

# Cerebral immune activation in chronic hepatitis C infection: A magnetic resonance spectroscopy study

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"This study supports previously published data showing that HCV infection is associated with CNS dysfunction, and it is the first MRS study to show a significant correlation between neurocognitive deficits and changes in MR-measurable metabolites in patients with minimal hepatic inflammation. The results thus echo previous studies which have demonstrated this link between MRS and cognition in HIV infection. Furthermore, we propose that cerebral immune activation is a key concept in understanding the issues surrounding the effect that HCV has on the brain. The difficulty in clinical practice is that  $\alpha$ -interferon-based treatments themselves exacerbate the situation, at least in the short to medium term. **Whether successful viral eradication eventually results in normalization of these changes is not known**, although improvements in health-related quality of life and fatigue scores after the end of antiviral therapy suggest that this **may** be the case. Longitudinal studies incorporating cognitive assessment and neuroimaging **may** in time provide the answer."

## Introduction

Patients with chronic hepatitis C (HCV) infection and human immunodeficiency virus HIV/HCV co-infection frequently report neuropsychological symptoms.

Numerous studies have documented high levels of fatigue, depression and impaired health-related quality of life (HRQL) in patients infected by HCV [1], [2], [3], [4], [5], [6], [7], [8], [9], [10].

A number of studies have also reported an **adverse impact of HCV on cognitive function** [11], [12], [13], [14], [15], [16].

Slowed processing speed and impaired working memory are the most common findings.

There has been considerable debate as to whether there is a biological basis to these findings and a number of studies have suggested that social and psychological factors are more important than the viral infection per se [17], [18], [19], [20], [21].

However, **a biological basis for neurocognitive impairment in HCV** infection is suggested by reports of both **HCV replication within the central nervous system** (CNS) [22], [23], [24] **and of altered cerebral metabolism**, as measured by in vivo proton magnetic resonance spectroscopy (1H MRS) [11], [15], [16], [25].

Using different techniques, four MRS studies have variously reported **elevations in basal ganglia and central white matter choline (Cho)-containing compounds** [11], [16], [25] and **reductions in grey and white matter N-acetyl aspartate (NAA)** [15], [16] in patients with histologically-proven **mild liver disease** due to HCV infection.

The studies to date have not been able to demonstrate an association between MRS findings and neurocognitive performance.

It should be noted that in vivo MRS is a readily available, standardized technique, which gives information on a number of cerebral metabolites, depending on the acquisition parameters employed. Cerebral 1H MRS has been readily applied to HIV-associated minor cognitive-motor disorder (MCMD) and HIV-associated dementia (HAD) more extensively than any other viral infection [26], [27]. In HIV disease, an early and common finding is increased myo-inositol (mI) in the frontal white matter [28], [29]. Other findings include elevated Cho and decreased NAA in both the white matter and basal ganglia [30]. These latter abnormalities parallel the findings to date in **HCV** infection.

myo-Inositol (mI) is an intracellular metabolite involved in the synthesis of phosphoinositides and it also plays a significant role as an osmolyte in the regulation of cellular swelling within the brain [31]. Increased mI is associated with microglial activation and astrogliosis [32], [33]. Elevated levels of mI in HIV infection are thought to relate to central nervous system (CNS) inflammation, which may underlie neuronal dysfunction [27], [29], [34], [35]. Of all readily measurable MR metabolites, elevations in white matter mI are the most consistently associated with abnormal cognitive processing in early HIV disease [36]. To date, there have been no reports of increased cerebral mI in HCV infection.

This in vivo cerebral 1H MRS study was designed to address the hypothesis that increases in cerebral mI occur in HCV-infected patients as a result of immune activation within the brain and that this cerebral metabolite abnormality is associated with mild cognitive impairment.

Twenty-five patients with histologically-defined mild chronic hepatitis C were recruited from a viral hepatitis clinic in a tertiary referral centre (St. Mary's Hospital, London). The patients had been referred for assessment of their liver disease. The mean age (standard deviation – SD) was 45.0 (8.3) yrs and the percentage of male subjects was 54%. Liver biopsy on all patients had been performed no more than 18 months prior to the study. All individuals had **mild inflammation only, in the absence of** cirrhosis or **significant fibrosis**. The median Ishak necroinflammatory score was 2 and the median Ishak fibrosis score was 1 [37]. All patients were viraemic at the time of the study, as defined by a positive PCR for HCV RNA (Roche Amplicor version 2). The method of viral transmission was related to injection drug use (IVDU) in 65% of cases and to infected blood products or undefined sources in 35% of cases. All patients were carefully questioned for a history of major recreational drug usage (MDU). Individuals were asked whether they had ever used any of five substances (heroin, methadone, LSD, cocaine, ecstasy). They were only classified as non-users if a history of MDU was unequivocally absent. None of the patients had used any recreational drugs in the year preceding the study.

## ABSTRACT

### Background/Aims

Abnormal cerebral metabolism and cognitive impairments have been reported in patients with chronic hepatitis C (HCV) but studies have failed to demonstrate a relationship between these findings.

## Methods

Twenty-five HCV-positive patients with **histologically-mild liver disease** were studied with cerebral proton magnetic resonance spectroscopy (MRS), using acquisition parameters to quantify myo-inositol (mI) and other metabolites in frontal white matter (FWM). Patients underwent automated attention and working memory tests (Cognitive Drug Research test system).

## Results

**The mean mI/creatine ratio in the HCV+ve patients (0.64, SD 0.21) was significantly higher (p=0.02) than in healthy controls (0.52, SD 0.10).**

On cognitive testing, the HCV+ve patients showed impairments in 2/4 composite scores, reflecting **working memory and attention, compared to normative data from healthy volunteers** (p<0.005) and HCV–ve controls (p=0.03). There was a significant association between elevated FWM mI/creatine and prolonged working memory reaction times (R=0.72, p=0.002).

## Conclusions

Elevated FWM mI/creatine is a feature of HIV-related minor cognitive-motor disorder. It is associated with **infection and immune activation of microglial cells**.

The similar findings in this study suggest that **cerebral immune activation may also occur in HCV infection**.

This may underlie the mild **neurocognitive impairment and neuropsychological symptoms observed in a proportion of patients**.

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## Results

The mean **mI/Cr in the HCV-positive (+ve) patients was significantly higher** (p=0.02) than in the healthy controls (Table 1).

Twenty percent of the HCV+ve patients had **mI/Cr ratios that were elevated** above the highest value in the control group (Fig. 2).

There was no significant difference in mI/Cr between HCV-infected patients with and without a history of MDU (MDU+ve 0.69, SD 0.24; MDU-negative (–ve) 0.57, SD 0.15, p=0.15).

There were no significant differences in any other metabolites (NAA, glutamine/glutamate (Glx) or Cho) (Table 1).

There were no significant associations between age and any of the measured metabolites.

A sub-group of 16 HCV-infected patients (62% male, mean age 40 years) underwent cognitive testing.

Cognitive testing facilities were not available for the other nine patients;

there were no demographic differences between those who did and did not undergo cognitive assessment.

Impairments were seen in 2 out of 4 composite scores, reflecting spatial and numeric working memory and attention, compared to normative data from healthy volunteers ( $p < 0.005$ ) (Table 2). The same impairments were seen when the patients were compared to the historical HCV-exposed but HCV-ve controls ( $p = 0.03$ ).

There was **a strong and significant association between elevated mI/Cr and prolonged working memory reaction times** ( $R = 0.72$ ,  $p = 0.002$ ) (Fig. 3).

There were no significant associations between the working memory or attention scores and the other cerebral metabolite ratios.

## Discussion

This is the first cerebral MRS study to significantly associate MR-measurable metabolite changes with the cognitive deficits in patients with histologically-mild HCV-related liver disease.

Our findings on mI are thus different from previous studies and the sophisticated data analysis methods that we used in this study may be of relevance in elucidating subtle metabolite changes, and in explaining the different results obtained from those in the literature.

The  $^1\text{H}$  MRS signal from intracellular mI forms part of a composite resonance and its measurement is dependent on accurate measurement of the other components of this resonance (Fig. 1).

A number of techniques have been employed for this, most commonly involving the measurement of peak areas by integration or line fitting of the Fourier-transformed signal in the frequency domain. Analysis of spectra, in either the time or frequency domain can be improved by the use of previously obtained information regarding the component characteristics of the spectrum [41], known as prior knowledge. The MRUI software package [38] uses the AMARES algorithm [41] to analyse spectra in the time domain, which confers a number of advantages. The method can cope with artefacts, such as baseline roll, which results from a short delay in data acquisition in the sequences used, and any underlying broad spectral component [42]. Furthermore, the only user input that MRUI requires is an initial estimate of parameter values. Starting from these values, it incorporates prior knowledge, reducing operator-dependent variability during operations such as phasing of spectra [43].

The use of the above techniques may explain why we detected elevated white matter mI/Cr in HCV-infected patients in contrast to two previous studies, which also measured mI [15], [16]. Both these studies measured metabolites or metabolite ratios in the frequency domain. Furthermore, unlike McAndrews and colleagues [16], we expressed metabolites as ratios, rather than estimating absolute concentrations, which requires an assumption that brain water concentrations remain unchanged in disease. In theory, our methodology may reduce the magnitude of any error, since there is a difference of several orders of magnitude between brain water and mI concentrations [44]. The current study did not show elevations in cerebral Cho/Cr, in contrast to our earlier studies, where the acquisition parameters were optimized for the measurement of Cho with a longer echo time (135ms).

The disadvantage of expressing metabolites as ratios is that it is not clear from the data whether the increased mI/Cr in the HCV patients is a numerator or denominator effect. The normal Cho/Cr and NAA/Cr levels in this study suggest that there are true elevations in mI concentrations. However, the stability of cerebral Cr, which represents high-energy metabolites, has been questioned. In HIV infection, Chang and colleagues measured the level of cerebral MRS metabolites at different stages of AIDS dementia complex (ADC), using an absolute quantification technique [36]. They found

increases in basal ganglia Cr in mild dementia, but reductions in more advanced disease. In contrast, there were elevations of white matter Cr in advanced AIDS dementia.

In our study, there were no statistical differences in mI/Cr between patients with and without a history of MDU. However, more patients with a history of MDU had mI/Cr values well outside the normal range. In the light of this, it should be noted that both 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and methamphetamine have been reported to increase white matter mI/Cr [45], [46]. We cannot therefore exclude the possibility of an interaction between a history of usage of these compounds and HCV infection to account for abnormal cerebral MRS. Such a relationship has been demonstrated in HIV infection [46] and has been suggested in HCV infection [47].

Elevations in cerebral mI in HIV infection have been postulated to be the MRS correlate of inflammatory pathways leading to neuronal dysfunction, mediated via pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-6 [33]. The findings in our study would be consistent with the hypothesis that there is immune activation within the CNS in a proportion of patients with HCV infection. Furthermore, the demonstration of a significant correlation between mI/Cr and the working memory index, supports a biological aetiology for the neurocognitive dysfunction observed in HCV infection, as opposed to a non-specific consequence of an emotional disorder. The mechanism for this remains unknown, although the CNS effects of peripheral immune activation with recombinant cytokine administration, such as  $\alpha$ -interferon, or lipopolysaccharide (LPS) provide a number of insights.

**Depression, fatigue and cognitive impairment are well described in patients treated with  $\alpha$ -interferon, both in chronic HCV infection** and other conditions such as melanoma [48], [49], [50].

The peripheral administration of LPS or pro-inflammatory cytokines, such as IL-1 to healthy laboratory animals results in sickness behaviour, characterized by reduced activity, such as foraging for food or social exploration [51], [52].

This has been interpreted as an expression of reduced motivation, which may, in certain situations, serve as an adaptive response [53].

Injection of IL-1 into the lateral ventricles produces the same physiological and behavioural results as the systemic injection of much higher doses, including fever, reduced feeding, social withdrawal and sleep [53].

This suggests that cytokine **receptors exist within the brain and that peripheral cytokines cross or signal across the blood–brain barrier (BBB)**.

Three mechanisms have been suggested:

- (1) saturable transport of cytokines across the BBB [54],
- (2) production of pro-inflammatory cytokines by perivascular macrophage-like cells in the circumventricular organs in response to circulating cytokines or viruses and
- (3) an action of peripheral cytokines on afferent nerves to the CNS such as the vagus nerve.

The latter mechanism is supported by animal experiments in which sub-diaphragmatic transection of the vagus nerve resulted in abrogation of sickness behaviour induced by intraperitoneal LPS injection, but not by intravenous injection of IL-1 [55].

This mechanism may be particularly relevant in HCV infection, where the cytokine milieu in the liver, innervated by the vagus nerve, is deranged.

Several studies have reported **elevated levels of circulating cytokines in chronic HCV infection, including IL-1 $\beta$ , and TNF- $\alpha$**  [56], [57], which may result in CNS immune activation through the first two mechanisms.

Although the relative importance of these different mechanisms remains unclear, it is established that one of the principle neurochemical consequences of peripheral immune activation within the CNS is induction of intracerebral cytokine production, mainly from glial cells (for example: intracerebral IL-1 $\beta$  production, following peripheral administration of LPS) [58].

It has also been postulated that HCV may enter and replicate within the CNS. In support of this, negative strand HCV RNA and CNS-constrained quasispecies have been isolated in post-mortem brain tissue. A number of lines of evidence suggest that microglial cells may be the site of HCV infection within the CNS.

Microglial activation and pro-inflammatory cytokine expression would be the expected consequence of this [59].

There are therefore a number of potential mechanisms for chronic immune activation within the CNS in HCV infection, which may underlie the MRS abnormalities reported in this study and others.

The effects of **intracerebral cytokine production include modulation of neuronal Ca<sup>2+</sup> and other ion channels, alteration of glutaminergic and cholinergic neurotransmission, stimulation of microglia to release inflammatory mediators such as NO and activation of the hypothalamic pituitary axis** [60].

There is also evidence that **interferon- $\alpha$  increases serotonin uptake in vitro [61], and reduces frontal cortex concentrations in a dose-dependent manner** [62].

This is consistent with the clinical experience of the use of serotonin specific reuptake inhibitors (**SSRIs**) to treat interferon-induced depression [63].

Moreover, in a clinical **SPECT** study, **reduced serotonin and dopamine transporter binding have been reported in patients with HCV** infection who were **not undergoing treatment with  $\alpha$ -interferon** [64].

These findings all suggest that cerebral immune activation may result in **changes in neurotransmission**, which may, in part at least, underlie the reported **neuropsychological dysfunction in chronic HCV infection**.

There has been considerable debate about **the importance of a biological mechanism underlying the symptoms of fatigue and cognitive dysfunction in HCV infection** [16]

Numerous studies have stressed the importance of psychosocial factors and the issue has tended to become polarized between biological and functional arguments.

The concept of cerebral immune activation may allow a unifying model, which incorporates both biological and psychosocial stressors.

Animal data suggest that psychogenic stressors and pro-inflammatory cytokines may result in similar outcomes, in terms of HPA activation and neurotransmitter activity [60].

Thus, there may be synergism between cytokines and psychogenic stressors e.g. the well-established exacerbation of depressive symptoms by  $\alpha$ -interferon treatment. Indeed, it has been suggested that there may be **sensitization of biological pathways as a result of chronic immune activation, which may increase the vulnerability to affective illness**.

This may account for the high prevalence of **anxiety and depression in chronic HCV infection**.

This study supports previously published data showing that HCV infection is associated with CNS dysfunction, and it is the first MRS study to show a **significant correlation between neurocognitive deficits and changes in MR-measurable metabolites in patients with minimal hepatic inflammation**.

The results thus echo previous studies which have demonstrated this link between MRS and cognition in HIV infection.

Furthermore, we propose that cerebral immune activation is a key concept in understanding the issues surrounding the effect that HCV has on the brain.

The difficulty in clinical practice is that  $\alpha$ -interferon-based treatments themselves exacerbate the situation, at least in the short to medium term.

**Whether successful viral eradication eventually results in normalization of these changes is not known, although improvements in health-related quality of life and fatigue scores after the end of antiviral therapy suggest that this may be the case.**

Longitudinal studies incorporating cognitive assessment and neuroimaging may in time provide the answer.

## Patients and methods

Twenty-five patients with histologically-defined mild chronic hepatitis C were recruited from a viral hepatitis clinic in a tertiary referral centre (St. Mary's Hospital, London). The patients had been referred for assessment of their liver disease. The mean age (standard deviation – SD) was 45.0 (8.3) yrs and the percentage of male subjects was 54%. Liver biopsy on all patients had been performed no more than 18 months prior to the study. All individuals had mild inflammation only, in the absence of cirrhosis or significant fibrosis. The median Ishak necroinflammatory score was 2 and the median Ishak fibrosis score was 1 [37]. All patients were viraemic at the time of the study, as defined by a positive PCR for HCV RNA (Roche Amplicor version 2). The method of viral transmission was related to injection drug use (IVDU) in 65% of cases and to infected blood products or undefined sources in 35% of cases. All patients were carefully questioned for a history of major recreational drug usage (MDU). Individuals were asked whether they had ever used any of five substances (heroin, methadone, LSD, cocaine, ecstasy). They were only classified as non-users if a history of MDU was unequivocally absent. None of the patients had used any recreational drugs in the year preceding the study.

### 2.1. Cerebral magnetic resonance spectroscopy

Cerebral proton ( $^1\text{H}$ ) MRS was performed using a 1.5T Eclipse™ spectroscopy system (Philips Medical Systems, Cleveland, OH, USA). An enveloping quadrature transmit/receive coil tuned to 64MHz was used for all examinations. T1-weighted MR images were acquired in the transverse plane in order to exclude structural brain disease and to position the voxel of interest (T1-weighted field echo, repetition time (TR) 21ms, echo time (TE) 6ms). A single 8cm<sup>3</sup>-sized voxel was positioned in the frontal white matter at the level of the centrum semiovale and  $^1\text{H}$  MR spectra were obtained using a short-echo automated PRESS sequence (TE 40ms, TR 1500ms). Spectral analysis was performed within the time domain using the MRUI software package (available from: [www.mrui.uab.es/mrui](http://www.mrui.uab.es/mrui)), incorporating the AMARES algorithm [38] and metabolites were expressed as ratios to cerebral creatine (Cr) (Fig. 1). The total examination time was 30min. MR spectra were analyzed by a single observer in each case, who was blinded to the clinical status of the patients. Metabolite ratios were compared against age- and sex-matched historical reference data, acquired from 17 healthy hospital staff, using the same parameters and equipment. The mean

age (45.1 years, SD 5.7) and the percentage of male subjects (40%) did not differ significantly from the test subjects.

### Cognitive testing

The Cognitive Drug Research (CDR) [39] computerized cognitive assessment system was administered to the subjects, under standardized conditions by a trained individual who followed a protocol. The CDR system measures the speed of responses (with millisecond accuracy), as well as the accuracy of responses on a range of simple cognitive tasks. The battery employed in this study comprised tests of attention and working memory, domains where cognitive impairments have previously been reported in this patient population (simple reaction time, choice reaction time, digit vigilance, numeric and spatial working memory tasks) [11]. The data were used to generate four composite scores, which have previously been shown to reflect the following processes: power of attention, quality of working memory, speed of memory processes and ability to sustain attention [40]. The results were compared to age-matched normal data from between 85 and 420 healthy subjects, depending on the individual subtest of the cognitive battery (provided by CDR Ltd., Goring on Thames, UK). Secondary comparisons were also made to previously reported data from 18 individuals who had been exposed to HCV, but who were no longer viraemic on repeated HCV RNA testing by PCR (40% male, mean age 42 years) [11].

### Statistical methods

Data were tested for normality using the Shapiro-Wilk test. Between group comparisons were made with the Student's t-test or the Mann-Whitney U test as appropriate. Correlations were tested with Spearman rank test. All tests were two-tailed. Statistical analyses were performed using SPSS version 9 (SPSS Inc., USA).

### Ethics

Ethical approval was obtained from the Local Ethics Committees of the Hammersmith Hospital, London (REC 4047/93) and St. Mary's Hospital, London (98/BG/303) and was in accordance with the 1975 Helsinki Declaration. All subjects provided written informed consent.

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