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Evaluation of brain volume alterations in HCV-infected patients after interferon-free therapy: A pilot study

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ABSTRACT

The study was performed to evaluate cerebral volume changes in HCV-infected subjects before and after interferon-free therapy with direct-acting antiviral agents (DAA). We aimed also to estimate the impact of successful DAA therapy on the neuropsychological state of patients. Eleven HCV genotype 1 (GT1) patients treated with ombitasvir/paritaprevir (boosted with ritonavir) and dasabuvir, with or without ribavirin underwent brain magnetic resonance (MR) before and 24 weeks after completion of therapy. All patients achieved sustained viral response. Precise automatic parcellation was made using the fully-available software FreeSurfer 6.0. Statistically significant volume deceleration six months after treatment was found in the subcallosal cingulate gyrus, transverse frontopolar gyri and sulci, anterior segment of the circular sulcus of the insula and horizontal ramus of the anterior segment of the lateral sulcus. After DAA therapy we found statistically significant improvement in the performance of all three tasks of the Rey Complex Figure Test that permits the evaluation of different functions (attention, planning, working,memory). Additionally, significant amelioration in Percentage Conceptual Level Responses in The Wisconsin Card Sorting Test (a neurocognitive test for assessing intellectual functioning) was also discovered. Successful interferon-free therapy may lead to transient cerebral atrophy, probably by reducing neuroinflammation and oedema. This is the first pilot study of the alterations in brain volume after successful interferon-free therapy in chronic HCV patients. Longitudinal follow-up study is needed to observe further effects of therapy on cerebral structures volume changes.

1. Introduction

The Hepatitis C virus (HCV) is a major cause of liver disease worldwide and a major challenge to healthcare services as a potential cause of substantial morbidity and mortality. According to the Global hepatitis report by the WHO, in 2015 an estimated 71 million people suffered from chronic HCV infection with the highest reported prevalence of 2.3% and 1.5% respectively in the WHO Eastern Mediterranean Region and the European Region [World Health Organisation, 2017]. Furthermore, hepatitis C infection remains heavily under-diagnosed largely due to the oligosymptomatic course of infection and lack of specific symptoms. It is estimated that only 20–50% of

Abbreviations: HCV, hepatitis C virus; MR, magnetic resonance; DAA, direct-acting antiviral agents; MRS, Magnetic Resonance Spectroscopy; HAI, histology activity index; CNS, central nervous system; WSCT, Wisconsin Card Sorting Test; SVR, sustained viral response; PCR, polymerase chain reaction; CVA, cerebrovascular accident; APRI, AST to Platelet Ratio Index; SWE, shear wave elastography; RPM, Raven's Progressive Matrices; CTT, Colour Trials Test; CVLT, California Verbal Learning Test; MMSE, Mini Mental State Examination (MMSE)

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individuals infected with HCV are aware of the fact that they are carriers of the disease and can take advantage of the new treatment options to limit the risk of further virus transmission as well as late consequences of chronic infection and liver cirrhosis [1].

The Hepatitis C virus infection is frequently accompanied by extrahepatic manifestations (EHMs) including disorders of the central nervous system such as chronic fatigue, subclinical cognitive impairment, psychomotoric deceleration, symptoms of depression, and neurocognitive disorders [2]. Several studies detected HCV RNA in brain samples or cerebro-spinal fluid as well as elevation of some neurometabolite ratios, which suggests that HCV infection may induce neuroinflammation and brain dysfunction [3,4].

The new, highly effective interferon-free (IFN-free) regimens with direct-acting antiviral agents (DAA) are a potent therapeutic option. These novel medications interfere with critical steps in HCV replication [5]. Compared with previous interferon-based therapies, the effectiveness of new treatment agents is amazing with sustained viral response (SVR) percentage close to 100% [6]. Furthermore, DAA exhibit a favourable tolerability profile, minimal toxicity and shortened treatment duration [7]. Based on these recent encouraging results there is a realistic hope that in the future HCV may be the first chronic viral infection to be limited or even eradicated worldwide.

However, data regarding the safety and efficacy of DAA in HCV patients with EHMs are very limited. This study was aimed at investigating the association between HCV eradication and brain volume changes in subjects treated with DAA. Additionally, possible fluctuations in neuropsychological state of the patients were also assessed.

This is a pioneering project on a global scale. Until now, there have been no previous reports about the impact of DAA on cerebral volumetric changes.

2. Materials and methods

The study was conducted in accordance with the 1964 Declaration of Helsinki for Human Research and its later amendments, and the protocol was accepted by the local Ethics Committee (opinion number: 1/BO/2014). All individual participants gave voluntarily informed consent prior to inclusion in the study.

The prospective one-centre observational study was carried out between January 2015 and May 2016.

2.1. HCV subjects

Eleven European Caucasian race adult patients (6 female and 5 male; mean age 52 years, range 34–70 years) with chronic HCV genotype 1 infection were recruited from a named patient programme created to enable IFN-free therapy in patients at risk of an unfavourable course of chronic HCV infection. Antiviral drugs were provided by the manufacturer (AbbVie).

Previous antiviral treatment with interferon alfa and ribavirin did not exclude subjects from the study. Seven patients were treatment-experienced with pegylated interferon alfa and ribavirin. Advanced fibrosis or liver cirrhosis with no history of liver function decompensation was identified in five patients.

Therapy with ombitasvir/paritaprevir (boosted with ritonavir) and dasabuvir, with or without ribavirin (OBV/PTV/r/DSV ± RBV; 3D ± RBV) was scheduled for 12 or 24 weeks in accordance with the label and the European Association for the Study of the Liver (EASL) recommendations current at that time. The dose of co-formulated OBV/PTV/r was respectively 25 mg/150 mg/100 mg daily and the dose of DSV was 500 mg daily, divided into two doses. Four patients received RBV of 1000 or 1200 mg daily, corresponding to body weight.

During and after therapy patients underwent routine clinical assessment and basic laboratory testing to monitor potential side effects. The liver function was scored in Child-Pugh and MELD. Liver fibrosis was evaluated at D0 and FU24. Real-time shear wave elastography (SWE) using the Aixplorer US system (SuperSonic Imagine S.A., Aix-en-Provence, France) with a convex broadband probe (SC6-1) was performed with liver stiffness measured according to the cut-off values of SWE validated for hepatitis C [3]. An AST to Platelet Ratio Index (APRI) was calculated based on laboratory tests performed routinely in a local laboratory with an APRI score > 0.7 as the cut-off value for predicting advanced fibrosis and 1.0 as the cut-off value for cirrhosis [8].

HCV RNA was measured by the real-time polymerase chain reaction method using the GeneProof Hepatitis C PCR Kit (GeneProof a.s., Brno, Czech Republic) and performed on a Rotor-Gene 3000 (Corbett Research, Australia; sensitivity(LoD) 7.87 up to 208.544 IU/ml; linear range 1010–25 IU/ml ± 0.5log) to assess effectiveness of the therapy.

Two measurement points with MR imaging, voxel-based morphometric assessment, neurological assessment and psychological evaluation were: baseline (D0) and 24th week (FU24).

2.2. Control group

Eighteen healthy subjects (10 women and 8 men; mean age 48 years, range 32–68) with no history of liver disorders were included as the control group. Volunteers were matched to HCV patients in terms of age, sex and level of education.

2.2.1. Exclusion criteria

In order to isolate any potential confounding factors, exclusion criteria for both HCV patients and controls included as follows: presence of any neurological disease (e.g., cerebrovascular disease, inflammatory changes, multiple sclerosis, post-traumatic and post-toxic changes, brain neoplasms; patients with symptoms of central neurological deficit), structural brain abnormality on conventional MR imaging, HIV or hepatitis B virus (HBV) infection, past or current alcohol or drug abuse.

Epidemiological and clinical characteristics of the patients and control group are presented in Table 1.

2.3. MR imaging protocols

All MR examinations were performed on a 1.5 T MR scanner (Signa Hdx, GE Healthcare). Conventional T1-weighted images of the brain were recorded to assess cerebral morphology for designed volumetry.

The MR protocol consisted of an axial fast spoiled gradient-echo (FSPGR) 3D T1-weighted (T1w) sequence, axial, sagittal and coronal T2-weighted images, axial FLAIR (fluid-attenuated inversion recovery

Table 1
Demographic and clinical characteristics of chronic hepatitis C patients (HCV +) and healthy controls (CG).

	HCV +	CG
Sex, M/F	5/6	8/10
Age, years:		
Mean	52	48
Range	34–70	32–68
HCV infection estimated duration (years)	9–43	NA
probable route of HCV transmission	4 PE, 7 HAI	NA
Previous IFN-based antiviral treatment	7 patients	NA
HCV RNA, IU/ml:		NA
Range	46,400–12,800,000	
Mean	371,090.9	
HCV genotype, 1a/1b	2/9	NA

M: male; F: female.
NA: not applicable.
Negative: no history on use of drugs affecting the central nervous system in a medical interview; estimated duration of HCV infection: based on medical interviews and dated from the possible exposure probable route of HCV infection: based on medical interviews; PE - professional exposure, HAI - hospital acquired infection.

sequence) images, as well as diffusion-weighted imaging (DWI).

On the basis of the volumetric Bravo sequence, brain segmentation was performed into two basic classes: grey matter and white matter, and subsequently individual subcortical structures were separated in order to measure their volume.

The high-resolution three-dimensional brain volume imaging acquisition sequence (3D-BRAVO) MRI provides excellent T1w contrast between grey and white matter and is good for showing anatomical details. Bravo is one of the basic acquisition protocols for Automated Brain Segmentation. Acquisition time: 5:15 Voxel size: $1.0 \times 1.0 \times 1.0\,mm^3$, Geometry: FoV 25.6 cm (256×256 matrix), 176 sagittal slices, slice thickness 1 mm. T1W images are also required for assessing the degree of contrast enhancement on post-contrast scans.

2.4. Voxel-based morphometric assessments

In order to investigate a potential association between DAA therapy and brain volume changes in HCV patients, we conducted a voxel-based morphometry analysis.

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite version 6.0, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications [9–16].

The preprocessing of T1-weighted images was conducted in accordance with the standard recommended protocols (http://surfer.nmr.harvard.edu/). Using fully-available software the grey and white matter segmentation was done (Fig. 1). Subsequently the volume of whole grey and white matter in a study group before and 6 month after completion of the treatment was calculated. After automatic processing the white matter, subcortical segments and cortical parcellation of each individual were visually checked according to FreeSurfer quality control procedures. The aim was to assess the volume of each region and

Fig. 2. Cortical Surface Reconstruction and Labeling.

statistical analysis of group morphometry differences. Freesurfer segmentation is based on standard international anatomical nomenclature (topographic Brodmann map) (Fig. 2). Furthermore, Freesurfer provides topographic reconstructions permitting 3D views of the human brain (Fig. 3).

Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths [17,18].

2.5. Neurological assessment

All subjects underwent physical and neurological examination. Subjects with no significant neurological abnormalities in standard questionnaires and with a Karnofsky score above 80 were included in the study.

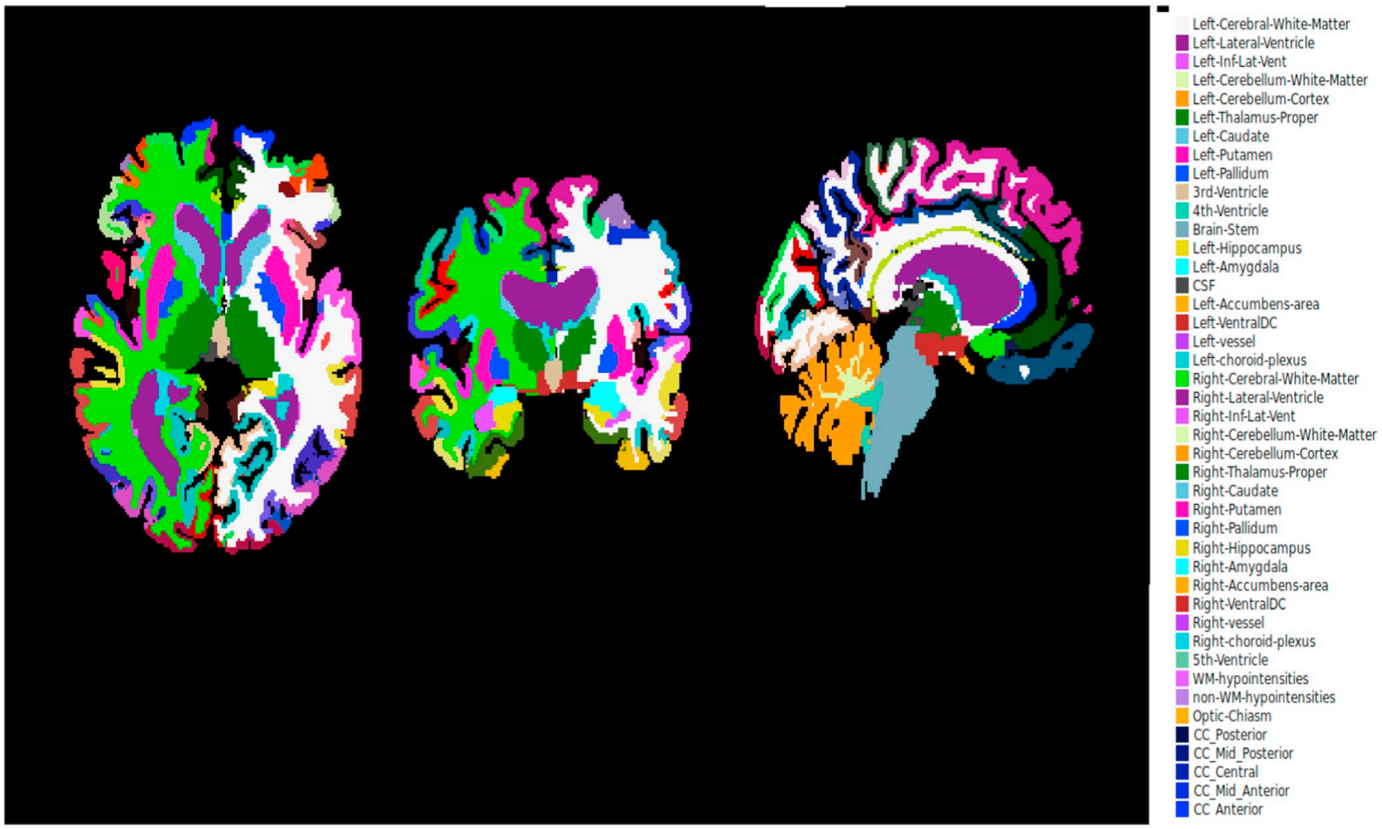

Fig. 1. Anatomical parcellation maps.

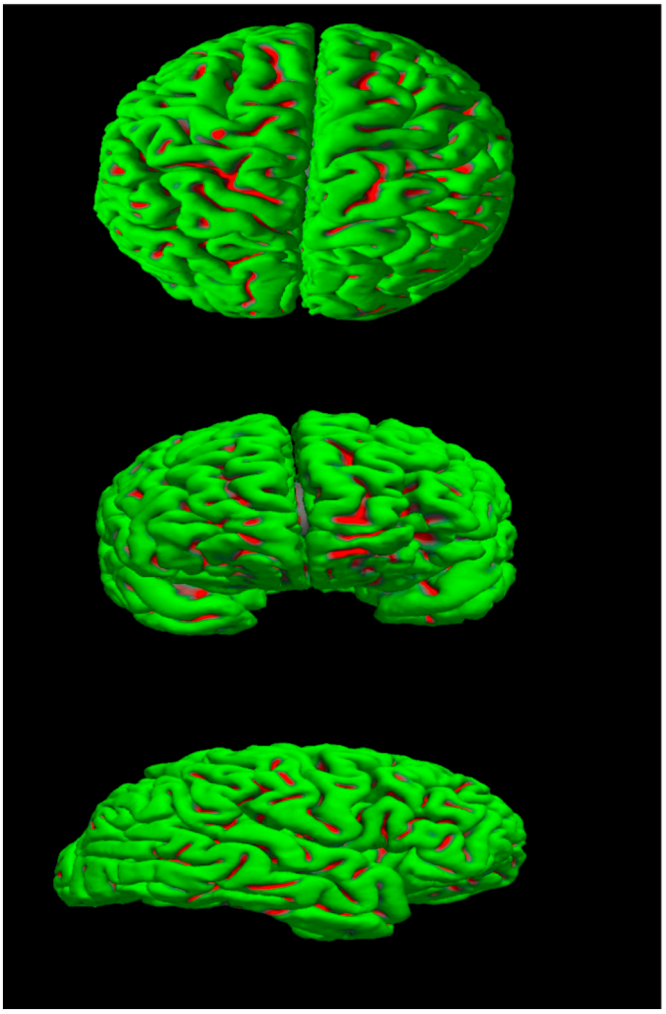

Fig. 3. 3D- Visualization of the whole brain from baseline MRI of an HCV patient.

2.6. Psychological evaluation

Neuropsychological assessment was performed using a series of seven validated, cognitive and neuropsychological tests as follows:

- Mini Mental State Examination (MMSE) to estimate the possible cognitive impairment,
- Raven's Progressive Matrices (RPM) to test abstract reasoning,
- California Verbal Learning Test (CVLT) to measure verbal learning and memory using a multiple – trial list – learning task,
- Wisconsin Card Sorting Test (WCST) to evaluate the effects of a frontal lobe injury, especially to assess cognitive reasoning,
- Colour Trials Test (CTT) as a measure of sustained and divided attention,
- Rey-Osterrieth Complex Figure Test, the purpose of which is to assess visuospatial spatial recall memory and constructional ability,
- Verbal Fluency (with Phonemic Fluency Tasks and Semantic Fluency Tasks) to examine the spontaneous production of words under restricted search conditions.

All assessments were conducted by the same clinician who was unaware of the patients' treatment outcome.

2.7. Statistical analysis

Statistical analysis was performed using Statistica v.12, computer software by StatSoft, Inc. Descriptive analyses were performed for demographic, virologic and MR volumetric measurements. The nonparametric Wilcoxon Matched Pairs Test was used to compare cognitive performance before and after treatment in all neuropsychological tests. The volumetric variables fulfilled the assumptions for parametric Student's *t*-test and was used to compare mean volumetric parameters before and after treatment (*t*-test for dependent variables), and to compare mean volumetric parameters between patients and controls (*t*-test for independent variables). A *p*-value of 0.05 was considered statistically significant in all the tests (two-sided).

3. Results

All patients achieved a sustained viral response measured as undetectable HCV RNA at 24 weeks after completion of antiviral therapy.

3.1. Cognitive tests

We reported statistically significant improvement in the performance of all three tasks of the Rey-Osterrieth Complex Figure Test – immediate replication of a figure ($Z = 2.20$ $p = .028$), replication after 3 min ($Z = 2.09$ $p = .037$) and after 30 min ($Z = 2.24$, $p = .025$).

Wisconsin Card Sorting Test (WCST) scores were not different before and after treatment, except for one of the scales - Percentage Conceptual Level Responses ($Z = 2.24$, $p = .02$) which gives a measure of insight into sorting principles. The Percentage Conceptual Level Responses is the key element for WCST assessment of intellectual functioning, which is a measure of executive functions.

There was no statistically significant difference between neurocognitive measurements before and after treatment in: Raven's Progressive Matrices (RPM), Colour Trials Test (CTT), California Verbal Learning Test (CVLT), Verbal Fluency Tasks and Mini Mental State Examination (MMSE).

3.2. Neurological examination

Before antiviral therapy neurological examination was normal in 5 patients (45.5%). One patient (9%) revealed symptoms from the central nervous system: left-side mouth asymmetry and weak deep tendon reflexes on the left side. Six patients (54%) showed symptoms from the peripheral nervous system: in one patient, left-side mouth asymmetry and weak deep tendon reflexes on the left side, and in 6 patients attenuation of superficial sensation in the distal parts of the legs as well as decreased knee and ankle reflexes.

In nine patients the neurological condition did not change significantly after therapy. In two patients we observed an improvement in superficial sensation in the distal parts of the lower limbs.

3.3. Magnetic resonance imaging and brain volume changes

Before therapy, there were no significant differences between HCV patients and healthy controls in total grey and white matter volumes. Furthermore, grey and white matter volume in the frontal, temporal, parietal, and occipital lobe did not differ between HCV patients and controls (Table 2).

Compared with healthy controls, HCV patients after treatment displayed significant volume loss in the subcallosal cingulate gyrus ($p = .029$), transverse frontopolar gyri and sulci ($p = .034$), anterior segment of the circular sulcus of the insula ($p = .01$) and horizontal ramus of the anterior segment of the lateral sulcus ($p = .044$) (Table 3; Fig. 4). These differences in mean volume values should be regarded as not very significant, due to the small size of the groups and low power calculations: about 0.5 for *t*-tests for dependent variables (comparisons before and after treatment), and about 0.25 for independent variables (comparisons with the control group).

Table 2
Mean total volume values of selected brain regions and *p* values.

Location	Mean volume			p-Values*	
	HCV-preT	HCV -postT	CG	HCV-preT/CG	HCV -postT/CG
Left-Unsegmented White Matter (LWM)	31,051.99	31,647.27	32,216.96	0.62	0.81
Right-Unsegmented White Matter (RWM)	31,966.55	31,742.22	32,739.75	0.75	0.66
left Cortical Volume (lCV)	218,490.88	219,925.57	229,855.76	0.29	0.35
right Cortical Volume (rCV)	219,784.97	220,028.16	229,630.33	0.33	0.34
Total Grey Volume (TGV)	570,544.78	572,805.36	593,876.61	0.26	0.30
Estimated Total IntraCranial Vol (TV)	1,404,726.57	1,422,944.48	1,457,471.86	0.32	0.49

* *t*-test.

4. Discussion

The Hepatitis C virus is a potential neurotrophic pathogen that can cause non-specific, mild to moderate neurological symptoms in a significant set of patients with chronic infection [19].

Although our knowledge is deepening regarding possible mechanisms of virus transmission to the central nervous system, in the diagnostics of neurological manifestations of HCV, in particular neurocognitive disorders, we lack the tools to assess them objectively. Asymptomatic patients chronically infected with HCV are not routinely directed to neurological tests to assess possible virus dependent neurological impairment. Over the past decade, several groups have studied possible cerebral effects of HCV using advanced MRI techniques such as MRS (Magnetic Resonance Spectroscopy) in non-symptomatic subjects [19].

In our previous report we noted a significant decrease of the NAA/Cr ratios in frontal and parietal white matter in HCV infected patients [20]. The discovered metabolic changes have been generally assumed to be due to microglial activation and astrocytic gliosis induced by HCV infection [21,22] and confirm the neurotoxicity of HCV.

Furthermore, we observed hyperperfusion in basal ganglia in HCV patients compared to the control group. It might be an indicator of the cerebral inflammation process ongoing in HCV-infected patients [23].

In the present study, we focus on specific aspects of DAA therapy on central nervous system manifestations. In the current literature we found some studies confirming an improvement of associated neuropsychiatric disorders in patients with sustained virology response after pegylated interferon with ribavirin therapy [24]. Nevertheless, there is lack of reports about the effect of new IFN-free therapy on CNS using advanced MR imaging.

Our report combines quantitative analysis of volumetric parameters and the whole spectrum of neuropsychological evaluation to detect pre-treatment and post-treatment cognitive functions in patients with chronic HCV infection. The aim of such a comparison was to understand the connection between brain damage and the extent of neuropsychiatric changes in treated patients.

In our study, we observed a statistically significant improvement in the performance of all three tasks of the RCFT and in the Percentage Conceptual Level Responses scale of WCST. It confirms an improvement in visuospatial and executive functioning in those individuals with efficient antiviral therapy. Significant improvement of an individual's competence in abstract reasoning measured with WCTS after treatment can indicate recovery and reorganisation in frontal lobes. RCFT measures visual problem solving and non-verbal memory. Significant improvement in RCFT results provides evidence of changes in the functioning of temporal lobes.

In summary, our findings indicate that successful DAA treatment leads to partial cognitive improvements in some domains measured by standard neuropsychological testing.

We did not find any statistically significant differences in executive functions measured with WCST, RPM, CTT, CVLT, VFT and MMSE.

Incidentally, neurological condition did not change significantly after therapy in most of the patients. Only 2 patients reported any improvement of superficial sensation in distal parts of the legs. Significant improvement in only two aspects of executive functions indicate that we should interpret these results with some caution.

In the literature we found some studies on anti-viral therapies with interferon with or without ribavirin, confirming that HCV eradication has had a beneficial effect on the cerebral metabolism and cognition. Byrnes et al. reported a significant reduction of choline and mioinozytol in basal ganglia in those who obtained permanent virus elimination after interferon/ribavirin therapy [24]. Barbosa et al. showed a significant improvement in the immediate and delayed verbal episodic memory, selective attention and phonemic fluency [25]. These results indicate that eradication of HCV may reduce cerebral dysfunction. Furthermore, the authors report significant improvements in verbal learning, memory and visuo-spatial memory in those patients [24]. This therefore suggests that changes in the central nervous system are potentially reversible.

These findings are consistent with our results showing increased Fractional Anisotropy (FA) values in all evaluated white matter tracts after DAA therapy, compared to values obtained before treatment, which is highly suggestive of white matter tracts recovery after successful interferon-free therapy in chronic HCV infection [26,27].

These results seem to confirm the hypothesis about the neuroprotective effect of DAA treatment.

The comparison of atrophy rates between the pre-treatment and post-treatment period showed significant reduction in brain volume rate 6 months after treatment completion. One possible explanation for the observed findings is that brain atrophy following the initiation of

Table 3
Mean volume values of the selected statistically significant areas in HCV-infected patients before treatment (HCV-preT), after treatment (HCV -postT) and the control group (CG).

Location	Mean volume			p-Values*	
	HCV-preT	HCV -postT	CG	HCV-preT/CG	HCV -postT/CG
Transverse frontopolar gyri and sulci	2578.91	2161.00	2340.53	0.044	0.029
The subcallosal cingulate gyrus	659.27	540.36	699.65	0.037	0.034
Horizontal ramus of the anterior segment of the lateral sulcus	550.00	494.00	503.06	0.021	0.01
Anterior segment of the circular sulcus of the insula	1043.09	962.00	957.12	< 0.001	0.044

* *t*-test.

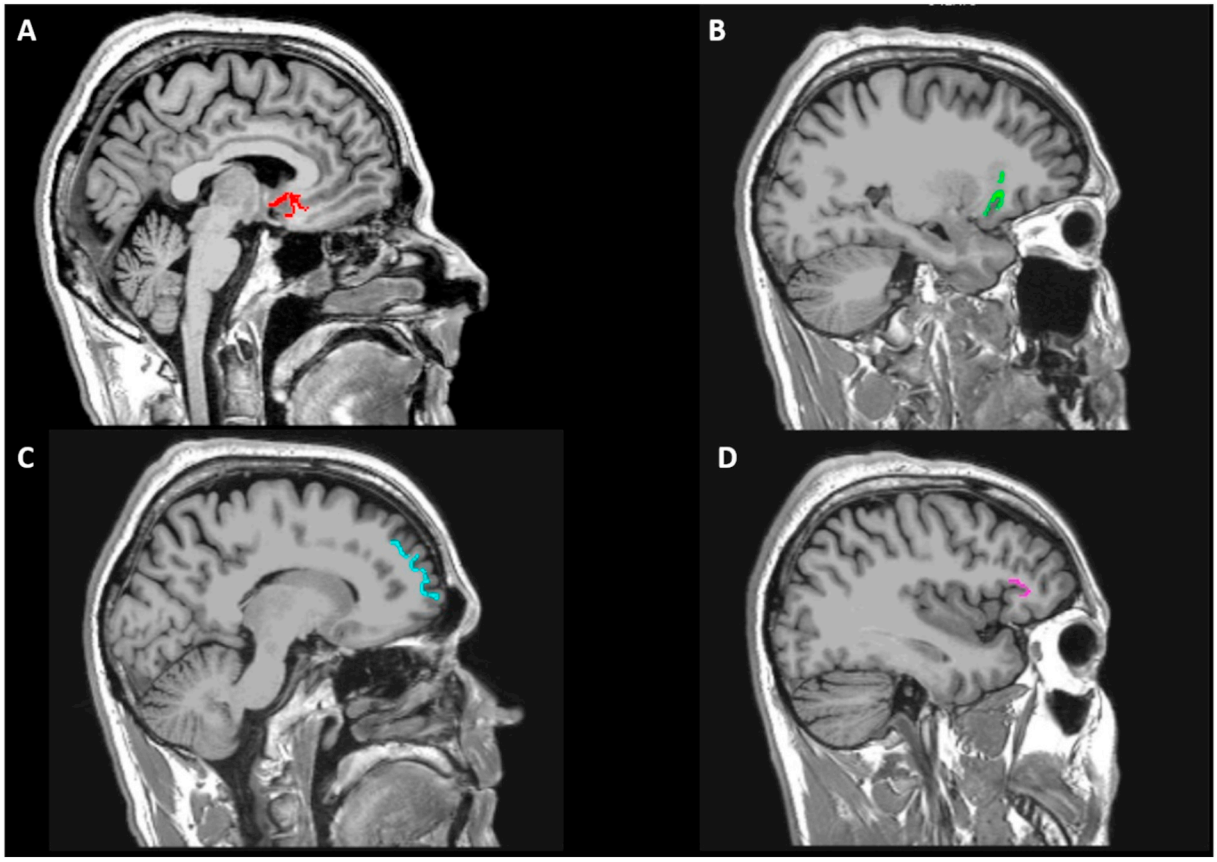

Fig. 4. Images show areas of the significant volume loss after DAA treatment represented by Freesurfer anatomical parcellation ROIs in 4 locations: subcallosal cingulate gyrus- marked in red (A), anterior segment of the circular sulcus of the insula – marked in green (B), transverse frontopolar gyri and sulci- marked in blue (C), horizontal ramus of the anterior segment of the lateral sulcus – marked in purple (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

therapy might be a consequence of reduced HCV-dependant oedema and inflammation [28].

Brain volume changes may also reflect changes in tissue water content due to disruption of the blood–brain barrier. Such a treatment-related, transient volume fluctuation is called “pseudoatrophy”, and should be distinguished from real tissue destruction and not be described as cerebral atrophy [29,30]. Furthermore, early brain atrophy after successful anti-inflammatory treatment seems to confirm the theory about brain inflammation in HCV patients [30,31].

Similar findings have already been reported by many studies in the literature. Chow et al. reported in their systematic review of neuroimaging studies a relationship between elevated levels of circulating cortisol with volumetric reduction of some brain structures such as the hippocampus [19].

The phenomenon of pseudoatrophy is well-described for therapies in patients with multiple sclerosis. According to them brain volume loss following the initiation of therapy was mainly confined to white matter and were described during the first year of therapy with natalizumab and interferon-beta [32,33]. The pathomechanism is referred to the resolution of inflammation and changes in the volume of inflammatory cells, such as microglia. It is necessary to distinguish it from true neurodegeneration, as it might complicate the interpretation of treatment effects. Thus the treatment effect might be delayed by at least several months [34].

Another study in an independent cohort of SM patients treated with interferon-beta have demonstrated that baseline inflammation estimated by the number of Gd-enhancing lesions was predictive of larger decreases in brain volume during the first year of therapy [32].

To the best of our knowledge, this report is the first study to explore the possible pseudoatrophy-like phenomenon after initiation of DAA treatment in HCV infected patients. Further follow-up is needed to assess atrophy rates over 6 months after DAA therapy compared with the pre-treatment state. It cannot be excluded that improvements may occur slowly after SVR achievement.

Nevertheless, it is important to note that the most important strength of our study is that for the entire sample there were no significant differences in age, gender, education or ethnicity between the groups (HCV and controls). We compared exactly the same group of subjects before and after treatment using exactly the same MR protocols. Therefore, we are convinced of the quality of our data.

What is of interest is that the volumetric parameters were measured as soon as 24 weeks after completion of the treatment. The potential hypothesis concerns the fact that improvements occur slowly over time. Thus follow-up is needed for understanding of the neuroanatomic effects of DAA and to check if these volume reductions will be stable over time.

However, our study has some limitations. The major limitation is the relatively small number of subjects. However, the subjects’ population is quite similar to other previous studies in this field. In the recently-published paper by Kleefeld et al. only 8 HCV-positive subjects have been included in the study in order to evaluate the effects of DAA therapy on cognition in HCV patients [35].

5. Conclusion

The neurologic manifestations of HCV are common and can adversely affect the quality of life. Thus, HCV-induced neurocognitive dysfunction assessment should be considered in all patients infected

with the hepatitis C virus when making treatment decisions, especially in those with mild disease. Therefore, for all patients infected with the hepatitis C virus, a diagnosis of HCV-induced neurological dysfunction should be undertaken considering hepatitis C treatment in the era of direct acting antivirals in not advanced liver disease. Without a doubt, the role of the new IFN-free therapy in the management of HCV-related neuropsychiatric disorders deserves further in-depth investigation, especially in the light of high recovery rates. HCV eradication has had a beneficial effect on selective aspects of neurocognitive function. Our findings from MR volumetry and neurophysiological research suggest that the basis of the mechanism of pseudoatrophy may be the phenomenon of partial brain volume loss. In conclusion, this report shows that further follow-up studies are desirable to observe for delayed benefits of HCV eradication on cerebral function. The point of interest is to determine whether treatment-induced viral clearance has the potential to abolish initial atrophy over a longer period of time. This is the first reported and therefore unique sample study of a therapeutic outcome for treatment with direct-acting antiviral agents using outcome parameters, including MR volumetry and neurocognitive function. The alterations of the brain volume and their associations with clinical status after successful DAA therapy bring us new insight and might be of great interest to clinicians as a biomarker of treatment results.

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