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REVIEW

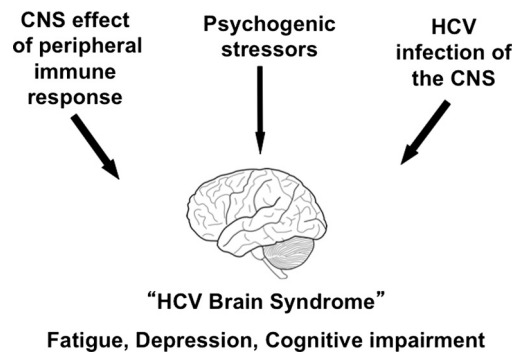# Hepatitis C virus infection, and neurological and psychiatric disorders – A review

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ABSTRACT

An association between hepatitis C virus infection and neuropsychiatric symptoms has been proposed for some years. A variety of studies have been undertaken to assess the nature and severity of these symptoms, which range from fatigue and depression to defects in attention and verbal reasoning. There is evidence of mild neurocognitive impairment in some patients with HCV infection, which is not fully attributable to liver dysfunction or psychosocial factors. Further evidence of a biological cerebral effect has arisen from studies using magnetic resonance spectroscopy; metabolic abnormalities correlate with cognitive dysfunction and resemble the

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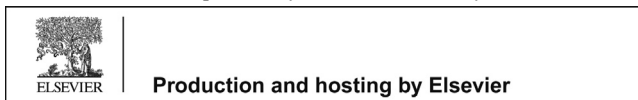
patterns of neuroinflammation that have been described in HIV infection. Recent research has suggested that, in common with HIV infection, HCV may cross the blood brain barrier leading to neuroinflammation. Brain microvascular endothelial cells, astrocytes and microglia may be minor replication sites for HCV. Importantly, patient reported outcomes improve following successful antiviral therapy. Further research is required to elucidate the molecular basis for HCV entry and replication in the brain, and to clarify implications and recommendations for treatment.

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Dr Daniel Forton PhD FRCP is a Consultant Hepatologist and Reader at St George's Hospital, London. He has performed basic scientific and clinical research into the CNS manifestations of HCV infection and was the first to demonstrate evidence of a biological effect of HCV on brain function. Awarded the Dame Sheila Sherlock Research Medal, he remains active in clinical research, performing phase I to III trials of new treatments in liver disease. He is Associate Medical Director, responsible for Research at St George's, sits on the board of the South London NIHR Comprehensive Research Network and is co-chair of the South Thames Hepatitis Network (STHepNet), the largest treatment network in England.

Introduction

Chronic hepatitis C virus (HCV) infection is important globally as a cause of liver-related morbidity and mortality with hepatic fibrosis, cirrhosis and hepatocellular carcinoma as the major clinical sequelae [1]. Hepatic encephalopathy as a complication of HCV-induced cirrhosis and portal hypertension, is the most obvious manifestation of (CNS) involvement, albeit indirect and non-specific [2]. CNS vasculitis can rarely result from HCV-associated mixed cryoglobulinaemia [3], which more commonly causes a peripheral sensory or motor

neuropathy [4]. The suggestion that chronic HCV infection itself might directly cause cerebral dysfunction came from initial, anecdotal observations that patients with HCV infection, but without cirrhosis or cryoglobulinaemia, frequently reported a range of non-specific symptoms [5]. For example, fatigue is the commonest symptom in HCV infection, affecting 53% of patients in one large series [6]. HCV infected individuals with minimal liver disease have also shown increased levels of depression and diminished abilities in the areas of concentration, attention, verbal learning, working memory, executive functions and psychomotor performance. A large number of studies have documented the prevalence of such symptoms and their impact on health related quality of life (HRQL) in cohorts of patients with HCV infection [7–13]. This paper will review the evidence for a link between HCV infection and CNS symptoms.

Health-related quality of life

HRQL refers to an individual's personal assessment or perception of his/her physical, mental and social well-being. Disease specific and generic HRQL questionnaires have been employed widely to study the impact of HCV infection on patients' well-being and the effects of anti-viral therapy. These data challenge the historical perception that HCV infection is an "asymptomatic" disease, with a consensus that HRQL is significantly reduced in HCV infected patients [10,11,14]. Even in patients without significant liver disease, HRQL is impaired and is driven by fatigue, depression and cognitive impairment [15,16]. One early study reported reduced Short-Form 36 (SF-36) HRQL scores in patients with HCV infection compared to patients with hepatitis B (HBV) infection. HRQL was more impaired in HCV patients and this was unrelated to the mode of acquisition (ie previous intravenous drug usage) [17]. These findings and other cohort studies, outlined in this review suggest a direct impact of HCV infection per se on HRQL. There are however other important determinants related to physical and psychiatric comorbidities, impact of a diagnosis and anxiety about prognosis, treatment and stigmatization [18–20]. Rodger and colleagues administered the SF-36 questionnaire to a cohort of subjects who were admitted to hospital in the 1970s with acute hepatitis, a proportion of whom were unaware of their diagnosis of chronic HCV infection. Those who were aware of their serostatus rated significantly worse on seven of eight SF-36 scales compared to population norms, whereas those who did not know their diagnosis scored significantly worse in only three scales [18]. The authors concluded that reduced HRQL might result from labelling as a consequence of the diagnosis. However, other prospective studies of blood donors have revealed impaired SF-36 scores in

HCV infected individuals prior to knowledge of their diagnosis, compared both to donors who tested HCV negative and to those who had a false positive result [21,22]. The data together suggest the presence of an independent effect of HCV infection on HRQL, which may be compounded by the impact of diagnosis. There are clearly numerous factors that may explain reduced HRQL in patients with HCV, ranging from physical to psychosocial influences and the question of the presence of a direct effect of HCV infection has become unnecessarily dichotomized in this context [23]. What has emerged from large studies in the era of directly acting antivirals is that there is a rapid and clinically important improvement in well-being, as viraemia is controlled [14,24]. Although previous interferon-containing treatments also demonstrated positive HRQL impacts over time [11], the improvements were delayed due to the adverse effects on interferon and/or ribavirin. In an analysis of 1952 patients treated in three major clinical interferon free trials of sofosbuvir and ledipasvir ± ribavirin, patient reported outcomes improved after just two weeks of treatment with the ribavirin free combination and coincided with viral suppression [24]. Improvements in at least ten patient reported outcomes (including the SF-36 physical and mental summary scales and the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) measures) exceeded the minimal clinically important difference in 37% of patients by week four and by 47% at the end of treatment. The study provides important evidence to support the hypothesis that HCV itself is responsible for a major component of HRQL impairments.

Fatigue

Fatigue is one of the most commonly reported symptoms of infection with hepatitis C, and can be persistent and debilitating [6,19,25–29]. It has been assessed using various qualitative and quantitative methodologies in the literature, with a reported prevalence ranging from 20% to 80% in different cohorts. Fatigue severity does not appear to correlate with the degree of biochemical hepatitis [7,12,19]. Huckans reported associations between neuropsychiatric symptoms including fatigue and depression and the inflammatory profile of 47 immune factors measured in plasma using a multiplex microbead methodology [30], suggesting that peripheral immune activation may potentially contribute to this symptom. However, fatigue is a multidimensional symptom and is influenced by multiple, interrelating social, psychological and physical factors [19,27,31], meaning that the relative contribution of a biological mechanism, if there is one, remains unclear. As a consequence of any potential cerebral effect of HCV infection, measured fatigue is unlikely to be a useful marker in mechanistic studies. However, as a patient reported outcome in clinical trials, marked improvements in treatment responders suggest a viral contribution [24,32,33].

Depression

Depression is a common finding in HCV-infected patients [19,20,34,35]. As well as being an important comorbidity, depression has limited the tolerability of and compliance with α-interferon based treatments [36]. The relationship between HCV and depression is undoubtedly complex. The prevalence of HCV in psychiatric populations is significantly increased above the general population (17.4% in those with serious mental illness in the USA [37], compared to a rate of around 1% in the general population), suggesting that those infected with HCV are more likely to suffer from problems such as depression in the first place. In Western Europe and the USA, HCV infection occurs predominantly in current or former injection drug users, who have a higher prevalence of depression [38]. Conversely, a reactive depression may relate to the diagnosis of HCV infection and associated anxiety over long term health or may it be secondary to symptoms such as fatigue and cognitive impairment [27]. Finally, a biological effect of HCV infection itself may underlie depression. Although there is a large literature that describes the pathogenesis of α-interferon induced depression [36], there is currently little evidence of an effect of endogenous cytokines. Pawlowski and colleagues measured gene transcripts from peripheral blood mononuclear cells of 28 cytokines and chemokines in patients before and during α-interferon based therapy [39]. Before treatment, neuroticism scores correlated with IL-3, IL-8 and M-CSF transcription levels. Six months after the end of therapy there was a correlation between depression and/or neuroticism scores and various pro-inflammatory cytokines. The authors concluded that there might be a pivotal role for immune cell activation in depression in chronic HCV infected patients.

Cognitive dysfunction

In 2001 cerebral magnetic resonance spectroscopy (MRS) was used for the first time in HCV infected patients with minimal liver fibrosis and demonstrated metabolic abnormalities in white matter and basal ganglia, compared to both patients with HBV infection and normal controls [40]. Subsequently there followed a large number of studies to determine whether there is evidence of neurocognitive impairment in HCV infection. The literature is heterogeneous and characterized by cross-sectional rather than longitudinal assessments of relatively small and selected cohorts. Potential confounding factors such as psychiatric disorders, injection drug use, recent interferon treatment or liver cirrhosis were controlled for to varying degrees. Furthermore, a variety of neuropsychological test batteries have been employed to test different domains of cognitive function, limiting the validity of comparisons between studies.

In order to control for impairment due to the presence of minimal hepatic encephalopathy (MHE), patients with cirrhosis should be excluded. The cognitive defects of MHE are well delineated, and include reductions in selective attention, visuoconstructive function and motor performance. Weissenborn [41] tested a cohort of 30 patients with HCV without cirrhosis and found that while levels of anxiety, depression, fatigue, attention and executive function were affected, motor performance and visuospatial function were relatively preserved, suggesting an independent effect of HCV infection. Moreover, these results were associated with MRS changes, which were qualitatively different from those encountered in hepatic encephalopathy.

Most studies have recruited patients from hospital clinic settings with the concomitant risk of selection bias and the true prevalence of cognitive impairment has not been defined.

Within these limitations, the majority of studies have demonstrated relatively mild impairments of attention, concentration and working memory. For example, in one study of patients with histologically mild liver disease, selective impairments of concentration and working memory were reported that were not seen to the same degree in patients, who had recovered from HCV infection, either spontaneously or after successful therapy. Furthermore, although patients with depression or former substance abuse were not excluded from the study, the presence of fatigue, depression or a history of substance abuse did not explain these findings [35]. Hilsabeck and colleagues performed two studies in this field [25,42] and reported that cognitive test performance was associated with fibrosis stage on liver biopsy. However impairments in attention and concentration were evident in HCV-patients with minor hepatic injury, affecting up to 50% of non-cirrhotic patients. The pattern of impairment was similar to the findings described by others and was thought to be consistent with frontal-subcortical dysfunction, similar to the pattern encountered in HIV-infection. In their second study, there were no associations between subjective complaints of cognitive dysfunction or fatigue and performance on the neuropsychological tests. In contrast, Weissenborn and colleagues found that distinct attention and higher executive deficits were more pronounced in patients with moderate rather than mild fatigue [41].

Some authors have attempted to control for confounding factors through exclusion. Lowry and colleagues studied a small, homogenous cohort of iatrogenically infected women, of whom 9 had spontaneously cleared HCV, and showed that PCR positive women had impaired memory and attention compared to normal controls and the PCR negative group [43]. McAndrews and colleagues applied strict exclusion criteria to reduce confounding factors. Attention and speed of processing were not impaired in this study, with only minimally poorer learning efficiency in a small proportion (13%) of HCV patients compared to controls [44]. Similarly, other studies have found no association between HCV infection and impaired cognitive function, although these have involved small groups of patients [42,45,46]. One such study recruited participants who were screened for infection with HCV at blood donation centres, thus removing several confounding factors [45], with the caveat that this group may have been self-selected for general good health and well-being. No cognitive impairments were seen in viraemic patients, except in patients with cirrhosis, which could potentially be explained by MHE.

It is also important to note that, higher cognitive reserve as evidenced by premorbid intellectual function and level of education, may be protective against HCV-related cognitive impairment [47,48]. One study divided patients into two groups with "high" and "low" cognitive reserve. The results differed between groups but were not shown to be statistically significant [47]. The other study examined cirrhotic patients and did not include controls [48]. Indeed, it is important to match patients and controls for IQ, since cognitive impairment in HCV infected patients is highly correlated with IQ and premorbid cognitive ability [49].

Human immunodeficiency virus (HIV) infection can lead to a range of neuropsychiatric manifestations, from asymptomatic cognitive impairment to overt HIV-associated dementia. Given that the patterns of impairment in some patients with HCV infection are similar to HIV-associated mild neurocognitive disorder, it has been postulated that there is an underlying pathogenesis common to both diseases. Indeed, patients co-infected with HIV and HCV are more likely to have worse cognitive function than those with HIV mono-infection [50], and this appears to be more than an additive effect, raising the possibility that co-infection potentiates the brain injury in these patients. Evidence for this comes from a number of studies, including a cohort of drug users who were classified based on serostatus for HIV and HCV [51]. Patients with HIV/HCV co-infection performed worse on a voice-activated reaction time version of the Stroop task, compared to monoinfected subjects who had demonstrable impairments compared to seronegative controls. The study did not control for cirrhosis and it is possible that co-infected patients were infected longer and had more severe liver disease. Studies from the Manhattan HIV Brain Bank cohort [52] also showed worse executive functioning in co-infection compared to individuals with advanced HIV monoinfection. The authors concluded that there was a detectable impact of HCV co-infection on neurocognitive functioning, despite the advanced stage of HIV disease of their cohort.

The literature clearly demonstrates evidence of neurocognitive impairment in patients with chronic HCV infection. However, for the reasons outlined above, it is not clear that these impairments can be attributed, wholly or in part, to the virus itself. For this reason a number of brain imaging studies have been reported, which have aimed to reveal a biological association between HCV infection and cerebral effects.

Neuroimaging

Magnetic resonance spectroscopy (MRS) [35,40,41,44,53,54], positron emission tomography (PET) [54,55] and single photon emission computed tomography (SPECT) [56] are imaging techniques that have been used to determine whether biological abnormalities are associated with the neuropsychological impairment that has been observed in HCV infection. More recently, MRI-perfusion weighted imaging and diffusion tensor imaging have also been deployed to provide information regarding cerebral blood flow and cellular microarchitecture respectively [57–59].

MRS allows measurement of metabolite concentrations in specific brain regions. Metabolite abnormalities have been reported in a number of conditions such as multiple sclerosis, brain tumours and HIV infection [60,61]. In HIV infection, increased frontal white matter concentrations of myoinositol (mI) and choline (Cho) are associated with increasing levels of HIV-associated cognitive impairment [62], findings consistent with CNS glial cell proliferation and cell membrane injury respectively. Choline is an osmolyte that acts as a marker for cell membrane synthesis and turnover [63]. In untreated patients with HIV infection, reduced levels of frontal white matter and basal ganglia N-acetyl aspartate (NAA), a marker of neuronal integrity, and increased levels of mI have been reported which reverse with anti-retroviral treatment [64]. This methodology has been used to investigate whether similar metabolite profiles are seen in HCV infection compared to HIV infection where viral penetration into the CNS occurs.

Increases in Cho relative to creatine (Cr) in the basal ganglia and frontal white matter have been reported in

HCV-infected patients with histologically mild liver disease [40]. This was not associated with previous substance misuse or seen in a control group of patients with chronic hepatitis B infection. This is distinct from hepatic encephalopathy where the Cho/Cr ratio is reduced [65], suggesting a different mechanism underlies the findings in HCV infection. Only a weak association between the abnormal metabolites and cognitive performance was reported in this study. Using the same technique but different acquisition sequences, Weissenborn and colleagues studied 30 HCV-infected patients with normal liver function using careful cognitive testing and MRS [41]. They reported reduced NAA/Cr ratios in occipital grey matter compared to healthy subjects but no alterations of Cho containing compounds or other abnormalities. Again there were no strong associations between MRS metabolites and cognitive scores, although in general, the deficits were more marked in moderate rather than mild fatigue. McAndrews and colleagues undertook MRS analysis on 37 HCV-infected patients with minimal hepatitis, a group of patients who were highly selected to exclude all possible relevant comorbidities and confounding factors [44]. They demonstrated elevations in cerebral Cho and reductions in NAA in voxels that were localized to the central white matter. These findings are in keeping with both the results from Weissenborn and Forton [35,40,41] and suggest a decrease in neuronal activity and/or glial inflammation and activation. In this study, there were only very mild cognitive impairments confined to poorer learning efficiency in 13% of subjects. More recently, Bladowska and colleagues reported similar findings in a study of 15 HCV positive patients with no neurological symptoms and normal plain MRI [57]. They found that HCV infected individuals had reduced NAA/Cr in frontal and parietal white matter.

Myoinositol (mI) is a cerebral osmolyte and a marker of CNS inflammation and gliosis. Elevated white matter mI/Cr ratios were reported that were significantly associated with deficits in working memory in a cohort of patients with mild liver disease [53]. Elevations in white and grey matter mI have also been described by Thames, who also demonstrated a positive correlation between frontal white matter mI and both fatigue and overall cognitive performance in a larger study of 76 patients with chronic HCV infection [59].

These findings contrast with the results reported by Bokemeyer and colleagues [66]. Their study quantitatively assessed metabolite levels in 53 HCV positive patients with mild liver disease using MRS. There were increased levels of white matter Cho and basal ganglia Cho and N-acetyl aspartate/N-acetyl-aspartyl glutamate (NN). Although mI concentrations were not increased in HCV patients, in contrast to Thames, they correlated negatively with fatigue scores. Similar patterns were seen for the other metabolites i.e. a negative correlation with fatigue. The reason for this is not fully understood but NAA is thought to demonstrate the number and function of neurons and to be coupled to glucose/glycogen metabolism. MRS studies of other cerebral pathologies have shown raised NAA in instances of neurocompensation. The authors concluded that this study may provide evidence for neurocompensatory mechanisms in HCV infected patients that underlie the variation in neurocognitive effects.

Studies have been limited by a number of factors including differences in MR parameters for data acquisition, data analysis techniques, and inconsistent methods of neuropsychological assessment. One can conclude however that definable MRS-measurable metabolic abnormalities exist in a proportion of HCV-infected patients with minimal or absent liver disease, who have demonstrated fatigue and cognitive impairment on formalized testing. Similar MRS findings are reported in HIV-related minor-cognitive disorder and are considered to represent neuronal dysfunction and immune activation of microglial cells. It is therefore possible that neuroinflammation may also occur in chronic HCV-infection.

More recent studies have used perfusion weighted imaging (PWI) techniques to assess blood flow, and diffusion tensor imaging (DTI) to interrogate cell microarchitecture in HCV patients. Cerebral blood flow has been shown to be increased in the basal ganglia and decreased in the cortices, showing correlations with metabolite findings [57,58]. Hyperperfusion in the basal ganglia may be an indicator of HCV associated neuroinflammation. DTI techniques used in two studies elicited differing results. Thames et al. found increased fractional anisotropy in the striatum and greater mean diffusivity in the fronto-occipital fasciculus and external capsule compared to controls [59]; this diffusivity in the fronto-occipital cortex was positively correlated with fatigue scores, which were also associated with increased white matter mI. The anatomical distribution suggested that HCV-associated neurological complications disrupted frontostriatal structures, which may result in fatigue and impaired performance of cognitive tasks that involve frontostriatal systems. Bladowska et al. however found increased diffusivity in certain white matter tracts but decreased fractional anisotropy. The significance of this is not yet clear [57,59].

Further evidence for neuroinflammation in HCV is provided by PET studies that used a ligand (PK11195) that binds to the peripheral benzodiazepine receptor, now termed translocator protein, which is mainly expressed on the outer mitochondrial membrane of activated microglia. Increased ligand binding in the caudate nucleus of HCV-infected patients was reported by Taylor-Robinson's group in a pilot PET study of patients with histologically mild liver disease. These findings, which correlated with viraemia, suggest the presence of neuroinflammation [55]. Using the same PET ligand, Pflugrad and colleagues studied the associations between cognitive impairment, fatigue and viraemia in HCV-exposed patients [67]. Patients reported greater fatigue and depression than healthy controls and performed worse in some cognitive domains including working memory and attention. However, in this study no differences were seen between HCV polymerase chain reaction (PCR) positive (+ve) and negative (−ve) patients on any scales or cognitive tests. Increased PK11195 binding was seen in PCR−ve patients in the frontal cortex, compared to both controls and PCR+ve patients. Furthermore, PK11195 binding in the basal ganglia and temporal cortex was inversely associated with performance in attentional tasks i.e. greater binding was seen in the least impaired patients. The authors suggested that microglial activation might be associated with neurogenesis and neuronal regeneration that, in some way, provides a neurocompensatory effect, thus explaining the negative correlation. However, the absence of any association with HCV PCR status is perplexing. One explanation might be that HCV exposure is sufficient to cause a CNS effect without detectable ongoing viral replication. Another might be that the findings in this study are unrelated to HCV exposure, the attention and PET abnormalities and relationships being due to another factor.

The same group has also previously studied the impact of HCV infection on neurotransmission [54] using single photon emission computerized tomography in 20 patients with minimal liver disease and fatigue. Pathological dopamine and serotonin transporter binding was seen in 50–60% of patients, which was associated with worse cognitive performance compared to controls. The investigators have studied a further cohort of 15 patients without cirrhosis but with longstanding HCV exposure secondary to anti D immunoglobulin administration 28 years previously [54]. Again, there was reduced striatal dopamine and midbrain serotonin transporter availability, which correlated with psychometric results and significantly reduced glucose metabolism, measured with (18)F-fluoro-deoxy-glucose PET, in the limbic association cortex, and in the frontal, parietal, and superior temporal cortices. This study is notable in that there were no significant differences in findings between those who were PCR+ve and the four patients who were PCR−ve. Patients were selected for demonstrating symptoms associated with "HCV encephalopathy". Although numbers were small, the study raises the intriguing possibility that CNS abnormalities may persist once virus is no longer detectable in blood. The replication of the earlier SPECT data, together with the association between striatal dopamine transporter binding and regional cerebral hypometabolism, suggests that cerebral dysfunction in HCV may be associated with alterations in dopaminergic neurotransmission. In particular, impaired mesotelencephalic dopamine projections to the frontal lobe may underlie abnormalities in executive function and working memory.

The neuroimaging studies share a number of limitations including small study sizes, heterogeneity and varying selection of patients, different neuropsychological assessment methods and varying degrees of control matching. However, there is convergent evidence to suggest the occurrence of neuroinflammation, hypometabolism and dopaminergic signalling abnormalities in the brains of some patients with HCV infection. The findings suggestive of neurocompensatory effects are in keeping with this and are likely to be an area of continuing research over the coming years.

Evidence for HCV infection of the CNS

HCV RNA has been detected in post-mortem brain samples and cerebrospinal fluid (CSF) using PCR based methods, but this alone does not prove productive infection or replication because of the possibility of brain-blood barrier breakdown after death [68–71]. However, low level HCV replication in the CNS is suggested by more sophisticated molecular techniques, including the detection of the negative strand of HCV RNA, which is considered a replicative intermediate [69] and distinct viral quasispecies within the brain samples [69–71]. HCV RNA is detected at a 1000–10,000 fold lower level in brain compared to liver, indicating that the brain is a minor replication site at most [70]. Unique sequences incorporating the HCV internal ribosomal entry site (IRES) were detected in the CNS [71]. The IRES mediates viral protein translation and when theses variants were incorporated into a reporter vector, there was a reduction in translational efficiency, which may represent a possible mechanism for CNS latency [71]. More recently, a deep sequencing approach has demonstrated genetic compartmentalization of HCV within the CSF, obtained from cognitively impaired patients with HCV infection [72]. Although the number of subjects was necessarily small, independent viral evolution of HCV envelope sequences was seen in the CSF of two cognitively impaired patients but was not observed in cognitively preserved patients, consistent with viral sequestration.

Immunohistochemical and molecular techniques have indicated that microglia and astrocytes are cellular targets for HCV infection [73,74]. Wilkinson and colleagues showed that cells that stained for HCV non-structural protein 3 (NS3), costained for CD68, a microglial marker. When these cells were isolated by microdissection and pooled RNA was amplified, there was expression of microglial markers and also of increased proinflammatory genes such as TNF-α and IL-12 [74]. Microdissected cells from HCV+ patients that were negative for NS3 did not express proinflammatory genes. These studies, which include HCV mono- and co-infected patients, are consistent with microglial immune activation, as a consequence of HCV infection and parallel the findings from the clinical PET studies using PK 11195 [55,67].

HCV core protein has been shown to trigger activation of the extracellular signal-related kinase (ERK)/signal transducer and activator of transcription 3 (STAT3) system via toll-like receptor 2 (TLR2) in the CNS [75]. Analysis of post mortem brain tissue, revealed increased phospho-ERK levels in HCV infected patients that was not seen in patients with HIV encephalitis or in HCV seronegative patients [75]. Using a neuronal cell line, HCV core protein was shown to activate ERK via TLR2 expression, which is thought to play a role in neurodegeneration. This group also investigated the direct effect of HCV core by introducing it to murine hippocampi. They demonstrated ERK activation, dendritic shortening, reduced neuronal density, astrogliosis and cytoskeletal disruption [75]. These findings are in concordance with those from neuroimaging studies of metabolite changes and microarchitectural breakdown.

The mechanism of HCV entry into the CNS has been postulated to be through infected monocytes, trafficking across the blood-brain barrier [5]. Astroglia cell lines do not express all the cell molecules required for classical HCV entry (tetraspanin, CD81 and tight junction proteins claudin-1 and occludin) [76] and attention has turned to the blood/brain barrier. Fletcher and colleagues have shown that brain microvascular endothelial cells (BMECs), are permissive to HCV infection in culture [77]. In a series of experiments, they demonstrated infection of BMECs by culture-derived HCV and release of particles that were subsequently infectious in Huh-7 hepatoma cells, this being the first demonstration of HCV replication in non-hepatocyte cell culture. Infected BMECs displayed apoptosis, which might result in reduced endothelial barrier activity. In this way, peripherally derived cytokines, viruses and immune cells might gain access to the CNS across an HCV-disrupted blood/brain barrier, resulting in immune activation within the CNS. In addition viral proteins might act directly to potentiate neurotoxicity, as suggested for HCV core protein by Paulino [75] and in a report where HCV core protein potentiated HIV-1 neurotoxicity in a mouse model [78]. It might therefore be expected that CNS symptoms correlate with levels

of peripheral cytokines but the studies to date have given mixed results [30,72,79].

Changes following successful antiviral therapy for HCV infection

Improvements in HRQL and reductions in fatigue levels occur in patients with a sustained virological response (SVR) after treatment with pegylated interferon and ribavirin [11]. The use of these drugs is itself associated with physical, mental and cognitive symptoms. The advent of the directly acting antiviral (DAA) drugs, which have minimal side effects, provides an opportunity to study the impact of viral eradication on HRQL. In a double blind placebo controlled randomized clinical trial of Sofosbuvir/Velpatasvir in 750 patients with hepatitis C, subjects completed questionnaires that assessed 25 patients reported outcomes (PROs) at baseline and every four weeks through treatment [80]. Those who achieved a sustained virological response were followed to 24 weeks after the end of treatment. By week four of treatment, statistically significant improvements were seen in general health, emotional well-being, and fatigue in patients on treatment compared to baseline in the patients on active treatment. These changes were not seen in the placebo group. By the end of treatment the improvement in PRO scores had continued with no improvement in the placebo group. The patients were unaware of their viral status when completing the assessments. A multivariable analysis was performed, which took into account baseline levels of the PROs in addition to clinical and demographic factors, and demonstrated that treatment emergent changes in the PROs, including fatigue, during and after treatment were independently predicted by receiving antiviral treatment as opposed to placebo. The data definitively show that viral suppression per se improves patient symptoms including fatigue, depression and anxiety. Similar results have been reported for other compounds [81].

The impact of successful HCV clearance on cognitive function and brain metabolism has also been studied. In a trial of 168 patients, Kraus and co-workers showed that successful HCV eradication with peginterferon and ribavirin was associated with improved attention, vigilance, and working memory, while virological non-responders showed no such improvements [82]. These improvements in cognitive function were demonstrated one year after the end of treatment to control for the known effect of interferon-based treatment on brain function during the treatment period. A much smaller pilot study [83] using brain proton MRS and cognitive assessments before, during and after interferon-based therapy, showed significant CNS metabolic changes towards normal levels in those patients who had an SVR. These findings were interpreted as improvement in cerebral immune activation associated with HCV clearance, although any conclusion is limited by the very small study groups. No convincing changes were seen in cognitive performance. More recently, a similar MRS study using interferon free treatment with Sofosbuvir and Ledipasvir, reported normalization of cerebral N-acetyl aspartate, in patients with viral suppression, which was interpreted as recovery of neuronal dysfunction [84]. These studies were small and need to be validated in a larger sample of patients undergoing treatment with DAA drugs to understand the relationship between viral eradication, cognitive function and brain imaging.

Conclusions

The clinical evidence for neuropsychological impairment in HCV infection has aroused interest but the clinical significance in terms of disease endpoints remains to be defined. For example, an association between HCV infection and Parkinson's disease has been reported in a very large epidemiological study in Taiwan [85]. Much of the evidence to date has been drawn from small, variably controlled studies, on a background of multiple confounding factors. HCV therapy has advanced rapidly, with the availability of highly effective, directly acting antiviral drugs without major side effects. Large prospective studies are required to determine whether these new treatment regimens reverse neurocognitive symptoms and brain abnormalities in HCV-infection. This will be the true test of whether there is a direct biological effect of this virus on the CNS. Finally, the presence of an immune privileged, extrahepatic site could be a potential source of late relapse after oral antiviral therapy and long-term surveillance is advised.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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