Hepatitis C and the Brain

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• Hepatitis C: epidemiology and natural history

  • Estimated global prevalence of 3%
  
  • Mild chronic hepatitis – normal ALT 25%
  • Mild chronic hepatitis – raised ALT 35%
  • Moderate or severe hepatitis 40%
  
  • Cirrhosis develops in 20-30% over 20 years
Hepatitis C: epidemiology and natural history

- serum from 8000 war veterans 1948 - 1954
- 17 positive for hepatitis C Ab
- 1/17 died of cirrhosis 44 years later
- 1/17 is alive with cirrhosis


- 384 women infected with HCV by contaminated rhesus immunoglobulin
- 17 years follow up
- 51% fibrosis
- 2% cirrhosis

*NEJM* 1999; 340:1228-33

HCV and Fatigue

Prevalence of fatigue = 50-80% in patients referred to treatment centres

Poynard T et al *J Viral Hep* 2002

No association between the severity of fatigue and the degree of hepatitis

Goh J et al *Eur J Gastroenterol Hepatol* 1999

Associated with medical comorbidity, the effect of the diagnosis (labelling), female gender, depression and poverty

Dwight MM et al *J Psychosom Res* 2000
McDonald J et al *J Gastroenterol Hepatol* 2002
Psychological Aetiology

Hepatitis C related fatigue

Biological Aetiology

Hepatitis C related fatigue
Biological Aetiology + Psychological Aetiology

Hepatitis C related fatigue

Minor psychological issue
No unique relevance

Major issue that affects quality of life
Reductions in all domains of HRQL

Foster GR et al. *Hepatology* 1998

• Major QOL issue in a proportion of patients
• Limits tolerability of antiviral therapy
• Insights regarding natural history of HCV infection?
Does Hepatitis C Cause Cognitive Impairment?

- Virus
- Psychosocial factors
- Liver Disease
- Immune Response
HCV and cognitive impairment

1. Study patients who are viraemic (PCR+ve)
2. a) Compare to patients who have been exposed but are not viraemic (PCR-ve)
   b) Examine the effects of viral eradication
3. Exclude patients with significant liver disease
4. Exclude or control for other risk factors for cognitive impairment

Cognitive Assessment in Chronic HCV Infection

Forton et al Hepatology 2002

40 viraemic patients with histologically mild hepatitis C
48% history of intravenous drug abuse

• 18 HCV antibody +ve, HCV PCR-ve individuals
  age and IQ (NART) matched
  61% history of intravenous drug abuse
Cognitive Assessment in Chronic HCV Infection
Forton et al Hepatology 2002

<table>
<thead>
<tr>
<th></th>
<th>HCV PCR+ve</th>
<th>HCV PCR-ve</th>
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<tbody>
<tr>
<td>2.0 [0-6]</td>
<td>0.5 [0-4]</td>
<td>p=0.009</td>
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</table>

HCV PCR+ve patients were impaired on more cognitive tasks than PCR-ve patients.

<table>
<thead>
<tr>
<th>Drug history</th>
<th>No Drug History</th>
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<tbody>
<tr>
<td>1.9 [0-6]</td>
<td>1.6 [0-6]</td>
<td>P=0.62</td>
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</table>

On multivariable analysis there were no associations with between performance and:

- major drug history
- depression
- fatigue
- health-related quality of life
Cognitive Assessment in Chronic HCV Infection
Forton et al Hepatology 2002

HCV viraemic patients were impaired in the domains of attention, concentration and working memory

Attention

* p<0.01

Speed of memory processes / ms

Power of concentration / ms

HCV-infected*     HCV-cleared

HCV-infected*     HCV-cleared

* p<0.03

P=0.001 v controls

medical outcome study cognitive function scale

P<0.001
Cognitive Assessment in Chronic HCV Infection

Weissenborn et al. J Hepatol 2004

• 30 HCV+ve patients with normal liver function
• 15 mild, 15 moderate to severe fatigue (FIS)
• Comprehensive Cognitive Battery
  • Attention, memory, learning ability, visual perception, visuoconstrutional abilities, motor speed, verbal function, concept formation and reasoning

• Distinct deficits in attention and higher executive function
• Worse in more fatigued patients
• More depression and anxiety in patient group (HADS)

Cognitive Assessment in Chronic HCV Infection

Weissenborn et al. J Hepatol 2004

Mean of the WAIS-R subtest results

Cognitive Assessment in Chronic HCV Infection

HCV patients performance impaired >1SD:
49% digit cancellation time
25% symbol digit modalities test
25% trailmaking A
21% trailmaking B

Hilsabeck et al. Hepatology 2002

Significant impairment in learning efficiency,
No association with depression or fatigue

McAndrews et al. Hepatology 2005

Cognitive Impairment in HCV/HIV Co-infection

Ryan et al. Neurology 2004

• 116 patients with advanced HIV infection:
  67 co-infected, 49 HCV-ve
• 29-72% prevalence of neurocognitive impairment
• Co-infected more likely to have impairments in executive functioning and AIDS dementia.
• Co-infected patients more likely to have history of opiate dependence
• No account taken of liver disease

Difficult to conclude that HCV itself has an additive effect in HIV associated cognitive impairment
Cognitive Impairment in HCV/HIV Co-infection

Additive effect of HIV/HCV coinfection over monoinfection with either virus on performance in voice-activated reaction time (RT) version of the Stroop task.

Martin et al. Neurology 2004

Significant impairments in electrophysiological measurements of psychomotor speed in monoinfected (HCV or HIV) and coinfected patients.

Von Giesen et al. J AIDS 2004

HCV augments cognitive deficits associated with HIV infection and methamphetamine.

Cherner et al. Neurology 2005

HCV coinfection has an additive effect on neurocognitive impairment, controlling for some other risk factors.

Richardson et al. AIDS 2005

Cognitive Impairment in HCV/HIV Co-infection

Drug Abuse and Hepatitis C Infection as Comorbid Features of HIV Associated Neurocognitive Disorder: Neurocognitive and Neuroimaging Features

Eileen M. Martin-Thormeyer and Robert H. Paul

Cognitive Assessment - Summary

• Impairments in attention, working memory, learning efficiency
• 20-30% prevalence in patients referred to hospital
• Related to HRQL (cognitive) and fatigue symptoms
• Multiple, complex interacting factors

Is there a biological aetiology?

Neuroimaging studies

Normal $^1$H MR spectrum of the Brain

NAA - N-acetylaspartate
marker of neuronal integrity

Cho - Choline
marker of cell membrane turnover

Cr - Creatine
### Cerebral proton MRS in mild chronic HCV infection

Elevated mean Cho/Cr ratio in the basal ganglia and white matter in HCV patients compared to normals and HBV patients

Forton et al *Lancet* 2001

![MRS spectra](image)

Patient

**PRESS TE 135ms, TR 1500ms**

Volunteer

---

### Cerebral Magnetic Resonance Abnormalities in Chronic HCV Infection

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Region</th>
<th>Abnormality</th>
<th>Imaging Sequence</th>
<th>Software</th>
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<tbody>
<tr>
<td>Forton</td>
<td><em>Lancet</em> 2001</td>
<td>Basal Ganglia</td>
<td>Increased Cho/Cr</td>
<td>PRESS 1500/135</td>
<td>Marconi proprietary software</td>
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<tr>
<td>Weissenborn</td>
<td><em>J Hepatol</em> 2004</td>
<td>Occipital Grey Matter</td>
<td>Reduced NAA/Cr</td>
<td>STEAM 1500/18</td>
<td>GE proprietary software</td>
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<tr>
<td>McAndrews</td>
<td><em>Hepatology</em> 2005</td>
<td>White Matter</td>
<td>Reduced NAA, Increased Cho</td>
<td>PRESS 2000/30</td>
<td>LC model with quantitation</td>
</tr>
</tbody>
</table>
Short echo (TE 40) MRS – Elevated white matter myo-inositol in HCV+ve patients

Forton et al J Hepatol 2006

Correlation between white matter myo-inositol and working memory speed

Forton et al J Hepatol 2006
Cerebral proton MRS and HIV

• Cerebral metabolite abnormalities in HCV infected patients have been reported in HIV infection

• Elevated frontal white matter ml/Cr is the most common MRS finding in early HIV infection and correlates with the deficits of HIV related minor cognitive-motor disorder

  Chang, Neurology 1999;52, 100-108

• It is associated with microglial infection and subsequent activation

Altered monoaminergic neurotransmission and cognitive function in HCV

Weissenborn, K et al. Gut 2006;55:1624-1630

• Cerebral SPECT study of serotonin and dopamine transporter binding capacity

• 20 HCV-Ab +ve patients with disabling fatigue and cognitive decline (16 PCR+ve, 4 PCR-ve)

• Reduced dopamine (60%) and serotonin (50%) transporter binding capacity

• 3/4 PCR-ve had abnormal results
Dopamine transporter binding (DAT) and serotonin transporter binding (SERT) in patients and controls.

Weissenborn, K et al. Gut 2006;55:1624-1630

Altered monoaminergic neurotransmission and cognitive function in HCV

Weissenborn, K et al. Gut 2006;55:1624-1630

• Reduced dopamine / serotonin transporter binding associated with impaired cognitive function
• No association with fatigue, HRQL or depression
• Findings seen in PCR-ve patients aswell
HCV and the Brain

- Clinical syndrome of sorts
- CNS metabolic changes, independent of HE
- Altered neurotransmission / transporter binding
- Mechanism

CNS effect of peripheral immune response?  HCV infection of the CNS?

“HCV Encephalopathy”
Fatigue, Depression, Cognitive impairment
HCV generates quasispecies due to limited fidelity of viral RNA polymerase

Distinct viral quasispecies in PBMC
inc. monocytes, B cells and CD8 T cells

(Okuda, Hepatology 1999;29:217-22)
Internal ribosomal entry site (IRES) Mediates cap-independent translation of viral poly protein

Most variable region
Interacts with CD 81

Hypervariable region 1 (HVR1)

Role in cellular tropism

Role of HCV IRES in cellular tropism

• AAA selected during viral passage through lymphoblastoid line

• AAA confers increased translational efficiency in lymphoblastoid lines

• AA sequences detected in PBMC and dendritic cells
  Laskus et al. J Infect Dis 2000;181:442-8
  Laporte et al. Blood 2003;101:52-7
The A (204), A (243) mutation - in brain-derived IRES clones

Brain HCV RNA detected in 2/3 HCV+ve, HIV-ve samples

*Forton et al J Virol 2004*

Identification of unique HCV quasispecies in *post-mortem* brain tissue

*Forton et al J Virol 2004*
Demonstration of HCV replication in brain matter

Detection of HCV negative-strand sequences in post-mortem brain tissue

Vargas et al. Liver Transpl. 2002

Detection of brain-specific HCV quasispecies

Laskus et al AIDS 2005
Fishman et al. J Inf Dis 2008 (7/13 HCV/HIV coinfected)
Murray et al J Neurovirol 2008 (6/10 coinfected, 1/3 monoinfected)

Cellular localisation of HCV within the CNS

Laskus J Virol 2009

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age [y]</th>
<th>gender*</th>
<th>Race</th>
<th>HIV status</th>
<th>HCV status</th>
<th>Brain pathology</th>
<th>Liver pathology</th>
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<tr>
<td>1</td>
<td>35M</td>
<td>Male</td>
<td>White</td>
<td>High</td>
<td>Pos</td>
<td>Nodular endophymitis, peri-vascular lymphocytic cell</td>
<td>Cirrhosis</td>
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<tr>
<td>2</td>
<td>55M</td>
<td>Male</td>
<td>White</td>
<td>Normal</td>
<td>Pos</td>
<td>Mild atherosclerosis, mild cerebral atrophy</td>
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<td>3</td>
<td>57M</td>
<td>Male</td>
<td>White</td>
<td>Pos</td>
<td>Pos</td>
<td>Hydropsphalus</td>
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<tr>
<td>4</td>
<td>55M</td>
<td>Male</td>
<td>White</td>
<td>Pos</td>
<td>Pos</td>
<td>Microinfarcts</td>
<td>Cirrhosis</td>
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<tr>
<td>5</td>
<td>67F</td>
<td>Female</td>
<td>White</td>
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<td>Pos</td>
<td>Microinfarcts, myelolipoma</td>
<td>Bridging veins, hemangioma</td>
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<td>44M</td>
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<td>Pos</td>
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<tr>
<td>7</td>
<td>46M</td>
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<tr>
<td>8</td>
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<td>9</td>
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<td>White</td>
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<td>Neg</td>
<td>Normal</td>
<td>Cirrhosis</td>
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<tr>
<td>10</td>
<td>50M</td>
<td>Male</td>
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<tr>
<td>11</td>
<td>52M</td>
<td>Male</td>
<td>White</td>
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<tr>
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<tr>
<td>13</td>
<td>52M</td>
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<td>14</td>
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<tr>
<td>16</td>
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<td>Intracranial hemorrhage</td>
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<td>Neg</td>
<td>Pos</td>
<td>Normal</td>
<td>Normal</td>
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</tbody>
</table>

* M: male; F: female.
**Pos: positive; Neg: negative.
Cellular localisation of HCV within the CNS

Laskus J Virol 2009

Laser Capture Microdissection

Viral load by rtPCR
(genomic equiv/400-650 cells)
Cellular localisation of HCV within the CNS

83-95% NS3+ cells costained for CD68+

4-29% NS3+ cells costained for GFAP

Cellular localisation of HCV within the CNS

NS5 staining of brain sections from 12 HIV/HCV coinfected individuals

Letendre S J Inf Dis 2007
Cellular localisation of HCV within the CNS

Western blot immunoreactivity in 8/12 patients

Letendre S J Inf Dis 2007

HCV Neuroinflammation Hypothesis

**HCV enters the brain** and causes **neuroinflammation** which may account for **neuropsychiatric symptoms**.
Microglial Activation

Resting microglia

Activated microglia

Peripheral Benzodiazepine Receptor (PBR)

[\(^{11}\text{C}\)](R)-PK11195
PET ligand for PBR

PK11195 PET - Study Objective

Determine if there is increased microglial activation in mild HCV demonstrated by increased activity of \([^{11}\text{C}](R)\)-PK11195 on PET imaging.

Grover et al (AASLD 2007)

PK11195: Alzheimer’s disease,
Multiple Sclerosis,
HIV-associated dementia (Hammoud et al. 2005)
Patient characteristics

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age</th>
<th>Sex</th>
<th>Mode of transmission</th>
<th>Genotype</th>
<th>Viral load (iu/ml)</th>
<th>Fatigue (0-160)</th>
<th>CDR (nr 4 to -2)</th>
<th>PHES (nr 6 to -4)</th>
<th>ALT (0-37)</th>
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<td>Subject 1</td>
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<td>1a</td>
<td>4,450,000</td>
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<td>Subject 2</td>
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<td>? Occ</td>
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<td>126,000</td>
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</table>

PET images

Axial PET image taken at the level of the basal ganglia from a healthy volunteer (A) and patient with mild hepatitis C (B). In the patient (B) binding is increased in the thalamus, while the healthy control (A) only shows constitutive PK11195 binding. Image C represents a T2 MR image from the same axial level.
PK binding potential mHCV vs controls

Statistically significant increase in the caudate: *$P < 0.05$
Mann-Whitney test

Genotype 1 mHCV patients vs healthy volunteers

Statistically significant differences in the caudate $P=0.01$ and thalamus $P=0.007$.
Mann-Whitney test
Thalamic binding potential for Genotype 1 mHCV subjects versus viral load

Spearman’s rho = 0.771, P = 0.07

Results

Increased microglial activation in caudate and thalamus of Genotype 1 mHCV

Trend between increasing viral load and PK11195 binding potential in the thalamus
Putative mechanism

- HCV infected PBMC’s cross BBB
- HCV spreads to microglia
- Infected macrophages release pro-inflammatory cytokines
  - Neurosteroids, TNF-α, IL-1, IL-6, NO
- Neurocognitive dysfunction

HCV-Encephalopathy

- Clinical syndrome of sorts
- CNS metabolic changes, independent of HE
- Altered neurotransmission / transporter binding
- Mechanism
  - evidence of CNS infection
  - in vivo evidence of microglial activation
“HCV Encephalopathy”
Fatigue, Depression, Cognitive impairment

**HOST GENETICS**

**CNS effect of peripheral immune response?**

**Psychogenic stressors**

**HCV infection of the CNS?**

---

**IFN induced gene expression in HCV**

- 50 patients undergoing pegylated IFN/Riba Rx
- Pre/post IFN gene expression measured with Affymetrix platform

- 16 Genes upregulated in IFN-induced depression
  - DYNLT1, DISC1, GCH1, TOR1B, ST3GAL3, MEF2A

*Schlaak J EASL 2009, AASLD 2008*
IFN induced gene expression in HCV

Schlaak J EASL 2009, AASLD 2008

Depression gene expression in brain in depression

Schlaak J EASL 2009, AASLD 2008
This study confirms that the genes that are associated with the development of depressive symptoms during IFN therapy in HCV patients are also relevant for IFN-unrelated depression.

The data also suggest that the IFN-system plays a pivotal role in endogenous depression.

Evidence of immune activation in brains from HCV+ve individuals.
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SD Taylor-Robinson
HC Thomas
P Karayiannis
J Allsop
B Grover

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M Gess
N Tatman
J Sheldon
D Holt