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Effects of anti-viral therapy and HCV clearance on cerebral metabolism and cognition

Valerie Byrnes[1], Anne Miller[2], Damien Lowry[4], Erin Hill[2], Cheryl Weinstein[2], David Alsop[3], Robert Lenkinski[3], Nezam H. Afdhal[1,*]

[1]*Department of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States;* [2]*Department of Psychiatry, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States;* [3]*Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States;* [4]*Liver Centre, Mater Misericordiae University Hospital, Dublin 7, Ireland*

Background & Aims: Chronic hepatitis C virus (HCV) infection is associated with altered cerebral metabolism and cognitive dysfunction. We aimed to evaluate the effect of pegylated interferon/ribavirin (PIFN/R) and HCV clearance on cerebral metabolism, and neuropsychological performance.

Methods: Fifteen non-cirrhotic HCV positive subjects underwent ^{1}H MR spectroscopy (MRS) before, during, and after treatment with PIFN/R. The metabolites of interest namely, *N*-acetylaspartate (NAA), choline (Cho), myo-inositol (MI), and the control metabolite creatine (Cr), were acquired from 3 different brain regions; left basal ganglia, left frontal cortex, and left dorso-lateral pre-frontal cortex. Coinciding with this, subjects also underwent a battery of neuropsychological tests to evaluate the domains of verbal learning, memory, attention, language, executive functioning, and motor skills. Seven HCV positive controls (not receiving anti-viral therapy) underwent MRS and neuropsychological testing at two time points, 12 weeks apart, to examine for variation in cerebral metabolites over time and the practice effect of repeat neuropsychological testing.

Results: Significant reductions in basal ganglia Cho/Cr ($p = 0.03$) and basal ganglia MI/Cr ($p = 0.03$) were observed in sustained virological responders (SVRs, n = 8), but not non-responders/relapsers (NR/R, n = 6), indicative of reduced cerebral infection and/or immune activation in those who cleared virus. SVRs demonstrated significant improvements in verbal learning, memory, and visuo-spatial memory. A small but significant improvement in neurocognitive function secondary to the practice effect was seen in both HCV controls and HCV subjects during treatment.

Conclusions: HCV eradication has a beneficial effect on cerebral metabolism and selective aspects of neurocognitive function and is an important factor when contemplating anti-viral therapy in HCV, especially in those with mild disease.

Keywords: Hepatitis C; HCV; Cognitive dysfunction; Neuropsychological; Metabolite abnormalities; Immune activation; Proton magnetic resonance spectroscopy; H MRS; Combination treatment.

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* Corresponding author. Address: Liver Center, Beth Israel, Deaconess Medical Center, 110 Francis St., Suite 8E, Boston, MA 02115, United States. Tel.: +1 6176321070; fax: +1 6176321065.

E-mail addresses: dlowry@mater.ie (D. Lowry), nafdhal@bidmc.harvard.edu (N.H. Afdhal).

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; ^{1}H MRS, proton magnetic resonance spectroscopy; Cho, choline; MI, myo-inositol; CHC, chronic hepatitis C; NAA, *N*-acetylaspartate; Cr, creatine; CNS, central nervous system; BIDMC, Beth Israel Deaconess Medical Center; HBV, hepatitis B; CVA, cardiovascular accident; IFN, interferon; MRI, magnetic resonance imaging; PEG-IFN, pegylated interferon; HVLT, Hopkins verbal learning test; ROCF, Rey-Osterreith complex figure; WAIS, Wechsler adult intelligence scale; D-KEFS, Delis Kaplan executive function system; BDI, Beck depression inventory; SF-36, short form 36; CARS, Connor's attention rating scale; RNA, ribonucleic acid; SVRs, sustained virological responders; NR/R, non-responders/relapsers.

Introduction

Until recently HCV related neurocognitive dysfunction was believed to be a consequence of cirrhosis associated hepatic encephalopathy [1]. Several studies subsequently demonstrated cognitive dysfunction in non-cirrhotic HCV patients, when compared to age- and education-matched controls [2–8]. Selective impairments in the neurocognitive domains of attention, concentration, and working memory appear to predominate across studies [1,2,8]. While it has been contended that poorer neuropsychological test performance may in fact be attributable to the many confounding factors associated with the heterogeneity of hepatitis C patient populations [1,9,10], there is a growing body of evidence supporting the hypothesis that the hepatitis C virus may adversely affect cognition through direct central nervous system (CNS) involvement [1,10–12].

HCV sequences have been demonstrated in CSF and brain tissue (at autopsy) in chronically infected individuals, suggesting that HCV may cross the blood–brain-barrier [12–14]. There is also evidence that HCV replicates within certain cell populations in the brain, namely macrophages and microglial cells [15]. Studies using proton MR spectroscopy have demonstrated altered cerebral metabolism in HCV, even in patients with mild liver disease [8–11]. Elevated choline (Cho) and myoinositol (MI) ratios have been found in the basal ganglia, central, and frontal white matter of HCV-infected patients [9–11]. These findings are reflective of glial cell inflammation or proliferation. In addition, decreased levels of *n*-acetyl aspartate (NAA) have been observed in the central white matter of HCV-infected patients, suggestive of reduced neuronal

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integrity or neuronal 'drop-out' [8,9]. Of particular interest is a recent finding by Forton *et al.* of increased MI/Cr ratios in the frontal white matter of HCV-infected patients that negatively correlated with working memory performance [11]. This was the first demonstration of a significant relationship between the observed metabolite abnormality, and a neurocognitive correlate, suggesting that a metabolite disturbance might in fact underlie a functional property of the brain. Another study demonstrated improved neurocognitive function following successful anti-viral treatment for HCV [16]. However, longitudinal studies on the effect of anti-viral therapy and viral clearance in HCV subjects are lacking.

This investigation sought to examine the cerebral effects of anti-viral therapy and HCV clearance in patients with mild HCV-related liver disease. We hypothesized that eradication of HCV would be associated with improved neurocognitive function as determined by MRS and neurocognitive testing, but that this may be offset by the adverse effects of PIFN/R. Demonstration of improvements in cerebral metabolism and cognition following viral eradication would strengthen the biologic link between HCV and cerebral dysfunction and may influence the criteria for patient selection for antiviral therapy in the future.

Materials and methods

Patients

HCV positive patients were recruited from a prospective series of liver clinics at Beth Israel Deaconess Medical Center (BIDMC). The Institutional Review Board of BIDMC gave ethical approval for the study and all individuals provided fully informed consent prior to participation. Subjects with cirrhosis, active alcohol or drug abuse, co-infection with human immunodeficiency virus (HIV), structural brain abnormality, past history of cerebrovascular accident (CVA), serious head trauma, and seizure disorder were excluded from the study due to the confounding influences these factors have on cognitive performance. Other exclusions were contraindication to PIFN therapy, current psychiatric disorder or contraindications to MRI e.g., pacemaker and claustrophobia.

To date, there are no published data on the change in levels or ratios of cerebral metabolites following initiation of anti-viral therapy to facilitate a sample size calculation, and as such, this study is exploratory. Fifteen HCV positive patients due to start PIFN/R met eligibility and were enrolled in the study. Seven HCV positive patients who either declined treatment with PIFN or were non-responders to PIFN in the distant past were enrolled to serve as a control arm to the study. The purpose of the control sample was twofold: to control for the variability of cerebral metabolites in HCV positive patients over time, and to control for the practice effect of repeat neuropsychological testing at an interval of 12 weeks. While the cognitive effects of interferon are reportedly reversed within 4–6 weeks of stopping treatment, [17] the effect of interferon on cerebral metabolites is unknown and as such an interferon 'wash-out' period of 2 years was required for eligibility in subjects previously treated with interferon alfa.

Procedure

Subjects underwent neurocognitive and MRI/MRS testing blocks on the same day. The order of blocks was randomly assigned. Neuropsychological examination took place in a private testing room within the hospital and lasted approximately 2 h.

MRI and ^{1}H MRS

Patients undergoing combination treatment for HCV had an MRS carried out at 3 time points. Time 1 was the baseline measurement i.e., within 1 week prior to starting PIFN, Time 2 was at week 12 of treatment with PIFN and Time 3 was 12 weeks following discontinuation of PIFN (i.e., 36 weeks and 60 weeks from baseline for genotype 2/3 and 1, respectively). Control participants had an MRS exam carried out on two separate occasions. Time 1 was at baseline and Time 2 was 12 weeks subsequent.

MRI and ^{1}H MRS were carried out on a 3T magnetic resonance scanner (Signa LX, General Electric, Waukesha, WI) using a birdcage head coil. Conventional T_1-weighted images of the brain were recorded using a high-resolution 3D MP RAGE sequence (5.5 min scan time, 256 × 256 matrix size) to assess cerebral morphology and to define regions of interest for the MRS study. Density-weighted images were recorded prior to each MRS scan using a fast gradient echo sequence (1 min scan time, 256 X160 matrix size, TR 150 ms) for verification of head and voxel position. The fiduciary marker was observed on both types of images and aided in reproducible MRS voxel positioning.

A single voxel technique was used to assay the metabolites of interest, namely *N*-acetyl aspartate (NAA), Choline (Cho), Myo-inositol (MI) and the control metabolite Creatine (Cr). Three brain regions were targeted to reflect those most implicated by other investigations. They included the left basal ganglia (dorsal head of the caudate nucleus), the left frontal cortex and the left dorso-lateral pre-frontal cortex, (DLPFC, Fig. 1). Single voxel (10.5 ± 1.1 cm^3) PRESS ^{1}H spectra were acquired with a repetition time of 2 s, time to echo of 35 ms, spectral width of 5000 Hz, 2048 time points, and 272 averages (9 min). Analyses of metabolite levels were performed using LC-Model, (Stephen Provencher Inc. Oakville, Ontario, Canada) embedded in SAGE (GE Medical Systems). The 3 metabolites of interest, NAA, Cho and MI were expressed as a ratio of the control metabolite Cr.

Neuropsychological assessment

Neuropsychological assessment was performed using a series of validated test measures. The order of test administration was the same for each participant to maximize internal validity. Test descriptions closely correspond to the order in which they were administered.

Verbal learning and Memory was assessed using the Hopkins learning trials (HVLT). The Rey-Osterrieth Complex Figure test (ROCF) was used to evaluate visuo-spatial abilities, memory, planning, and working memory. Digit Forward and Digit Backwards tests in addition to the Letter Number sequencing subtests of the WAIS-III were used to assess attention and working memory for information presented in the auditory modality. Delayed verbal and visual recall, were tested using the HVLT and ROCF respectively. The structured ROCF then allowed for the examination of visuo-spatial memory more directly, as the executive functioning component of organization was removed from the task. Motor skills were assessed by the Grooved Peg Board test (dominant and non-dominant hand). The Delis Kaplan Executive Function System (D-KEFS) Color-Word Interference test assesses directed attention and cognitive flexibility for information received visually. Trails A and B provided information on visual scanning, information processing speed, attentional switching, and executive functioning. Further executive functioning capabilities such as accessing semantic knowledge and word retrieval were assessed using The Verbal Fluency FAS and Verbal Fluency Animals tests.

Self-report measures

All study participants were also asked to complete the Beck's Depression Inventory (BDI-II) to evaluate self-reported levels of depression, the short form 36 (SF-36 version 1) evaluating health-related quality of life, and the Connor's Attention Rating Scale (CARS), a self-rating questionnaire of cognitive dysfunction. SF-36 scores were normalized (SF-36 Health Outcomes Scoring software from QualityMetric, Lincoln, RI).

Statistical analysis

Data was analyzed using SPSS version 13 and the frequencies programme was used for data verification. Demographic information was evaluated using descriptive statistics. For the purpose of analysis, each subject served as his or her own control. Differences in metabolite ratios, cognitive performance, BDI, CARS, and SF-36 scores for each subject at each time point were compared using paired-*t* tests or the Wilcoxon sign rank test for parametric and non-parametric data, respectively. Group based comparisons were also conducted via the Mann–Whitney *U* test. Baseline metabolite levels were correlated with hepatitis C viral load using Spearman's rho correlation co-efficient.

Results

Clinical characteristics during longitudinal follow-up

Twenty-two patients were enrolled in the study, 15 treatment candidates and 7 HCV controls. Patient characteristics are presented in Table 1. Mode of HCV acquisition in the treatment group was as follows; blood transfusion (n = 5), injection drug use (n = 5),

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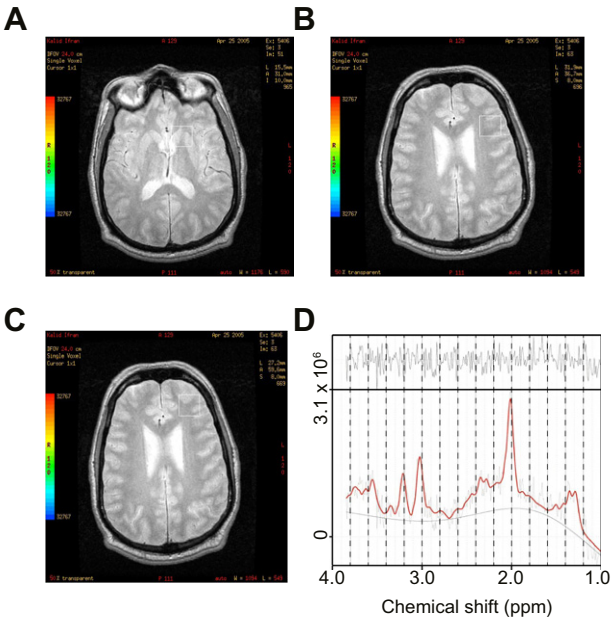

Fig. 1. MRS showing voxel positioning in the basal ganglia (A), left frontal cortex (B), left dorsolateral prefrontal cortex (C) and spectral analysis (D).

occupational (n = 1), tattoos (n = 1), cocaine snorting (n = 1), and unknown (n = 2). Genotype 1 was the predominant genotype in 12/15 patients while the remaining 3/15 had genotype 3.

One patient could not complete the MRI component of the study due to undiagnosed claustrophobia and another could not complete the neuropsychological assessment accurately due to non-proficiency in the English language. Another stopped PIFN due to intolerable side effects, dropped out of the study after 12 weeks, and was lost to follow up. Thus, complete MRS data was available on 14/15 subjects at time 1 and 2, but 13/15 subjects for all 3 time points. Similarly, complete neuropsychological assessment was available for 13/15 at all 3 time points. All controls completed both MRI/MRS and neuropsychological testing at the 2 time points.

Of the 15 treated patients, 13 had undetectable HCV RNA by quantitative testing (<600 IU/ml) at week 12 of treatment (Time 2). One patient had a reduction in HCV RNA from 395,000 to 5500 IU/ml, and the remaining patient had no change in HCV RNA level and discontinued PIFN after a total of 24-week treatment. By Time 3, 8/13 patients were persistently HCV RNA negative and proved to be SVRs. The remaining 5 tested HCV RNA positive.

MRS

Table 2 provides a breakdown of cerebral metabolite ratios (NAA/Cr, Cho/Cr, MI/Cr) in the basal ganglia, frontal cortex, and dorso-lateral pre-frontal cortex (DLPFC), for all study subjects. There was no significant difference in baseline measurements of cerebral metabolites between HCV treated patients and controls. In addition, cerebral metabolite levels remained constant in the control group, indicating a lack of significant change over time.

A significant reduction was observed in Cho/Cr in the basal ganglia of SVRs at Time 3 when compared to baseline (−32%, $p = 0.03$), this effect was not seen in NR/R (−11%, $p = 0.8$) (Fig. 2). Likewise, a significant reduction in MI/Cr was observed in the basal ganglia of SVRs at Time 3 when compared to baseline (−11%, $p = 0.03$), whereas basal ganglia MI/Cr slightly increased in NR/R (+2%, $p = 0.7$) (Fig. 3). Non-significant reductions were also noted in Cho/Cr ratio measured from the DLPFC in SVRs (−24%, $p = 0.3$) and NR/R (−5%, $p = 0.6$) at Time 3 when compared to baseline Table 3. Interestingly, the significant decrease in basal ganglia Cho/Cr from baseline was observed as early as Time 2 (i.e., 12 weeks on treatment) in subjects who subsequently achieved and SVR, although the decline did not reach significance, there was a trend towards significance (−32%, $p = 0.06$). No differences in NAA/Cr were observed between any of the time points examined.

Neuropsychological

Minor differences were observed between HCV controls and HCV treatment candidates at baseline on select measures of working memory (Letter number sequencing; $U = 14.0$, $z = -2.21$, $p = 0.03$) in addition to immediate visuo-spatial planning and memory (ROCF copy, $U = 15.0$, $z = -2.57$, $p = 0.01$; sROCF copy, $U = 8.5$, $z = -3.0$, $p = 0.002$ respectively), indicating poorer performance in the HCV control group Table 4. It must be noted that while differences appear between groups, case summary analysis reveals no HCV candidate scored within a clinically impaired range on letter number sequencing whereas 2 (both HCV controls) exhibited impaired scores on the ROCF copy.

At the time of last follow up (Time 3), SVRs had significant improvements in total verbal learning recall ($z = -2.02$, $p = 0.04$), verbal memory recognition ($z = -2.21$, $p = 0.03$) and visuo-spatial memory ($z = -1.99$, $p = 0.04$) when compared to baseline. This trend was not matched in non-responders/relapsers. Moreover, while improvements across verbal recognition and visuo-spatial memory domains were within a 'healthy'

Table 1. Baseline characteristics of study participants.

Characteristics		HCV controls (n = 7)	HCV treatment group (n = 15)	*p*
Age	M (SD)	50.6 yr (7.9 yr)	48.1 yr (5.8 yr)	n.s.
Years of education	M (SD)	14.3 yr (2.2 yr)	11.78 yr (3.0 yr)	n.s.
Genotype 1 2 and 3	*f* (%)	7 (100%)	11 (73) 4 (27)	n.s.
Fibrosis (Metavir score)	Med (R)	1 (0-2)	2 (0-3)	n.s.
Viral load (IU/ml)	M (SD)	10,267,000 ± 9919,288	3258,986 ± 4450,660	n.s.
ALT (IU/ml)	M (SD)	112 ± 98	151 ± 149	n.s.

M, mean; SD, standard deviation; *f*, frequency; Med, median; R, range; n.s., not significant.

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Table 2. Levels of the 3 cerebral metabolites choline (Cho), *N*-acetyl aspartate (NAA), and myoinositol (MI) from each brain region at the each study time point i.e., baseline (T1), week 12 (T2), and for treatment candidates, 12 weeks post treatment with PEG-IFN and ribavirin (T3).

Measure	Treatment group			Controls		
	Cho/Cr	MI/Cr	NAA/Cr	Cho/Cr	MI/Cr	NAA/Cr
Basal ganglia						
T1	0.42 + 0.26	0.78 + 0.19	1.26 + 0.27	0.30 + 0.04	0.78 + 0.19	1.28 + 0.54
T2	0.28 + 0.05	0.71 + 0.11	1.25 + 0.23	0.28 + 0.05	0.72 + 0.21	1.07 + 0.13
T3	0.34 + 0.18	0.75 + 0.13	1.09 + 0.17	n.a.	n.a.	n.a.
p (scan 1 and 2)	0.08	0.3	0.9	0.4	0.9	0.6
Frontal cortex						
T1	0.29 + 0.12	0.68 + 0.14	1.73 + 0.29	0.28 + 0.03	0.77 + 0.23	1.55 + 0.49
T2	0.26 + 0.03	0.75 + 0.32	1.74 + 0.25	0.29 + 0.08	1.19 + 0.68	1.72 + 0.67
T3	0.29 + 0.06	0.71 + 0.36	1.75 + 0.37	n.a.	n.a.	n.a.
p (scan 1 and 2)	0.7	0.4	0.6	0.7	0.1	0.6
DLPFC						
T1	0.31 + 0.14	0.79 + 0.19	1.72 + 0.43	0.27 + 0.05	0.78 + 0.18	1.61 + 0.31
T2	0.25 + 0.04	0.75 + 0.22	1.65 + 0.28	0.27 + 0.06	0.86 + 0.13	1.60 + 0.29
T3	0.26 + 0.04	0.93 + 0.41	1.78 + 0.28	n.a.	n.a.	n.a.
p (scan 1 and 2)	0.3	0.3	0.3	0.5	0.4	0.9

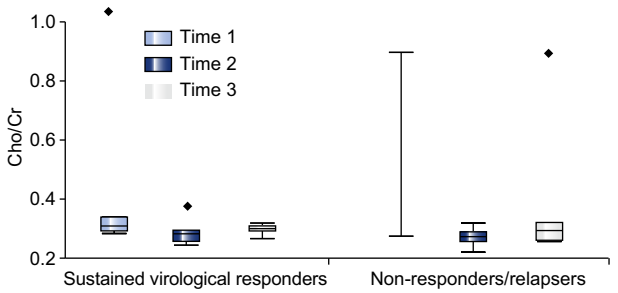

Fig. 2. MRS showing basal ganglia choline/creatine ratios across study time points for sustained virological responders and non-responders/relapsers.

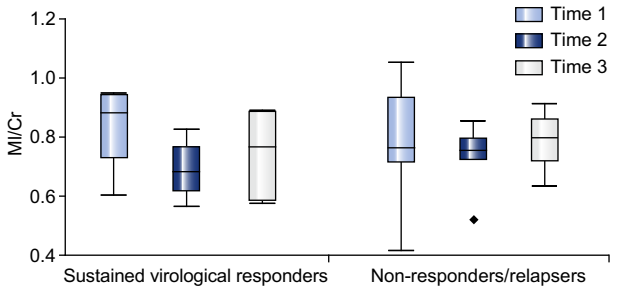

Fig. 3. MRS showing basal ganglia myo-inositol/creatine ratios across study time points for sustained virological responders and non-responders/relapsers.

normative range, the median scores for total verbal learning recall were 2 SDs below the normative mean in the case of HCV controls and 1.4 SDs below in the case of HCV treatment recipients. Case summary analyses revealed between 50% and 71% of all HCV study participants exhibited impaired scores on verbal learning domains at baseline. Additionally, retrospective analysis showed that little difference in cognition existed between SVRs and NR/R at baseline with NR/Rs performing worse relative to SVR counterparts only on a measure of visuo-spatial memory ($U = 6.5$, $z = -2.143$, $p = 0.035$, $r = 0.59$).

Furthermore, significant improvements among HCV controls were observed across 6 neurocognitive test subsections from baseline to Time 2. These include improvements in total verbal learning ($z = -2.19$, $p = 0.03$), delayed verbal recall ($z = -2.04$, $p = 0.04$), visuomotor construction ($z = -2.19$, $p = 0.03$), visuo-spatial immediate and delayed memory ($z = -2.37$, $p = 0.02$; $z = -2.12$, $p = 0.03$) in addition to cognitive flexibility ($z = -2.06$, $p = 0.04$) likely due to the practice effect of repeat neuropsychological testing. When all HCV participants undergoing PIFN treatment were grouped together, they were also seen to improve in four areas from baseline to Time 2, namely verbal recognition ($z = -2.59$, $p = 0.01$), visuo-spatial immediate and delayed memory ($z = -2.85$, $p = 0.004$) as well as working memory ($z = -2.00$, $p = 0.04$) further suggesting the effect of practice from Time 1 to Time 2.

Subjective health questionnaires

Depression scores increased in subjects following 12 weeks of PIFN therapy when compared to baseline ($z = -2.28$, $p = 0.02$). Median scores increased from 6 at baseline to 14.5 on treatment reflecting clinically significant levels. Analyses also revealed significant reductions on treatment in composite score estimates of physical and mental functioning ($z = -1.96$, $p = 0.05$; $z = -2.52$, $p = 0.01$). Restored 'healthy' levels of reported depressive symptomatology, physical, and mental health functioning were observed following the cessation of treatment, (Time 3).

Discussion

This study sought to investigate the cerebral effects of anti viral therapy in patients with mild chronic HCV. Our findings failed to demonstrate adverse effects of PIFN on cerebral metabolism or cognition. What we found instead were improvements in the spectroscopic markers of cerebral inflammation (reductions in Cho and MI) in addition to improvements in selective cognitive

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Table 3. Cerebral metabolite levels of choline (Cho), *N*-acetyl aspartate (NAA), and myoinositol (MI) expressed as ratios to creatine (Cr) from each brain region at baseline and Time 3 for all HCV patients who underwent treatment.

Measure	SVRs			NR/R		
	Cho/Cr	MI/Cr	NAA/Cr	Cho/Cr	MI/Cr	NAA/Cr
Basal ganglia						
T1	0.42 + 0.28	0.82 + 0.14	1.2 + 0.27	0.44 + 0.27	0.77 + 0.23	1.4 + 0.29
T3	0.29 + 0.02	0.73 + 0.14	1.02 + 0.18	0.39 + 0.27	0.78 + 0.11	1.17 + 0.13
p (scan 1 and 3)	0.03[a]	0.03[a]	0.13	0.75	0.75	0.17
Frontal cortex						
T1	0.26 + 0.04	0.63 + 0.15	1.7 + 0.23	0.32 + 0.18	0.73 + 0.14	1.8 + 0.32
T3	0.29 + 0.04	0.58 + 0.34	1.7 + 0.31	0.29 + 0.06	0.84 + 0.36	1.9 + 0.41
p (scan 1 and 3)	0.3	0.9	0.35	0.5	0.35	0.92
DLPFC						
T1	0.32 + 0.15	0.83 + 0.23	1.82 + 0.57	0.29 + 0.15	0.75 + 0.17	1.61 + 0.26
T3	0.24 + 0.04	0.96 + 0.53	1.71 + 0.31	0.28 + 0.06	0.89 + 0.25	1.85 + 0.26
p (scan 1 and 3)	0.3	0.87	0.5	0.6	0.17	0.35

Data presented as mean + standard deviation (SD). SVR, sustained virological responders; NR/R, non-responders/relapsers; DLPFC, dorsolateral prefrontal cortex.
[a]Significant reduction in metabolite levels, statistical significance deemed to be $p < 0.05$.

domains, in patients who cleared virus following treatment with PIFN/R, an effect that was not observed in those who failed to respond to therapy. This appears to be the first demonstration of improved cerebral inflammation and healthier neurocognitive function in SVRs and is directly attributable to the successful eradication of the virus.

Cho and MI are putative markers for glial cell inflammation and activation. Elevated levels of Cho in HCV positive subjects is believed to reflect cellular proliferation due to infection or inflammation [2,9]. MI is found only in glial cells and is also a constituent of membrane lipids [18]. Increased levels are believed to reflect glial cell activation and increased cell membrane turnover [11,19,20]. While the exact pathogenesis of glial activation in HCV is unclear, HCV RNA has been found in brain tissue and within the CSF of HCV infected individuals supporting direct infection of the central nervous system [21–25]. One hypothesis is that HCV may be introduced to the CNS via infected monocytes ('Trojan Horse' mechanism) and can infect brain microglial cells, which are essentially tissue resident macrophages of blood monocytic origin [26]. Alternatively, glial activation and inflammation in HCV may occur indirectly due to the mediation of peripherally derived pro-inflammatory cytokines such as 1L-6, IL8, IL12, and TNF-α [27–30]. Our finding of statistically significant reductions in Cho/Cr and MI/Cr in the basal ganglia following HCV eradication is suggestive of reduced glial cell inflammation ('gliosis') and adds support to the biologic link between HCV and cerebral metabolism as suggested by numerous other studies [1,8,10,30]. Trends towards significance exist in relation to the decline in basal ganglia Cho/Cr following 12 weeks of PIFN/R in both SVRs and the HCV patient group as a whole. The latter observation is likely due to the high proportion of patients who were serum HCV RNA negative at this time point. Interestingly, significant reductions in basal ganglia MI and non-significant reductions in basal ganglia Cho have also been reported in HIV positive subjects following initiation of aggressive anti-retroviral therapy [20]. We did not observe increased cerebral NAA following HCV eradication. Two studies have shown reduced NAA in HCV positive subjects when compared to HCV-negative controls, suggestive of reduced neuronal viability [8–9]. No observed 'restoration' of NAA in the current study may be due to voxel positioning, which did not include the occipital grey matter, a site of reduced NAA in prior studies. Moreover, in spite of the etiology of neuronal loss, neuronal regeneration, as indicated by increased levels of NAA, is an unlikely expected occurrence over the time frame of this study if it at all [20].

Coinciding with a reduction in the markers of cerebral inflammation, we found statistically significant improvements in neurocognitive performance among SVRs at the time of last follow up when compared to baseline, a trend not observed among NR/R. Areas of improvement fall particularly within verbal memory domains but also visuo-spatial abilities. It is likely that these improvements are attributable to the successful eradication of virus as NR/R exposed to the same testing procedures did not exhibit such improvements, although it is conceded that larger group sizes would strengthen such conclusions.

It must be noted that significant improvements in test performances were observed across a number of cognitive domains among both HCV controls and treatment recipients at Time 2 compared to baseline. While HCV treatment recipients reported significant deterioration in mood and HRQoL functioning, it was not associated with any deleterious effect on cognition, contrary to our working hypothesis at the outset. In fact, the opposite proved true with a number of significantly improved performances following 12 weeks of PIFN/R treatment. It would appear improvements at this juncture were likely to result from a practice effect as demonstrated by concomitant improvements in the HCV control group. These findings signal caution around interpreting data from repeat neuropsychological testing of patients using short interim periods. Validity of neurocognitive test outcomes can be optimized when test sittings occur after a minimum of 6 months.

This study is not without its limitations. Firstly, its small sample size may have precluded the finding of a treatment effect in the NR arm. Secondly, we also employed the use of multiple comparisons, increasing the likelihood of type I errors. We felt that the use of a Bonferroni correction for such a pilot study would have been too conservative increasing the risk of incorrectly failing to find a treatment effect. In spite of these limitations and the preliminary nature of this study, the improvements in the variables examined were consistent across the SVR group and absent from the NR/R group, but larger studies are required to validate this.

In conclusion, this study provides a substantial link between HCV and cerebral dysfunction by demonstrating a reduction in spectroscopic markers of cerebral inflammation and an improvement in cognition, following HCV eradication. While further

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Table 4. Complete breakdown of neurocognitive test performances at each time point.

Measures	Variable		Time point	HCV Controls (n = 7)	HCV Treatment Group (n = 15)	SVRs (n = 7)	NR/Rs (n = 4)
HVLT	Verbal learning total	M (SD)	T1	-2.0 (1.01)	-1.43 (0.71)	n.a.	n.a.
			T2	-1.16 (0.79)	-0.78 (1.23)	n.a.	n.a.
			T3	n.a.	-0.7 (0.79)	-0.56 (0.97)[b]	-0.95 (0.26)
	Verbal learning delay	M (SD)	T1	-1.86 (1.05)	-1.4 (0.98)	n.a.	n.a.
			T2	-0.84 (0.81)	-0.44 (1.4)	n.a.	n.a.
			T3	n.a.	-0.69 (1.05)	-0.75 (1.17)[c]	-0.58 (0.95)
	Verbal learning recognition	M (SD)	T1	0.2 (0.56)	-0.49 (1.08)	n.a.	n.a.
			T2	0.54 (0.46)	0.44 (0.61)	n.a.	n.a.
			T3	n.a.	0.78 (0.18)	0.78 (0.21)[b]	0.8 (0.14)
ROCF	Copy	M (SD)	T1	-1.0 (1.31)[a]	0.34 (0.49)	n.a.	n.a.
			T2	0.05 (1.22)	0.15 (0.77)	n.a.	n.a.
			T3	n.a.	0.33 (0.52)	0.48 (0.5)	0.08 (0.5)
	Immediate recall	M (SD)	T1	-0.42 (1.11)	-0.55 (1.02)	n.a.	n.a.
			T2	0.48 (0.42)	0.31 (0.99)	n.a.	n.a.
			T3	n.a.	0.39 (0.78)	0.71 (0.56)[c]	-0.18 (0.84)
	Delayed recall	M (SD)	T1	-0.67 (1.5)	-0.62 (0.97)	n.a.	n.a.
			T2	0.52 (0.81)	0.34 (1)	n.a.	n.a.
			T3	n.a.	0.43 (0.79)	0.78 (0.55)[c]	-0.09 (0.88)[c]
sROCF	Copy	M (SD)	T1	-0.43 (0.57)[a]	0.54 (0.62)	n.a.	n.a.
			T2	-0.24 (0.78)	0.08 (0.68)	n.a.	n.a.
			T3	n.a.	0.26 (0.26)	0.28 (0.29)	0.22 (0.19)
	Immediate recall	M (SD)	T1	0.76 (1.05)	0.49 (0.82)	n.a.	n.a.
			T2	1.33 (0.72)	1.03 (1.03)	n.a.	n.a.
			T3	n.a.	1.2 (0.79)	1.48 (0.42)[b]	0.55 (1.17)
	Delayed recall	M (SD)	T1	0.86 (1.05)	0.25 (0.99)	n.a.	n.a.
			T2	1.24 (0.5)	0.97 (1.13)	n.a.	n.a.
			T3	n.a.	1.29 (0.63)	1.39 (0.68)	0.99 (0.45)
Rey	Rey recognition	M (SD)	T1	0.86 (0.38)	0.58 (0.52)	n.a.	n.a.
			T2	0.86 (0.38)	0.75 (0.45)	n.a.	n.a.
			T3	n.a.	1 (0)	1 (0)	1 (0)
Trails	Timing A	M (SD)	T1	33.85 (12.69)	30.1 (11)	n.a.	n.a.
			T2	32.88 (12.19)	37.3 (22.1)	n.a.	n.a.
			T3	n.a.	28.7 (9.8)	28.3 (8.8)	29.1 (11.9)
	A errors	M (SD)	T1	-0.07 (1.28)	0.37 (1.11)	n.a.	n.a.
			T2	0.22 (1.28)	-0.32 (1.83)	n.a.	n.a.
			T3	n.a.	0.49 (1.04)	0.38 (0.97)	0.61 (1.21)
	Timing B	M (SD)	T1	88.75 (27.1)	69.4 (28.1)	n.a.	n.a.
			T2	90.54 (33.3)	76.2 (26.9)	n.a.	n.a.
			T3	n.a.	80.5 (35.3)	91.8 (39.7)	63.6 (21.8)
	B errors	M (SD)	T1	-0.55 (1.52)	0 (1.22)	n.a.	n.a.
			T2	-0.63 (1.58)	-0.32 (1.46)	n.a.	n.a.
			T3	n.a.	-0.58 (1.71)	-1.3 (1.8)[c]	0.49 (0.87)

All scores with the exception of Trails Timing scores are in Z score format. Timing scores are raw data.
SVR, sustained virological responders; NR/R, non-responders/relapsers; HVLT, Hopkins Verbal Learning Test; ROCF, Rey-Osterreith Complex Figure; sROCF, structured Rey-Ostererith Complex Figure; D-KEFS, Delis-Kaplan Executive Function System.
[a]Reduced performance by HCV controls compared to HCV treatment group as determined by Mann Whitney *U* Test (significance at $p < 0.05$ level).
[b]Statistically significant improvement at Time 3 compared to baseline as determined by Wilcoxon signed rank test (significance at $p < 0.05$ level).
[c]Trend towards significance $p < 0.08$.

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Table 4 (continued)

Measures	Variable		Time point	HCV Controls (n = 7)	HCV Treatment Group (n = 15)	SVRs (n = 7)	NR/Rs (n = 4)
Digit sequence	Working memory	M (SD)	T1	-0.33 (1.11)	0.17 (1.03)	n.a.	n.a.
			T2	-0.39 (0.94)	0.45 (0.94)	n.a.	n.a.
			T3	n.a.	0.40 (1.07)	0.11 (1.02)	0.84 (1.11)
Digit symbol	Processing speed	M (SD)	T1	0.05 (0.81)	0.18 (1.25)	n.a.	n.a.
			T2	0.0 (0.89)	0.29 (1.26)	n.a.	n.a.
			T3	n.a.	0.87 (1.37)	0.95 (1.18)	0.75 (1.03)
Letter number sequencing	Working memory	M (SD)	T1	-0.67 (0.3)[a]	0.24 (1.07)	n.a.	n.a.
			T2	-0.29 (0.71)[a]	1.06 (1.22)	n.a.	n.a.
			T3	n.a.	-0.13 (1.10)	-0.33 (1.27)	0.17 (0.88)
FAS	Verbal Fluency	M (SD)	T1	-0.33 (1.1)	-0.38 (0.97)	n.a.	n.a.
			T2	-0.05 (1.21)	-0.48 (1.01)	n.a.	n.a.
			T3	n.a.	0.02 (1.13)	-0.43 (1.06)	0.81 (0.84)[c]
Animals	Semantic knowledge	M (SD)	T1	0.23 (1.8)	0.56 (1.26)	n.a.	n.a.
			T2	0.26 (1.33)	0.31 (0.88)	n.a.	n.a.
			T3	n.a.	0.45 (1.05)	0.42 (1.23)	0.52 (0.62)
PEG test	Motor coordination (dominant)	M (SD)	T1	0.16 (1.25)	0.33 (1.18)	n.a.	n.a.
			T2	0.27 (1.05)	-0.24 (0.91)	n.a.	n.a.
			T3	n.a.	0.29 (0.79)	0.33 (0.99)	0.24 (0.28)
	Non-dominant	M (SD)	T1	0.36 (0.73)	0.16 (1.02)	n.a.	n.a.
			T2	0.11 (0.81)	0.74 (1)	n.a.	n.a.
			T3	n.a.	-0.07 (1.06)	0.12 (0.79)	-0.41 (1.5)
D-KEFS Color-word interference	Directed attention/ cognitive flexibility	M (SD)	T1	0.14 (0.42)	0.43 (0.82)	n.a.	n.a.
			T2	0.43 (0.46)	0.3 (0.67)	n.a.	n.a.
			T3	n.a.	0.48 (0.5)	0.33 (0.54)	0.75 (0.32)

larger-scale studies are required to confirm these findings, the cerebral benefit of HCV clearance should be recognized and considered an integral part of any anti-viral therapy dialog.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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