New use of an old drug: Chloroquine reduces viral and ALT levels in HCV non-responders (A randomized, triple-blind, placebo-controlled pilot trial- NCT02058173)

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| Keyword: | chronic hepatitis C, non-responsive, Chloroquine, pilot study, autophagy |
New use of an old drug: Chloroquine reduces viral and ALT levels in HCV non-responders (A randomized, triple-blind, placebo-controlled pilot trial NCT02058173)

Running Title: Chloroquine therapy for non-responsive HCV genotype 1 patients

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Abstract

HCV infection induces autophagy, but the virus assimilates the autophagic response into its own life cycle. Chloroquine (CQ) is an autophagy inhibitor that is clinically used to treat malarial. The aims of this pilot clinical trial were to evaluate the therapeutic potential and short-term safety of CQ in patients with chronic HCV genotype 1, who were unresponsive to a combination of pegylated interferon alpha and ribavirin. Ten non-responders to previous antiviral treatment(s) were randomized to receive either CQ (150 mg daily for 8 weeks) or placebo, and were followed for 4 weeks after CQ therapy. HCV RNA load and plasma alanine transaminase (ALT) levels were measured at baseline, week 4 (initial response), week 8 (end-of-treatment response) and at the end of 12 weeks. A significant decrease in HCV-RNA after the treatments (week 8) was observed in all patients in the CQ-group ($p=0.04$). However, HCV RNA levels increased within 4 weeks after discontinuation of CQ-treatment although they were still lower than baseline. In addition, the ALT normalized during treatment in the CQ-group. However, this response was also lost after treatment cessation. This study provides preliminary evidence that CQ is possibly a safe treatment option for HCV non-responders.

Trial registration: ClinicalTrials.gov NCT02058173

Keywords: chronic hepatitis C, non-responsive, Chloroquine, pilot study, autophagy
Introduction

Hepatitis C virus (HCV) infection remains one of the most prevalent and serious clinical and public health concerns globally. It is estimated that as many as 170 million people or approximately 3% of the world's population is living with HCV, which represents a large economic burden (S. M. Alavian et al. 2010; Alter et al. 1992; Di Bisceglie 1998). This burden is expected to increase significantly during the next 20 years, given that the percentage of patients with HCV-related diseases is predicted to almost double (S. M. Alavian et al. 2011; Davis et al. 2010). HCV infection-related end-stage liver disease is the leading reason for liver transplantation worldwide (S. M. Alavian et al. 2009).

The goal of HCV infection treatment is permanent elimination of the virus to prevent fibrosis progression in chronic hepatitis and liver-related complications in cirrhotic patients (Bruno et al. 2007; Poynard et al. 2002). Currently, pegylated interferon-α and ribavirin (PEG+RBV) therapy is a common standard therapeutic procedure to achieve viral eradication (Schvarcz et al. 1995). Novel oral treatments for HCV have been introduced recently with more promising results, but because of cost limitations, PEG+RBV is still the most common treatment for this infection (M. A. P. S. A. S. M. Alavian 2014; August-Jorg et al. 2003). Among all factors that influence the outcome of antiviral therapy, the HCV genotype is considered one of the most important predictive elements in determining the appropriate treatment strategy (Dalgard et al. 2008). For example, PEG+RBV therapy gives a higher sustained virologic response (SVR) rate (70%-90%) in HCV genotype 2- and 3-infected individuals, while the SVR rate in those infected with genotype 1 is 40%-55% (Fried et al. 2002; Manns et al. 2001).
According to the European Association for the Study of the Liver (EASL) guidelines (Clinical Practice Guidelines: Management of HCV Infection), non-responding patients are defined as those who fail to achieve a $2 \log_{10} (\text{IU/ml})$ reduction in HCV RNA after 12 weeks of treatment, or those who never achieve undetectable HCV RNA levels during a treatment period of at least 24 weeks (European Association for Study of 2014; Schvarcz et al. 1995). In the most recent clinical trial studies (ISRCTN53821378), PEG+RBV re-treatment of HCV genotype 1-infected non-responder patients had very low success rates of only 4%-14% (Teuber et al. 2003; Veldt et al. 2003). In difficult-to-treat populations of HCV-infected patients, several other strategies have also been assessed including re-treatment with higher dose regimens of IFN-α alone or in combination with ribavirin or amantadine. However, despite these strategies, 30%-50% of the treated patients still remained non-responders to most antiviral treatments (Veldt et al. 2003). To overcome treatment failures, novel anti-HCV medications have recently been introduced. However, they are expensive drugs that are often associated with adverse effects, which may lead to discontinuation of treatment (M. A. P. S. A. S. M. Alavian 2014). Thus, new therapeutic drugs, which are less toxic, more efficacious and more affordable, are required.

Autophagy is a highly regulated catabolic process that involves lysosomal degradation of cytoplasmic organelles and protein aggregates (Ghavami et al. 2014; Klionsky and Emr 2000; Mizushima 2007; Yoshimori 2004). In addition to its central role in cellular homeostasis, autophagy has emerged as an important antimicrobial host defense against diverse infections (S. M. Alavian et al. 2011; Orvedahl and Levine 2009). Interestingly, a group of viruses have evolved to evade, subvert, or use
autophagy for their own benefit (Dreux et al. 2009; Tanida et al. 2009; Tian et al. 2011). Among them, positive-strand RNA viruses such as HCV induce autophagy and exploit it as a platform for their own replication (Dreux et al. 2009; Ke and Chen 2011; Richards and Jackson 2013; Tanida et al. 2009). Thus, interference with HCV-activated autophagy can suppress HCV replication, which makes inhibition of the autophagy pathway a potentially attractive and novel therapeutic strategy for HCV patients.

Chloroquine (CQ) is a lysosomotropic agent that inhibits lysosomal and endosomal acidification (Poole and Ohkuma 1981), thereby preventing completion of the autophagy. Because lysosomes have an essential role in proper function of autophagic machinery, CQ also appears to exert antiviral effects during replication of several viruses including HCV (Mizui et al. 2010; Rolain et al. 2007; Savarino et al. 2003). In an in vitro study using Huh-7 cells, CQ treatment ($10^{-7}$–$10^{-3}$ M) effectively inhibited HCV replication in a dose-dependent manner (Mizui et al. 2010). However, the therapeutic efficacy and safety of CQ has not yet been studied in HCV patients. Therefore, the aims of the present randomized, triple-blind, placebo-controlled pilot trial were to evaluate the therapeutic efficacy and short-term safety of CQ in patients with chronic HCV genotype 1 infection who were not responding to previous antiviral treatments. The overall aim was to determine an effective safe and affordable treatment for HCV in patients who are nonresponsive to standard therapy.

**Patients and Methods**

**Patient selection**
We prospectively enrolled 10 non-responder patients with chronic HCV at the Motahhari Hepatology Clinic in the Shiraz University of Medical Sciences (SUMS), from March 2014 to June 2014 (ClinicalTrials.gov Identifier: NCT02058173). Enrolled patients were male, between 18 and 60 years old with confirmed chronic hepatitis C genotype 1 infection, who were non-responsive to previous PEG+RBV treatment. All patients were informed about the trial, and after understanding the study procedures, signed informed consent forms. Patients with decompensated cirrhosis i.e scores equal or more than 7 [decompensated cirrhosis were characterized based on Child-Pugh criteria which has been previously described (Tarantino et al. 2009a; Tarantino et al. 2009b) who were receiving anti-neoplastic, anti-viral or immunomodulatory drugs during 6 months prior to the study, patients showing co-infection with hepatitis A, B, D viruses or HIV, patients infected with multiple genotypes of HCV, active alcohol users or those who had used alcohol regularly in the recent 2 years, and mentally-impaired patients were excluded from this study. Patients with hepatocellular carcinoma, aminotransferase levels 5 times above the normal range and those with a history of any adverse reaction to CQ or drugs with similar structure were also excluded. Finally, patients who were already being treated for HCV, who planned to initiate HCV treatment, or who discontinued HCV treatment within the previous 6 months were also excluded. The study was approved by ethics review board (ERB) of the Shiraz University of Medical Sciences and performed in accordance with the Declaration of Helsinki and Good Clinical Practices (GCP) guidelines. The intervention was discontinued if a patient developed any adverse drug reactions (including seizures, hear reactions: deafness or tinnitus. Gastrointestinal reactions: nausea, vomiting, diarrhea,
abdominal cramps, and anorexia, mild and transient headache, skin reactions: itchiness, skin color changes, hair loss, and skin rashes, unpleasant metallic taste chloroquine retinopathy, hypotension and electrocardiographic changes, pancytopenia, aplastic anemia, reversible agranulocytosis, low blood platelets, and neutropenia) or if they wanted to discontinue the treatments for personal reasons. All of the patients were followed-up and monitored for a period of 12 weeks after starting the treatment.

### Study Design

This trial was a randomized, triple-blinded, active control, single center pilot study that enrolled non-responder patients with HCV genotype 1 infection. The patients were randomized into two groups at a ratio of 1:1 using random allocation software (RAS)(M. 2004). Patients in the treatment group received 150 mg CQ once daily (6 patients) and patients in the control group received placebo once daily (4 patients) for duration of 8 weeks. Placebo and CQ were similar to each other in shape, weight and appearance. The randomization codes remained concealed until all patients had completed their follow-up and the database had been verified and closed. There is no emergency case that required breaking the blind on randomization.

Blood samples were obtained at baseline, week 4, week 8, and at the end of the follow-up (week 12) and immediately centrifuged. The plasma was then stored at -70°C until assayed at qualified laboratories. HCV viral load and biochemistry tests were performed and the results were compared between groups. The baseline characteristics
of the enrolled patients are summarized in Table 1. All patients were assessed for safety, tolerance and efficacy in an outpatient setting at the end of weeks 4, 8, and 12.

**Quantitative HCV-RNA viral load**

Initial and follow-up (week 4, week 8 and week 12) HCV-RNA viral loads were quantitatively measured in all samples (COBAS TaqMan HCV test, version 2.0, Roche, Branchburg, NJ) after 'high pure' viral nucleic acid purification. This test has a standard deviation of 0.004 to 0.388 IU/mL HCV RNA, a lower limit of detection is 25 IU/ml, and a linear range of 43 to $6.90 \times 10^7$ IU/mL.

**HCV genotype**

HCV genotype was determined using the HCV database at SUMS (Shiraz University of Medical Sciences) on the date when the patients were diagnosed with HCV infection.

**IL28 genetic polymorphism analysis**

Genomic DNA was extracted from peripheral blood samples using a commercial extraction kit (DNP plus DNA Extraction Kit, Cinagene, Tehran, Iran). Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to detect rs12979860 variant alleles. The primer sequences were:
Forward 5′-CGCCAGGGCCCCTAACCTCT-3′

Reverse 5′-CCCAGCAGGCGCCTCTCCTA-3′

To determine the different genotypes, PCR products were digested using Hpy166II (Biolab, New England) for 16 hours at 65°C. The digested fragments were electrophoresed in 3% agarose gels. Samples from each genotype were sequenced using a genetic analyzer (Applied Biosystems, Foster City, CA) that confirmed the PCR results. Allele and genotype frequencies were estimated using the gene counting (GC) method. The observed genotype frequencies were compared with the expected genotype frequencies according to the Hardy–Weinberg equilibrium, using Arlequin software (version 3.1, CMPG, University of Berne, Switzerland).

Adverse events and Pharmacoepidemiology evaluation

Safety was assessed by evaluation of the adverse effects at the 4- and 8-week time points during treatment and at 12 weeks, 4 weeks after completion of treatment. Plasma HCV levels also were determined at baseline, 4, 8 and 12 weeks. Any life-threatening adverse events or progression to HCV resulted in CQ treatment withdrawal.

Statistical analysis

All statistical analyses were performed using SPSS software for Windows (Version 10 SPSS Inc, Chicago, IL, USA). P-values <0.05 were considered statistically significant. The repeated measures ANOVA (Friedman’s Repeated Measures ANOVA)
test was used to detect HCV RNA and ALT changes during the follow-up period within the groups. The Wilcoxon sign rank test was used to compare the HCV RNA and ALT level differences between the follow-up visits. The Kruskal-Wallis analysis was used to simultaneously compare the relationship between IL28 polymorphism and HCV RNA response to CQ therapy.

Results

Trial profile and patient characteristics

Figure 1 shows the trial profile and patient flow, based on the CONSORT-statement (http://www.consort-statement.org) criteria. A total of 40 patients were screened and 10 male subjects with chronic hepatitis C genotype 1 infection who were not responding to previous antiviral treatment (European Association for Study of 2014) were enrolled into the trial. The demographic and clinical characteristics of patients according to their age, body mass index (BMI), weight, risk of transmission, ALT levels, HCV RNA load and histology are shown in Table 1. There were no significant differences among cohorts. However, history of blood transfusion and drug abuse was more frequent in patients in the CQ group compare to placebo group. As seen in Figure 1, 80% of the patients (8 out of 10) completed the treatments and the follow-up period according to the protocol.

Efficacy
**Virologic response**

Measurements at the end of the first follow-up (week 4) showed a rapid decrease in median HCV RNA levels in plasma samples from CQ-treated patients (1,709,410 at baseline to 891,507 at week 4), while levels increased in the placebo group from 900,440 at baseline to 2,297,330 at week 4 (Table 2, Figure 2). HCV RNA measurements also showed that median HCV RNA levels in the CQ group remained low until the end of treatment (811,185 at week 8), which was followed by an increase at the end of follow-up (1,241,740 at week 12, 4 weeks after termination of CQ therapy; Table 2, Figure 2). Median HCV RNA levels in the placebo group showed a rapid decrease from 2,297,330 at week 4 to 855,170 at week 8; however, levels increased again to 1,873,280 by the end of the follow-up.

Although a persistent decrease in HCV RNA levels at the end of the 12-week follow-up period was not observed in any of the patients in either group, the median HCV RNA levels at the end of the follow-up were lower (1,241,740) in the CQ group compared to baseline (1,709,410), and there was a significant reduction of HCV RNA in the CQ group when compared with the placebo group ($P=0.04$; Table 2, Figure 2). Within the CQ group, a significant reduction ($P=0.04$) in the HCV RNA level was observed between baseline and the first follow-up, and between the first and second follow-up visits (Table 2, Figure 2).

**Biochemical response**
During the CQ-treatment ALT-levels decreased in the treated group, whereas they increased in the placebo-treated group. ALT levels in plasma samples from patients treated with CQ showed a rapid decrease at the end of the first follow-up, from 57 U/l at baseline to 50 U/l at week 4, while the median ALT levels increased in the placebo group from 44 U/l at baseline to 59 U/l at week 4 (Table 2, Figure 3). Median ALT levels in the CQ-group also remained low until the end of treatment (42 U/l at week 8) followed by an increase ($P=0.03$) at the end of follow-up, 4 weeks after discontinuation of CQ-therapy (46 U/l at week 12; Table 2, Figure 3). Median ALT levels in the placebo group remained high until the end of the treatment (60 U/l at week 8 and 56 U/l at week 12; Table 2, Figure 3).

**IL28 genetic polymorphism analysis**

A single nucleotide polymorphism (SNP) upstream of the interleukin 28B (*IL28B*) gene is associated with hepatic responsiveness to interferon therapy in the HCV genotype 1 patients (Chen et al. 2011; Derbala et al. 2012). Thus, in this study, we also analyzed *IL28B* gene polymorphism. The distribution of the rs12979860 IL28B alleles and genotype frequencies in HCV patients have been identified as follows: C: 8 (40%), T: 12 (60%), CC: 1 (10%), CT: 6 (60%) and TT: 3 (30%). The PCR results were also confirmed by HCV genotyping. Overall, our results showed that the T-allele was the most common allele in HCV individuals. The T-allelic frequency was 60% in patients in our study.
**IL28 polymorphisms and HCV RNA response**

Genome-wide association studies consistently linked HCV RNA response to IL28 polymorphisms in various populations. The IL28 polymorphism was not associated with HCV RNA load in response to CQ-treatment (Table 3).

**Adverse events and pharmacoepidemiology evaluation**

The spectrum and frequency of adverse events were similar in both treatment and placebo arms. Adverse events that were reported were usually mild and reversible. Severe adverse events (World Health Organization (WHO) grade IV) including severe diarrhea (1 patient at the first, second and third follow-up), severe thinning hair (3 patients at the first, and 2 patients at the second and third follow-up), severe paresis (2 patients at the first, 3 patients in second and 1 patient at the third follow-up) were observed more frequently in the CQ-treatment group. Paresis was observed with the same frequency in both groups at all follow-ups. One patient in cohort 2 (CQ-treatment group) experienced severe thinning hair at the second and last follow-up.

**Discussion**

Despite improvements in treatment of HCV infection in the past two decades, many patients still do not respond to PEG+RBV, and novel treatments with oral agents are costly (European Association for Study of 2014; Schvarcz et al. 1995). Thus,
development of more efficacious, safe and affordable anti-HCV therapies remains a medical priority, because large populations of HCV-positive patients inhabit countries with a low gross domestic product (GDP) per capita. The assimilation of autophagy into the HCV-life cycle makes the autophagy pathway a potential target for the development of new therapeutic strategies for HCV patients (Shrivastava et al. 2011; Tanida et al. 2009). Thus, using an in vitro model of cultured mammalian cells, autophagy can be inhibited either through silencing of the ATG genes or by treatment with pharmacological inhibitors of autophagolysosomes maturation such as CQ and Bafilomycin A1, repressed HCV replication (Ashfaq et al. 2011; Mizui et al. 2010; Savarino et al. 2003). In addition, reduced HBV replication has been shown in mice with liver-specific Atg5-knockout (Shrivastava et al. 2011; Tanida et al. 2009), which provides a rationale for further studies to explore the targeting of autophagic machinery for the treatment of hepatitis virus-infected patients.

CQ and its derivative hydroxychloroquine are well-known inhibitors of autophagic protein degradation via neutralization of the lysosomal pH, and are often used as antimalarial, anti-lupus erythematosus and anti-rheumatoid arthritis agents (Rolain et al. 2007; Verbeeck et al. 2005). It is worth mentioning that besides its direct hepatocytopathic injury, HCV virus is well known for its lymphotropic effects (Conca and Tarantino 2009). Lymphotropic HCV has been shown to be responsible for the pathogenesis of virus-related immunological disorders (Zignego et al. 1992), and is probably involved in wide non-organ-specific autoantibody production (Conca and Tarantino 2009). CQ has been shown to modulate the immune system and reduce the severity of several autoimmune disorders via dendritic cells (Thome et al. 2014).
Therefore the immunomodulatory properties of chloroquine and possible effect on lymphotropic HCV could be one of the mechanisms of inhibitory effects of CQ on HCV infection.

The antiviral effects of CQ on other RNA viruses, as well as its safety, tolerability and efficacy, have been previously demonstrated in different studies (Savarino et al. 2003). Thus, the current pilot clinical trial study was designed to evaluate the therapeutic efficacy and short-term safety of CQ in patients with chronic hepatitis C genotype 1 infection who were not responding to previous antiviral treatments. Our results showed a reduction in plasma ALT and HCV RNA levels with CQ-treatment, which was associated with the duration of the treatment. After discontinuing CQ, viral load and ALT levels increased, although both were still lower than baseline, 4 weeks after completion of CQ-treatment.

Whether a longer duration of CQ-treatment could have more sustainable effects needs to be examined in future studies. In addition, in this study we selectively analyzed non-responder patients with HCV genotype 1, who are more resistant to PEG+RBV. Thus, extending this study to additional genogroups is also warranted. In this small preliminary study, we also observed large variations in the HCV RNA levels in the placebo group. Chronic hepatitis C with fluctuating or persistently-elevated HCV RNA is a common feature of chronic hepatitis because of cyclic viremia, which has been reported previously (Halfon et al. 1998; Kaiser et al. 2006; Mosley et al. 2008). Large spontaneous fluctuations were observed daily, weekly and monthly, and this sometimes raises doubts about the clinical value of a single assessment of pretherapeutic viremia (Halfon et al. 1998; Kaiser et al. 2006; Mosley et al. 2008). In our study, two of four
patients in the placebo group showed an increase in HCV RNA, while the other two showed a decrease, leading to variation observed in the HCV RNA load in this group.

The pattern of the adverse events was somewhat different in both groups. Adverse events including diarrhea, thinning hair and paresis were more frequent in the patients treated with CQ. However, dose modifications were not necessary for any patients because most of the events were usually mild and reversible.

There are limitations to the role and interpretation in this exploratory study. Like many other pilot studies, the main limitation for the current study was the small sample size. Moreover, we had only male subjects in this study. Thus, we cannot generalize results obtained in this study to female patients. However, our results demonstrate a significant decrease in HCV RNA and ALT levels during treatment with CQ, as well as show that CQ has a good safety profile.

In conclusion, the results of this exploratory pilot study reveal that CQ may have a role as a potential new anti-HCV agent for the treatment of HCV genotype 1-infected patients who are unresponsive to PEG+RBV. It has an encouraging efficacy, safety and tolerability profile. CQ may play an important role in treating patients with HCV by targeting autophagic proteolysis. Prospective randomized trials with more patients and with those infected with other genotypes, as well as patients who have failed previous anti-viral therapy or who are treatment-naïve, are warranted to fully explore the role of CQ in the treatment of patients with HCV.

**Abbreviations**
Acknowledgments

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Competing interest

No conflicts of interest to declare.

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Author contributions

All the authors have significantly contributed to the work and the involvement of each author was as follows:

Lankarani KB. (1–8), Peymani P. (1-8), Yeganeh B. (3-5, 7-8), Ghavami S. (1, 2, 4, 5, 7, 8), Sabour S. (1, 2, 3, 6, 8), Geramizadeh B. (2, 3, 7, 8), Fattahi MR. (2, 5, 7, 8), Keyvani H (2, 3, 7, 8), Azarpira N. (2, 3, 5, 7), Coombs KM. (4, 5, 7, 8). [(1) Study concept and design; (2) Acquisition of data; (3) Analysis and interpretation of data; (4) Drafting of the manuscript; (5) Critical revision of the manuscript for important intellectual content; (6) Statistical analysis; (7) Administrative, technical, or material support; (8) Study supervision.] All authors critically reviewed the manuscript, providing suggestions for revision where necessary. All authors reviewed and approved the final version of the paper.
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Figures Legends

Figure 1. Trial profile flow diagram. A trial profile corresponding to the Consolidated Standards of Reporting Trials (CONSORT criteria) is shown, indicating the course of the pilot study.

† On the start day of the trial, the patient declined to participate in the study

‡2 patients were lost to follow-up at the third (last) follow-up visit. Six patients in the treatment and 4 patients in the placebo group completed the trial from baseline to the second follow-up visit, but 2 were lost to follow-up at the third (last) follow-up visit (1 patient in the intervention group and 1 patient in the placebo group).

Figure 2. Trend for the HCV RNA load from baseline to the end of follow-up

Figure 3. Trend for the ALT level from baseline to the end of the follow-up
# Tables

## Table 1. Demographic and clinical baseline characteristics of non-responders with HCV

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<td>0 (0)</td>
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<tr>
<td>Mild (%)</td>
<td>1 (25)</td>
<td>5 (84)</td>
</tr>
<tr>
<td>Moderate (%)</td>
<td>2 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Severe (%)</td>
<td>1 (25)</td>
<td>1 (16)</td>
</tr>
</tbody>
</table>

1. values are presented as the median (minimum-maximum)
2. this parameter is a multiple choice question
2 **Table 2.** HCV RNA load and ALT response (start and end of treatment and follow-up)

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n=4)</th>
<th>CQ-Treatment Group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HCV RNA Load (IU/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>900,440 (390780-2,941,705)</td>
<td>1,709,410 (138,390-10,580,020)</td>
</tr>
<tr>
<td>First follow-up</td>
<td>2,297,330 (16870-3,303,050)</td>
<td>891,507 (21,440-1,978,065)</td>
</tr>
<tr>
<td>Second follow-up</td>
<td>855,170 (205,110-4,747,670)</td>
<td>811,185 (69,800-1,267,125)</td>
</tr>
<tr>
<td>Third follow-up</td>
<td>1,873,280 (1,120,080-3,183,720)</td>
<td>1,241,740 (371,955-4,751,695)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference between time Trent and HCV RNA (Friedman test)

<sup>b</sup> Significant difference of HCV RNA between second follow-up (weeks 8) and third follow-up (weeks 12) (Wilcoxon sign rank test)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALT (U/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>44 (32-56)</td>
<td>57 (13-95)</td>
</tr>
<tr>
<td>First follow-up</td>
<td>59 (39-76)</td>
<td>50 (14-70)</td>
</tr>
<tr>
<td>Second follow-up</td>
<td>60 (26-98)</td>
<td>42 (15-72)</td>
</tr>
<tr>
<td>Third follow-up</td>
<td>56 (42-86)</td>
<td>46 (25-95)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference between time Trent and HCV RNA (Friedman test)
Significant difference of HCV RNA between second follow-up (weeks 8) and third follow-up (weeks 12) (Wilcoxon sign rank test)

1. values are presented as the median (minimum-maximum)
Table 3. HCV RNA load and IL28 polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n=4)</th>
<th>CQ-Treatment Group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCV RNA Load (IU/ml)</td>
<td>IL28 Polymorphism</td>
</tr>
<tr>
<td></td>
<td>CC (n=1)</td>
<td>TT (n=1) CT (n=2)</td>
</tr>
<tr>
<td>Baseline</td>
<td>2941705</td>
<td>961610</td>
</tr>
<tr>
<td></td>
<td>(390780-839270)</td>
<td>(138390-2630270)</td>
</tr>
<tr>
<td>First follow-up (weeks 4)</td>
<td>3303050</td>
<td>16870</td>
</tr>
<tr>
<td></td>
<td>(1554030-3040630)</td>
<td>(1554030-3040630)</td>
</tr>
<tr>
<td>Second follow-up (end of the treatment) (weeks 8)</td>
<td>4747670</td>
<td>712340</td>
</tr>
<tr>
<td></td>
<td>(205110-998000)</td>
<td>(205110-998000)</td>
</tr>
<tr>
<td>Third follow-up (end of the follow-up) (weeks 12)</td>
<td>3183720</td>
<td>1873280</td>
</tr>
<tr>
<td></td>
<td>(1120080-1120080)</td>
<td>(1120080-1120080)</td>
</tr>
<tr>
<td>P-value†</td>
<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a Values are presented as the median (minimum-maximum).

† Not statistically and clinically significant difference between time Trent and HCV RNA (Kruskal–Wallis)
Figures

Figure 1.
Figure 2.

![Graph showing HCV RNA Viral Load (UL/ml) vs Follow Up (Weeks) for Placebo and Chloroquine groups.](https://mc06.manuscriptcentral.com/cjpp-pubs)
Figure 3.

![Graph showing ALT level over time for Placebo and Chloroquine groups.](image-url)