidant enzyme activities in patients affected by HCV genotype 1 versus genotype non-1, sofosbuvir-based versus sofosbuvir-free treatment, RVR versus non-RVR, and no/mild fibrosis (F0/F1) versus significant fibrosis (F3/F4). We did not find any significant difference between subgroups related to genotype, treatment regimen, or RVR. On the contrary, when the two-way ANOVA analysis was performed in subgroups of patients with different fibrosis stage, a significant effect of fibrosis resulted for the serum 8-OHdG levels ($F = 80$, $p < 0.0001$) and CAT activity ($F = 1162$, $p < 0.0001$), while an interaction effect (time × fibrosis) was observed for serum 8-OHdG ($F = 274.2$, $p < 0.0001$), SOD activity ($F = 139.2$, $p < 0.0001$), and CAT activity ($F = 602.4$, $p < 0.0001$). Of interest, we observed that the serum 8-OHdG level was markedly reduced in the F3/F4 group as compared with F0/F1 patients at the end of treatment and at SVR12 (Figure 5A). No differences were described for the circulating levels of both the HNE- and the MDA-protein adducts. We also observed that the SOD activity was higher in the PBMC from F3/F4 patients rather than F0/F1 ones at SVR12 (Figure 5B), but the CAT activity was higher in the F0/F1 group with respect to F3/F4 at baseline, at 4 weeks after initiation of therapy, and at SVR12 (Figure 5C).

A subgroup analysis including patients previously treated with PEG-IFN was performed and we did not observe any significant differences in comparison to overall data.

Relationship among viremia, cytokine levels and circulating markers of oxidative stress

To assess the relationship between changes in viremia and redox balance during and after the DAA treatment, a linear regression model was designed which correlated the variation of HCV-RNA (independent variable) and the variation of serum HNE- and MDA-protein adducts, and 8-OHdG. A significant positive relationship was observed among changes registered in serum HCV-RNA and 8-OHdG between 4wks and baseline (Figure 6A). We further designed a linear regression model to study the relationship between the variation of circulating TNF-α and serum markers of oxidative stress. Interestingly, a positive association was found among changes in serum TNF-α and HNE-protein adducts between SVR12 and baseline (Figure 6B).

Discussion

The present study demonstrates for the first time that DAA treatment restores circulating redox homeostasis in patients affected by chronic hepatitis C. This beneficial effect occurs irrespectively of the HCV genotype and of antiviral regimen.

Liver injury associated with chronic HCV infection is caused by the specific immune response and a direct impairment in infected cells (24, 25). Among the cellular mechanisms by which HCV causes liver injury, oxidative stress plays a determinant role (6, 26). Indeed, oxidative stress has been long considered a hallmark of chronic hepatitis C (12). Oxidative stress is determined by an extensive production of reactive species, which damage biological macromolecules such as lipids, DNA and proteins. Lipid peroxidation produces high levels of reactive aldehydes, such as HNE and MDA, which in turn form adducts with proteins – including enzymes and signaling molecules – with consequent structural and functional alterations (27).

On the other side, high reactive species can oxidize DNA base or induce single and double-stranded DNA breaks, with consequent modifications that contribute to carcinogenesis. Oxidative damage to DNA increases the risk for patients affected by chronic hepatitis C to develop hepatocellular carcinoma (28).

Our data show that 4 weeks after starting DAA therapy, HCV replication is completely suppressed and antioxidant enzymes SOD and GPx are over-expressed both in terms of mRNA and enzymatic activity. Very interestingly, a clear reduction in the level of 8-OHdG, a fine marker of oxidative DNA damage, was observed that persisted several weeks after treatment discontinuation. We also analyzed typical markers of lipid oxidation by measuring HNE and MDA adducts to serum proteins and observed a significant reduction similar to 8-OHdG but starting later.

Catalase and GPx expression and activity seem recover early after starting DAAs therapy whereas lipid peroxidation reduction become evident later. Very interestingly, SOD showed a reduction in

terms of mRNA expression and in terms of activity 4 weeks after starting DAAs with a significant increase at the end of therapy that persisted three months later.

It is worth to note that HCV may directly induce the oxidation of DNA and the impairment of DNA repair enzymes (29).

This evidence strengthens the hypothesis that DAA therapy reverses this harmful effect in part by reducing the HCV-mediated production of reactive species. On the other side, HCV is sensitive to lipid peroxidation since the oxidative membrane damage induces modifications in the membrane-bound replicase components (17).

This could partially explain the observed latency in the reduction of circulating HNE- and MDA-protein adducts, with respect to 8-OHdG, in our patients.

Further to be a direct consequence of viral replication, oxidative stress in chronic HCV infection is also mediated by pro-inflammatory mediators produced by lymphoid cells, since the virus may cause cytopathic effects to circulating lymphocytes and monocytes (30).

PBMC in chronically infected HCV patients present with increased lipid peroxidation products and SOD activity, while no modification of CAT and GPx activity (31).Our data show that, with respect to baseline, SOD activity and gene expression decreased after 4 weeks of DAA treatment, to raise up at the end of treatment. Conversely, we observed a constant increase of both CAT and GPx activity and gene expression after the initiation of DAA treatment. SOD dismutates superoxide anion to hydrogen peroxide, which in turn is catabolized by CAT and GPX. The balance between antioxidant enzymes is crucial to prevent and counteract oxidative stress. An augmented activity of CAT or GPx with respect to SOD would be protective against oxidant-induced damage (32, 33).

Oxidative stress induces the proliferation of hepatic stellate cells promoting collagen synthesis and the consequent development of fibrosis in chronic hepatitis C (12). Indeed, oxidative markers level increases parallel to fibrosis stage, suggesting that their detection is important for monitoring the progression of HCV-related hepatitis (10). Our data show that, at baseline, high fibrosis stage CHC patients showed higher oxidative DNA damage and lower SOD and CAT activities as compared to

low fibrosis stages. Very interestingly, the effect of DAA treatment on circulating redox balance was significant higher in F3/F4 as compared to F0/F1 patients. These data confirm that HCV-infected patients with high fibrosis stage get the most favorable treatment efficiency with DAA. Further basic research is required to completely elucidate the biomolecular mechanisms underlying such observations.

In conclusion, treatment with DAAs improves the circulating redox status of patients affected by chronic hepatitis C. In addition, the most benefit is reported in patients with high fibrosis stage and is independent on the treatment regimen used. By modulating the antioxidant status DAA therapy may prevent morbidity and liver complication in chronic HCV patients.

Author contribution

conception and design of the study: GS

acquisition of data: RV, FB, FC, RT, MA, VNB, DBG, GS

analysis and interpretation of data: RV, FB, FC, RT, MA, VNB, DBG, GS

drafting the article or revising it critically for important intellectual content: RV, FB, GS

final approval of the version to be submitted: RV, FB, GS

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Table 1. Demographic and clinical characteristics of the study population

N=196 patients		**N, %**
Age (years)		63 (35-90)
Gender (M/F)		112/84 (57/43)
Cirrhosis		94 (48)
Child-Pugh Class	A	76 (81)
	B	18 (19)
HCV Genotype	1a/1b	120 (61)
	2	38 (20)
	3	20 (10)

	4	18 (9)
DAA treatment	Sofosbuvir-based regimen	96 (49)
	Sofosbuvir-free regimen	100 (51)
DAA treatment	Sofosbuvir + simeprevir	19
	Sofosbuvir/ledipasvir	13
	Sofosbuvir + daclatasvir	21
	Sofosbuvir + ribavirin	29
	Sofosbuvir + velpatasvir	14
	Ombitasvir/paritaprevir/ritonavir ± dasabuvir	42
	Glecaprevir/pibrentasvir	58
Treatment duration	24 weeks	59 (30)
	< 24 weeks	137 (70)
Liver function test	ALT ≤ ULN	88 (45)
	ALT > 2 ULN	45 (23)
Log_{10} HCV-RNA (UI/mL)		5.58 (2.3-6.9)
Previous treatment	PEG-IFN + ribavirin	53 (27)
	DAAs (1^{st} or 2^{nd} generation)	10 (5)
	Naïve	133 (68)
Cryoglobulinemia		10 (5)
Diabetes		47 (24)
Rapid Virological Response (RVR)		61 (31)
HCV-RNA undetectable at the EoT		196 (100)
Sustained Virological Response (SVR)		190 (97)

DAA, direct antiviral agent; ALT, alanine aminotransferase; ULN, upper limit of normal; PEG-IFN, pegylated interferon.

Figure legends

Figure 1. HCV viremia during treatment and after the end of treatment. At week 4 DAAs induce a rapid decrease in median HCV RNA levels.

Figure 2. Hydroxynonenal (HNE) and malondialdehyde (MDA) – protein adducts (panels A and B), and 8-hydroxydeoxyguanosine (8-OHdG, panel C) serum levels at baseline (T0), during antiviral treatment (4wks and EoT) and after the end of therapy (SVR12). Data are expressed as mean ± standard error of the mean. Statistical differences were assessed by the one-way analysis of variance for repeated measures and the Tukey as post hoc test. * = $p < 0.05$ vs T0; *** = $p<0.001$ vs T0; · = $p < 0.05$ vs 4wks; T0, baseline; 4wks, after 4 weeks of therapy; EoT, end of treatment; SVR12, 12 weeks after the end of treatment.

Figure 3. Cytokine levels at baseline, during antiviral treatment, and after the end of therapy.

Interleukin-6 (A) and interleukin-8 (B) serum levels did not change over time. IL-10 (C) and TNF-α (D) concentration decreased progressively during treatment and reached statistical significance at the end of treatment.

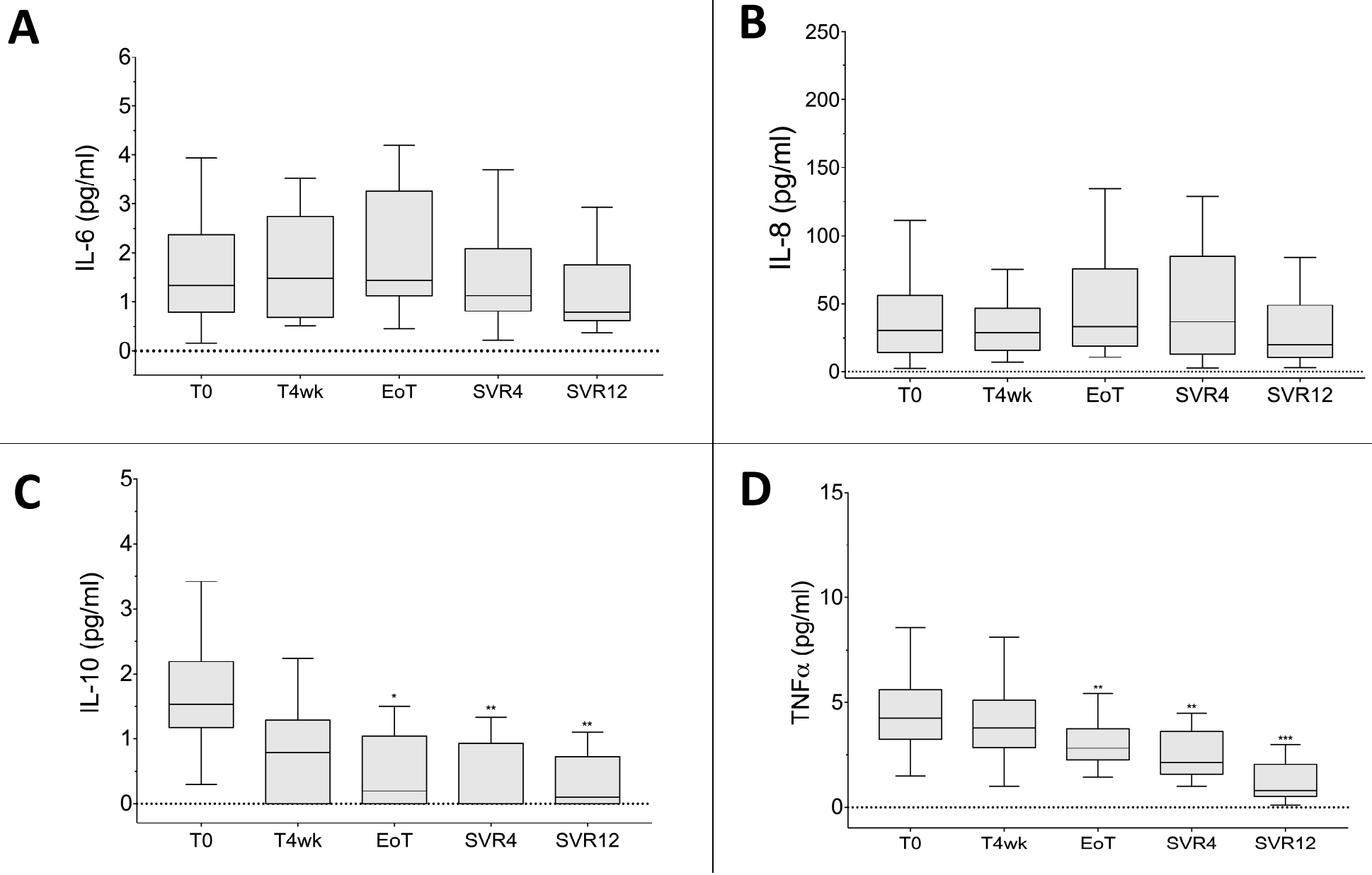
Values are shown as the median and interquartile range (IQR). Outliers are not reported. T0: baseline; T4wk: after 4 weeks of therapy; EoT: end of treatment; SVR4: 4 weeks after the end of treatment; SVR12: 12 weeks after the end of treatment.

* = $p < 0.05$ vs T0; ** = $p < 0.01$ vs T0; *** = $p<0.001$ vs T0

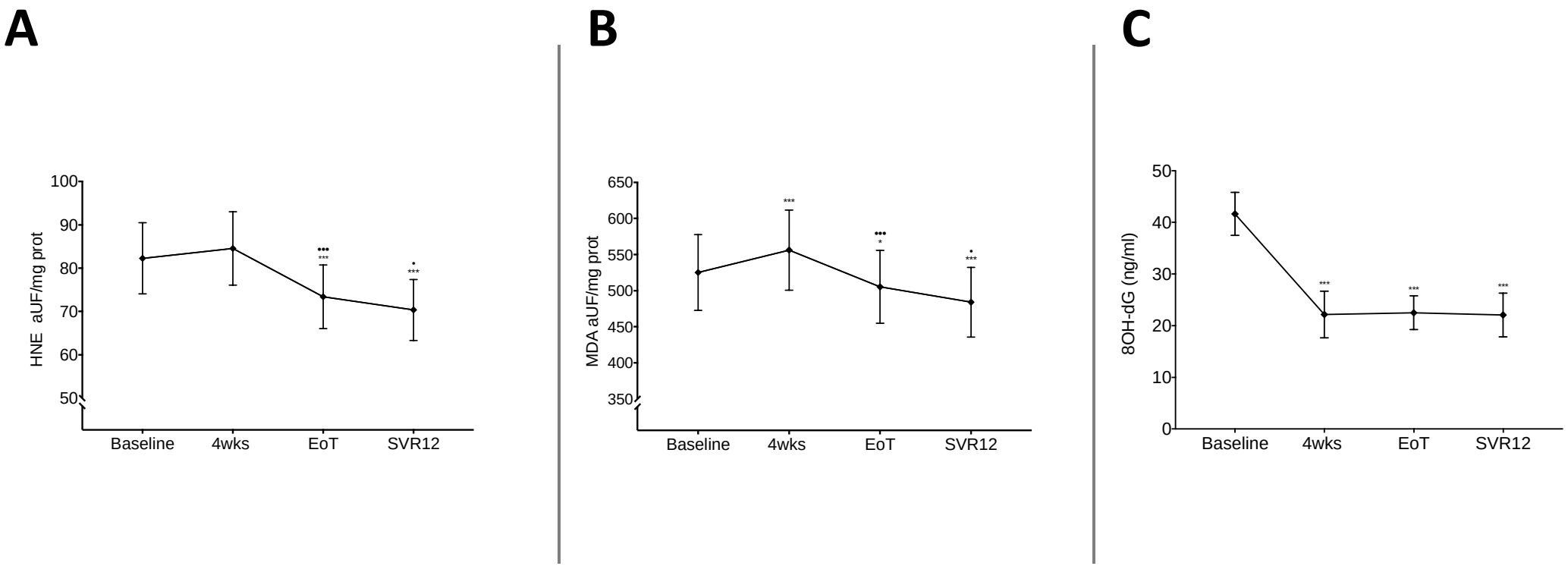
Figure 4. Superoxide dismutase (SOD, panel A), catalase (CAT, panel B) and glutathione peroxidase (GPx, panel C) activities, and mRNA expression (panels D-F) in peripheral blood mononuclear cells (PBMC) isolated from patients at baseline (T0), during antiviral treatment (4wks and EoT) and after the end of therapy (SVR12). Data are expressed as mean ± standard error of the mean. Statistical differences were assessed by the one-way analysis of variance for repeated measures and the Tukey as post hoc test. * = $p < 0.05$ vs T0; *** = $p<0.001$ vs T0; · = $p < 0.05$ vs 4wks; ·· = $p < 0.01$ vs 4wks; ··· = $p < 0.001$ vs 4wks. T0, baseline; 4wks, after 4 weeks of therapy; EoT, end of treatment; SVR12, 12 weeks after the end of treatment.

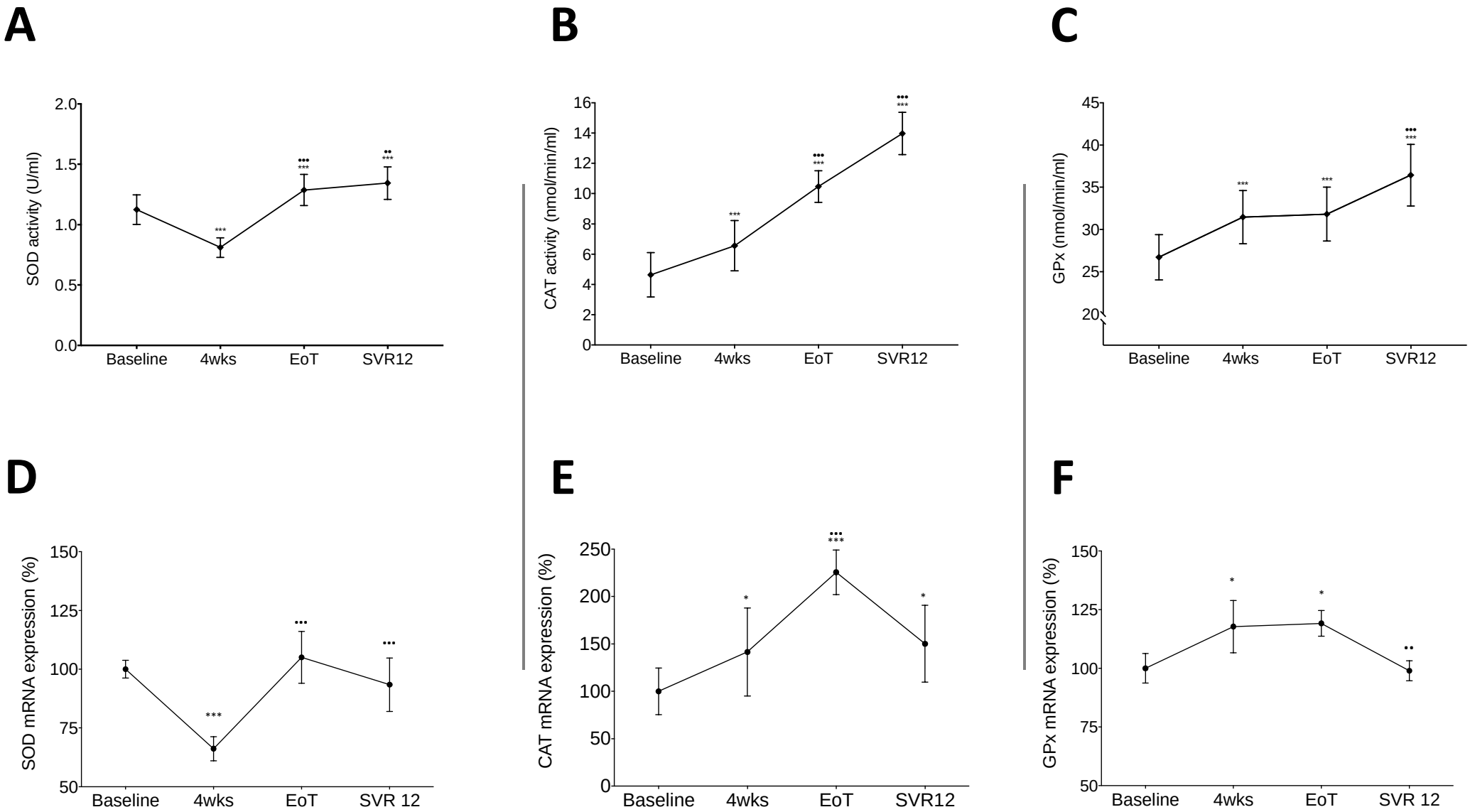
Figure 5. Serum 8-hydroxydeoxyguanosine level (8OH-dG, panel A), superoxide dismutase activity (SOD, panel B), and catalase activity (CAT, panel C) in peripheral blood mononuclear cells, in patients at baseline (T0), during antiviral treatment (4wks and EoT) and after the end of therapy (SVR12), according to mild (F0-F1) or severe (F3-F4) liver fibrosis. Data are expressed as mean ± standard error of the mean. Statistical differences were assessed by the two-way analysis of variance for repeated measures, and the Sidak as post hoc test. *** = $p<0.001$ F0-F1 vs F3-F4 .T0, baseline; 4wks, after 4 weeks of therapy; EoT, end of treatment; SVR12, 12 weeks after the end of treatment.

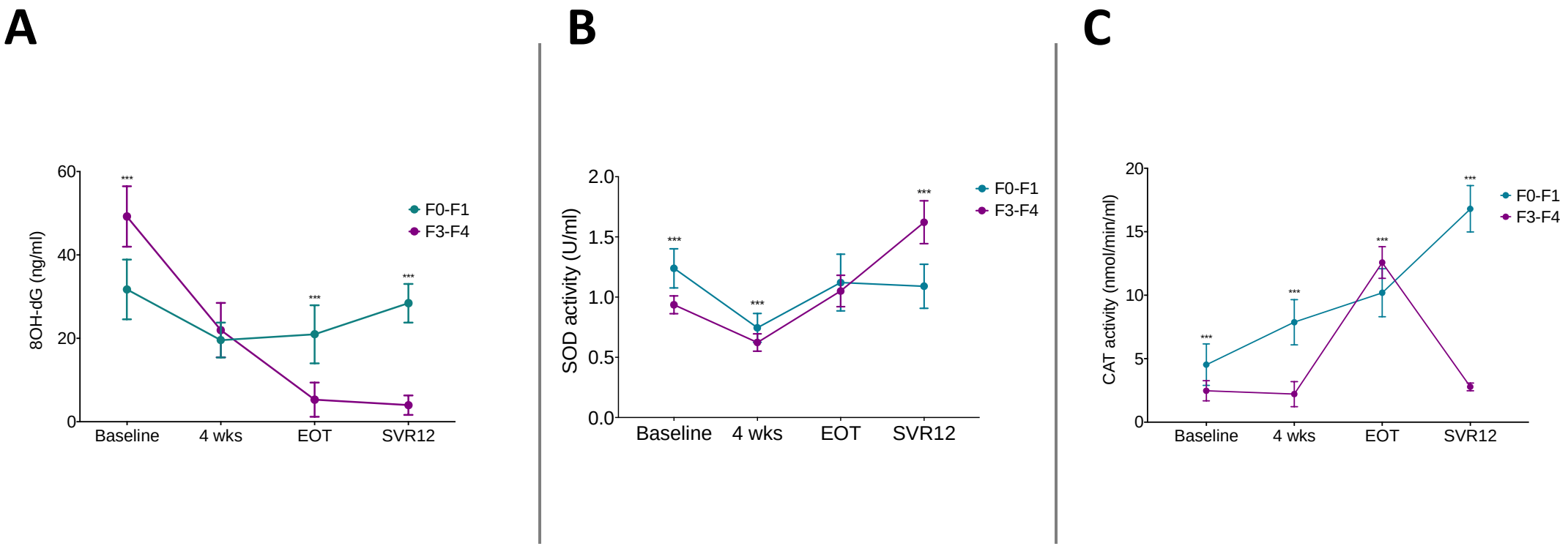
Figure 6. Linear regression analysis between the variation of serum HCV-RNA and 8-OHdG from baseline to 4wks (panel A), and between the variation of serum HNE-protein adducts and TNF-α levels from baseline to SVR12 (panel B).

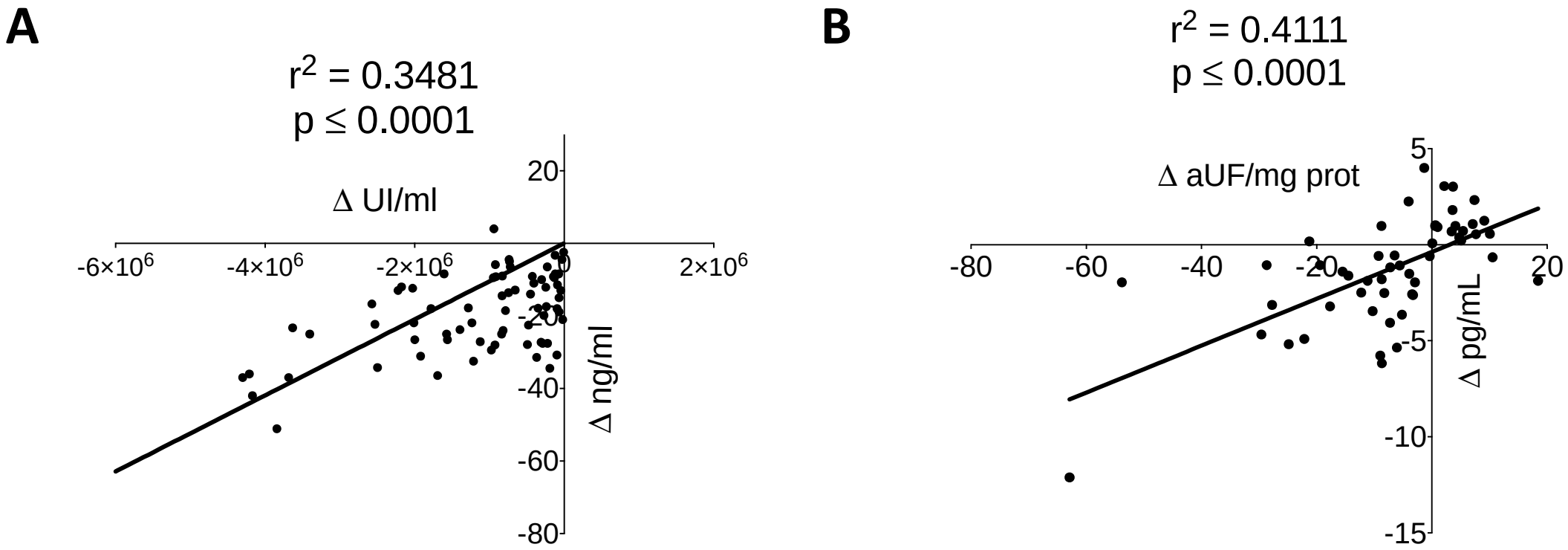

A
IL-6 (pg/ml)
6
5
4
3
2
1
0
T0
T4wk
EoT
SVR4
SVR12
B
IL-8 (pg/ml)
250
200
150
100
50
0
T0
T4wk
EoT
SVR4
SVR12
C
IL-10 (pg/ml)
5
4
3
2
1
0
*
**
**
T0
T4wk
EoT
SVR4
SVR12
D
TNFα (pg/ml)
15
10
5
0
**
**
***
T0
T4wk
EoT
SVR4
SVR12

HCV RNA (Median UI/ml)
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
T0
T4wk
EoT
SVR4
SVR12


A
HNE aUF/mg prot
100
90
80
70
60
50
Baseline
4wks
EoT
SVR12
B
MDA aUF/mg prot
650
600
550
500
450
400
350
Baseline
4wks
EoT
SVR12
C
8OH-dG (ng/ml)
50
40
30
20
10
0
Baseline
4wks
EoT
SVR12


A
SOD activity (U/ml)
2.0
1.5
1.0
0.5
0.0
Baseline
4wks
EoT
SVR12
B
CAT activity (nmol/min/ml)
16
14
12
10
8
6
4
2
0
C
GPx (nmol/min/ml)
45
40
35
30
25
20
D
SOD mRNA expression (%)
150
125
100
75
50
SVR 12
E
CAT mRNA expression (%)
250
200
150
100
50
0
F
GPx mRNA expression (%)


A
8OH-dG (ng/ml)
60
40
20
0
***
***
***
Baseline
4 wks
EOT
SVR12
F0-F1
F3-F4
B
SOD activity (U/ml)
2.0
1.5
1.0
0.5
0.0
***
***
***
Baseline
4 wks
EOT
SVR12
F0-F1
F3-F4
C
CAT activity (nmol/min/ml)
20
15
10
5
0
***
***
***
***
Baseline
4 wks
EOT
SVR12
F0-F1
F3-F4



Highlights

- Oxidative stress is an important mechanism of disease progression in HCV patients
- DAAs improve circulating redox status in patients with chronic hepatitis C
- Viral eradication associates with reduction of oxidative stress
- DAA treatment restores enzymatic activity of the antioxidant enzymes