Resting-State fcMRI analysis in HIV and HCV co-infection subjects. A pilot study

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Abstract

Background and Purpose: HCV co-infection's role on cognitive impairment of HIV patients is still debated and functional neuroimaging evaluation on this matter is lacking. To provide further insight about HCV's neuro-effects on HIV associated neurocognitive disorder (HAND), we performed a pilot resting state (RS) functional connectivity magnetic resonance imaging (fcMRI) study to find eventual functional connectivity alteration that could reflect HCV related cognitive performance degradation.

Methods: Eighteen patients (8 HIV, 10 HIV-HCV), either impaired or not impaired, were assessed with RS fcMRI. A statistic model including cognitive testing results was elaborated during data processing to evaluate brain networks alteration related to actual cognitive status in patients.

Results: Statistically significant different patterns of connectivity were found: HCV co-infection modified 17 ROIs' connectivity with 45 supra-threshold connections (p-FDR min 0.0022, max 0.0497). ROIs most involved were right pallidum, brainstem, vermian lobules 1-2 and right cerebellar lobule 10. Graph theory analysis did not demonstrate significant difference between networks, but HCV related modifications at ROI's local level were found, with particular involvement of ROIs of frontal lobe, basal ganglia and cerebellum. Increased fronto-striatal dysfunctions have been already reported as consequences of HCV infection and could reflect an additive effect. Cerebellar alterations are associated with HIV and HAND, but not with HCV infection, suggesting a synergic effect of HCV.

Conclusion: Our study demonstrates RS fcMRI can help to understand the interactions between HIV and HCV co-infection, and our preliminary results suggest synergic effects of HCV in HIV-related brain functional modification.

Keywords:

Resting state functional connectivity, hiv, hcv.

1 Introduction

HIV-associated neurocognitive disorder (HAND) is a frequent co-morbidity of HIV infection, and its prevalence is still high, affecting about 50% of HIV infected individuals despite combination antiretroviral therapy (cART) [1]. HAND includes variable degrees of impairment, from a sub-clinical stage defined asymptomatic neurocognitive impairment (ANI), to mild neurocognitive disorder (MND) and a last stage called HIV-associated dementia (HAD) [2,3,4].

Several social, physiologic and pathologic factors can affect diagnosis and managing of HAND, i.e., education, poverty, substance abuse, age, neurologic, psychiatric and cardiovascular disease. [4]

An important co-morbidity is the hepatitis C virus (HCV) co-infection which is likely due to shared primary modes of transmission via intravenous drug use and sexual contact. Depending on the HIV population, co-infection with HCV can be found in up to 75–85% in high-risk groups, such as intravenous drug users, and up to 25% on overall HIV population [5].

HCV co-infection implies some specific issues in HIV individuals: it influences the course and management of HAND, i.e. increasing the risk of antiretroviral drug-induced hepatotoxicity and with potential synergic cognitive effects even if there is no agreement for considering HCV status an independent risk factor for cognitive abnormalities in HIV patients. Several studies reported that both HCV and HIV are independently associated with cognitive impairment [6, 7].

In addition, co-infected patients showed attention and concentration difficulties and greater global impairment [7, 8]. Recent studies instead reported no significant cognitive differences between HIV mono-infected and HIV-HCV co-infected patients after careful control of confounding variables such as drug use and hepatopathy, or when HCV viral load is undetectable. Functional imaging was not included in any of these studies [9,10].

RS-fcMRI has proved to be a sensitive tool to detect early and asymptomatic cognitive changes in HAND and demonstrated that HIV infected patients hyper-activate brain regions involved in attention, memory, and executive functioning, supporting brain reserve theory, with particular evidence of fronto-striatal dysfunction [11].

Analogously, to provide insight into the aetiology of HIV-HCV associated brain dysfunction, aiming to identify potential brain functional differences related to HCV co-infection, in a cohort of individuals with chronic HIV infection, with a sub-group of HCV co-infected patients, we examined RS-fcMRI connectivity measures and we correlated those with cognitive abilities evaluated by the Repeatable Battery for the Assessment of Neuropsychological Status [12]. To our knowledge, our paper is the first work that analyses this matter from a perspective of functional connectivity and graph theory.

2.Materials and methods

2.1 Sample

Our sample included 18 HIV+ patients, 10 with HCV co-infection, in cART treatment; it is important to note that all patients were treated with cART because it is related to different expression of impairment [13].

Inclusion criteria were being at least 18 years of age, with normal neurological exam. Exclusion criteria included history of seizure disorder, previous cerebrovascular events, demyelinating disease or any other non-HIV neurological disease. Demographic and clinical factors of the two populations were compared with Student's t-test to evaluate statistically significant differences.

2.2 Neuropsychological evaluation

All patients were assessed for cognitive impairment, using the RBANS and Frascati criteria. In particular, examination included immediate and delayed memory, visuoconstruction skills and visuo-perception ability, attention, and language functioning. All scores were normalized to age [2,12].

2.3 MR exam protocol

All RS fcMRI were performed in our radiology department with a Philips MR scanner Ingenia dStream 1.5 T (Philips Healthcare, Eindhoven, NL). All participants received instructions for the exam (not to think of something in particular and not to fall asleep). After the localizing sequences structural T1 3D TFE and T2 TSE were performed to exclude significant brain pathology. The FE-EPI sequence had the following parameters: 2D acquisition with slice thickness of 5 mm, RT 3000 ms, ET 50 ms, FA 90°, EPI factor 37. All the scans have been considered negative for the presence of intracranial significant pathology. DICOM data were converted in NII format with dcm2niigui (software created by Chris Rorden) and then loaded in the MATLAB® suite CONN: functional connectivity toolbox (MathWorks, INC. https://it.mathworks.com/products/matlab.html) (Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC) http://www.nitrc.org/projects/conn) for pre-processing and processing [14]. CONN software implements a strategy for noise source reduction that doesn't rely on global signal regression allowing for interpretation of anticorrelations as there is no regression of the global signal; denoising of fMRI signal can be also performed by means of removal of movement artefacts, characterization and removing of temporal covariates, and temporal filtering of the residual blood oxygen level-dependent (BOLD) contrast signal. Then, first-level estimation of multiple standard

functional connectivity magnetic resonance imaging (fcMRI) measures, and second-level random-effect analysis for resting state as well as task-related data can be performed with CONN tools. We set a band-pass filter of 0,0001-0,1 Hz, because low-frequency and extremely low frequency resting state networks (< 0.1 Hz) reveal coherent, spontaneous fluctuations characterising the functional architecture of the human brain [15].

Then, we used CONN default pre-processing pipeline for volume-based analyses (direct normalization to MNI-space), with smoothing FWHM of 8 mm and intermediate settings (97^{th} percentile in normative sample in functional outlier detection sample). First level analysis (single subject) and second level analysis (group analysis) were performed according to the CONN software pipeline; data processing at second level has been performed by means of the ROI-to-ROI functional correlation approach to obtain connectivity maps of interest. Finally, graph theory measurements were performed in order to evaluate from the metric and topological point of view brain network alteration induced by HCV co-morbidity.

2.4 Statistical Analysis

Using the statistical tools included in the CONN software ANCOVA-based models, aimed to underline connectivity effects of HCV on HIV population, were developed and tested; RBANS total score values were included into the models to evaluate the influence of actual cognitive impairment as possible covariate. Results were corrected with FDR threshold (P=0.05), which calculates the number of potential false positives related to the multiple comparisons performed. Finally, graph theory parameters were calculated for the networks exhibited in our data in order to evaluate nodes and vertex alteration of interest, according to the CONN analysis procedures.

3. Results

3.1 Sample analysis

Demographic and clinical factors are summarized in table 1

Considering the two population of our sample we can see that sex, age, education and cognitive test score are well-matched, while HAND diagnosis are balanced (3 ANI, 1 MND per group) but with different prevalence (50% impaired in HIV vs 40% in HIV-HCV.) Using Student's T-Test we found significant difference about years of HIV infection, nadir and actual CD4 count.

An overall prevalence of cognitive impairment of 45% (50% in HIV+ group and 40% in HIV-HCV group) with 33% of ANI (37% and 30%), 11% of MND (12,5% and 10%) and 0% of HAD was found.

3.2 Functional connectivity results

We found that HCV co-infection is associated to a statistically significant increase of connectivity in 17 interconnected brain regions in HIV+ group. These 17 regions are organized in a network characterized by 45 supra-threshold connections (size) with an intensity of 208,47 (intensity is defined as sum of absolute T-values over these supra-threshold). The ROI most modified by HCV infection are right pallidum (size 10, intensity 41,53), brainstem (size 6, intensity 26,55), vermis 1 2 and cerebellum 10 right (both size 5, intensity respectively 23.76 and 21,09).

All network and ROI's findings are reported in table. 2

3.3 GRAPH THEORY RESULTS

Another finding of our study shows that HCV co-infection did not altered graph theory's global properties of HIV+ brain network, but it did locally, affecting not only the ROI included in the different sub-network. In particular, even if without multiple comparison correction, we found local significant differences in:

 DEGREE (p-unc min 0,004 max 0,0497): in order of T value, reduced for middle frontal gyrus left, precentral gyrus left, pars triangularis of inferior frontal gyrus right, posterior division of cingulate gyrus; increased in subcallosal cortex, left planum polare and frontal orbital cortex right.

It is noteworthy that many of this ROI with reduced degree belong to frontal lobe, suggesting more disrupted connections in HCV coinfected population.

 COST (p-unc min 0,004 max 0,0497): in order of T value, reduced for left middle frontal gyrus, left precentral gyrus, posterior division of left cingulate gyrus, pars triangularis of right inferior frontal gyrus; increased in subcallosal cortex, left planum polare and frontal orbital cortex right.

 GLOBAL EFFICENCY (p-unc min 0,007 max 0,0494): in order of T value, increased in left cerebellum 4 5, subcallosal cortex, left planum polar, lingual gyrus right, vermis 7, temporo-occipital part of left middle temporal gyrus, left cerebellum 3, right occipital fusiform gyrus, inferior division of left lateral occipital cortex, right cerebellum 3, anterior division of right parahippocampal gyrus, temporo-occipital part of left inferior temporal gyrus, and left pallidum.

 AVERAGE PATH LENGTH (p-unc min 0,003 max 0,0423): in order of T value, increased in left supracalcarine cortex, left intracalcarine cortex, triangular part of inferior frontal gyrus, anterior division of left supramarginal gyrus and left postcentral gyrus.

 CLUSTERING COEFFICIENT(p-unc min 0,015 max 0,0404): in order of T value, increased in vermis 10, right cerebellum 4 5, right cerebellum 10, right cerebellum crus 2, posterior division of left parahippocampal gyrus, vermis 6, right cerebellum 6, vermis 8, left cerebellum 8 and right cerebellum crus 1.

 LOCAL EFFICENCY(p-unc min 0,006 max 0,0481): in order of T value, increased in vermis 10 and 6, right cerebellum 10, 6 and 2,posterior division of right parahippocampal gyrus, right cerebellum 3,4 5, vermis 7, right cerebellum 1, left cerebellum 8 and brainstem.

 BETWEENNESS CENTRALITY (p-unc min 0,002 max 0,0447): in order of T value, increased in inferior division of left lateral occipital cortex, right supracalcarine cortex, left pallidum, right postcentral gyrus, Left Heschl gyrus, posterior division of left middle temporal gyrus and left occipital pole; reduced in right thalamus, right cerebellum 10, anterior division of left inferior temporal gyrus and left superior parietal lobule.

Table 3: summary of graph theory parameters local modifications caused by HCV on HIV functional network

4. Discussion

Analysis of our sample showed prevalence of HAND and its sub-categories consistent with literature [3]. Significant differences in years of HIV infection, nadir and actual CD4 count could be relevant, even if actually there is no consensus about possible effect of these factors on functional brain imaging (e.g. *Ipser et al* found that differences in HIV duration, plasma viral ribonucleic acid (RNA) occupancy, nadir and current CD4 count did not predict variability in theirs RS fcMRI study

[16]. It is worth noting that most of recent studies of HAND with task based and resting state fcMRI show similar sample's limitation [11]

Our paper is the first work that analyses HCV effects on HIV population from a functional connectivity and graph theory point of view.

This imply that we cannot compare our findings with previously published papers. To better understand and compare our findings, we evaluated recent HIV and HCV-related brain imaging studies, focusing, when possible, on RS-fcMRI results.

4.1 HIV-related brain alterations

Before cART, HIV was associated with primarily subcortical dysfunction [13] while with actual therapy, HIV show cortical and subcortical dysfunction [1]. Neuropsychological studies have suggested that brain regions involved in attention, working memory and episodic memory may be particularly affected in HIV+ patients, generating several fcMRI studies that evaluated detection of neuronal dysfunction in HIV infected people. Visual task based studies focused on attention demonstrated an increase in activation in the attention network (including bilateral dorsolateral prefrontal regions (right greater than left), as well as bilateral parietal (right greater than left) and medial cerebellar regions) when attentional load increases [11]. In particular, one of this task based study of attention included a longitudinal evaluation, finding that at baseline, the HIV individuals (with normal cognitive performance) showed greater BOLD activation in cerebellar/occipital and right prefrontal regions than seronegative controls only on the task with the greatest attentional load. After 1 year, HIV infected patients showed further increases in BOLD signals for all tasks, primarily in the right prefrontal and posterior parietal cortices, and the cerebellum bilaterally. These studies suggest that HIV patients have an attention deficit, and it can be counter balanced, at least for a certain degree, using brain reserve capacity [11]. It is remarkable that these studies frequently

reported frontal and cerebellar alterations in HIV population, because our study suggests that these areas are particularly involved in HCV co-infection. Working memory task based studies showed increase in activation in the lateral prefrontal cortex and/or parietal regions in the HIV-positive group [11]. Studies investigating attention and working memory suggest an altered function of hippocampal-prefrontal regions that could explain HIV-related memory deficit. Functional connectivity analyses on resting state data consistently suggested dysfunction in fronto-striatal network [16]. Fronto-striatal network consists of neural pathways that connect frontal regions with the basal ganglia, and these circuits are involved in executive functioning, decision making, task switching and memory. Plessis *et al.* reviewed twenty-one studies (468 HIV+, 270 HIV- controls), of which six (105 HIV+, 102 controls) utilized task based fcMRI with paradigms engaging the fronto-striatal-parietal network. Their meta-analysis revealed consistent functional differences in the left inferior frontal gyrus and caudate nucleus between HIV infected patients and healthy subjects [17]. Our study suggests that fronto-striatal dysfunctions are more pronounced in HCV co-infected populations, with functional connectivity alteration in basal ganglia and graph theory modifications in several frontal ROIs. In particular, most of the ROI with reduced cost and degree belong to frontal lobe, likely reflecting disrupted connections related to HCV in coinfected population involving fronto-striatal network and this is in accord with reduced fronto-striatal connectivity reported in literature. Volumetric/morphologic MRI studies also have investigated HIV-related brain modification.[18,19]

One of this studies reported that HIV positive people had smaller grey matter volume and abnormal white matter microstructure. [18]

Sanford et al found HIV related statistically significant reductions in volume within the thalamus, caudate, putamen, and globus pallidus at time 0 and after 2 years. Cortical modelling revealed that HIV-infected patients had thinner cortices in the temporal and frontal lobes on both hemispheres, right primary motor and sensory cortex, posterior cingulate, orbitofrontal cortex, and left anterior

cingulate cortex. HIV is associated with regionally specific patterns of reduced thalamic and brainstem volumes and reduced cortical thickness in the orbitofrontal cortex, cingulate gyrus, primary motor and sensory cortex, temporal, and frontal lobes. Nadir CD4 count showed no correlations with cortical thickness but correlates with reduced white matter in brainstem, globus pallidus, internal capsule, caudate, and right frontal lobe. [19]. Volumetric studies confirm that HIV can modify the brain regions that in our study showed modified connectivity.

4.2 HCV-related brain alterations

There are not many studies that focused on fcMRI brain changes caused by HCV infection (not mediated by hepatic encephalopathy). One of this study reported higher EC (eigenvector centrality, measure of centrality) in a cluster in the right anterior superior parietal lobule in HCV individuals. Further connectivity analysis showed increased connectivity of this cluster with primary and secondary somatosensory cortex, and temporal and occipital lobes in HCV infected patients [20]. We too found altered centrality values of several ROIs that could reflect modification of information flow and paths. In-between centrality should be correlated with other centrality measures (*i.e.,* eigenvector centrality), because HCV could modify some hubs of brain network.

Whole- brain voxel- wise comparison of GMV (grey matter volume) between HCV- positive patients and healthy controls, corrected for confounding effect of age, did not result in any significant differences [20]

A proton magnetic resonance spectroscopy and diffusion tensor imaging study examined neuroeffects of HCV with results suggesting that HCV-associated neurologic complications disrupt fronto-striatal structures, with consequent increased fatigue and poorer cognitive performance, particularly in those cognitive tasks regulated by fronto-striatal regions. In particular were reported

microstructural abnormalities in the striatum, external capsule, and fronto-occipital fasciculus, in accord with previous DTI studies of HCV and results among HIV infected patients [21,22]

This confirms that HCV can affect some regions that are involved in HAND pathogenesis, with possible additive/synergic effect. In addition, our results suggest an increased cerebellar segregation related to HCV in terms of clustering coefficient and local efficiency. We found modifications of average path length and global efficiency too, implying potential local alteration of integration mediated by HCV.

4.3 Interpretation of results.

Our study shows that HCV has an effect on HIV seropositive brain networks, causing different patterns of activation and modifying local properties of the network's nodes. Absence of neurocognitive test differences in our sample could be explained involving still available brain reserve [11].

Our hypothesis is that connectivity differences between the two groups reflect HCV infection, and while basal ganglia alterations were expected due to known cortico-striatal dysfunction in both HIV and HCV), cerebellum involvement was unexpected, even if it is consistent with emerging cognitive importance of cerebellum itself: executive function tasks were found to activate Crus 1 bilaterally, left Crus 2, right lobule VI and midline lobule VII. Language tasks activate bilaterally in lobules VI, midline lobule VIII, left Crus 1 and right Crus 2 [23]

In addition, as reported by neuro-pathological studies in HIV-HCV co-infected patients, HCV RNA is present in the frontal cortex, basal ganglia, and subcortical white matter, but not in the thalamus, cerebellum, and brainstem[24,25].

This is very important because functional connectivity and networks modifications we found affect even brain regions where there is no HCV replication and are usually spared by HCV, supporting

the hypothesis that cerebral alterations are more likely related to an increased inflammation, or other indirect factor, mediated by HCV than viral direct damage and suggesting a synergic effect of HCV in HAND pathogenesis.

To better understand our findings, it is useful to remind that pathogenesis of HAND is not fully understood. Neural injury occurring before cART initiation, toxicity of antiretroviral therapy or comorbid conditions likely all contribute to persistent impairment, but low-level CNS inflammation seems to be the most relevant factor. Activated infected and non-infected macrophages and microglia play a prominent role in the development and progression of disease through the secretion of chemokines, cytokines, and other inflammatory mediators that are, directly or indirectly, neurotoxic. Additionally, infected macrophages and microglia may spread virus to non-infected cells and release viral proteins that contribute to prolonged brain inflammation and neurotoxicity. This suggests an important role of both the HIV virus and inflammation in HAND pathogenesis. Inflammation, rather than virus replication in the brain, underlies the milder and severe forms, considered that degree of monocyte activation as well as the level of microglial activation has proven to be a more reliable correlate with HIV related dementia than viral load or even active viral replication [4, 9, 17, 26].

Several factors could explain synergic inflammation in HIV-HCV co-infection: both viruses can increase the permeability of the brain blood barrier and the expression of pro-inflammatory cytokines and chemokines, including IL-6 and IL-8. In addition, both can infect and replicate in microglia [27].

Microbial translocation, which is the passage of live bacteria and/or bacterial products across an intact intestinal barrier, seems an important factor in HIV-related chronic immune activation and may contribute to HAND pathogenesis. Microbial translocation can result from reduced host immunity and/or increased permeability of the intestinal lining and has also been shown in hepatitis C [4, 28].

Another possible synergic mechanism could be related to the Minimal Hepatic Encephalopathy (MHE, a common complication of cirrhosis characterized by cognitive dysfunctions that can be diagnosed only through specific neurological or psychometric tests.

Although we have not information about MHE diagnosis in our sample, considering the high prevalence in cirrhotic patients (30%-50%) and the presence of hepatopathy in 60% of our HCV-HIV group, it is possible that MHE could have contributed to our findings. Consistent with this hypothesis are RS fcMRI evidence suggesting that an alteration of cortico-striato-thalamic pathway may play an important role in HE[29]. In particular, even if with different RS-fcMRI approach (Granger causality), *Qi et al*. found increased connectivity to right pallidum from the right precuneus, right SMA, bilateral MFG, left MTG, right ITG and bilateral cerebellum partially, overlapping our results (increased connection of right pallidus with ,among the others, bilateral inferior temporal gyrus, right medial temporal gyrus and cerebellum 3 bilaterally)[30].

This could mean that our results could be at least partially related to MHE, and that RSfcMRI could be sensible to it, even in a contest of brain injury as neuroHIV, with intriguing perspectives in the MHE diagnosis. Obviously, dedicated studies are required to evaluate this possibility.

5. Limitations

Sample sizes employed in this study were small, limiting power to detect between group differences. Potential influence of M/F ratio, duration of ART theraphy and HIV and HCV infection, CD4 count, HAND prevalence and others clinical parameters on fcMRI results should be examined using larger samples. All participants in this study were receiving antiretroviral medication at the time of the scan. While this increases the relevance of our findings to the HIV population in the cART era, it was not possible to dissociate the relative contribution of potential

neurotoxic effects of antiretroviral medication and the HIV and HCV viruses on disruptions on intrinsic connectivity.

6. Conclusions

RS fcMRI can help to understand the interactions between HIV and HCV co-infection and their neuropsychological expression, leading to develop better models of pathogenesis of HIV and HCV.

Accordingly, with known usefulness of RS-fcMRI in assessing abnormalities earlier respect to neuropsychological testing, our work showed connectivity differences between HIV+ and HIV-HCV groups with equal cognitive performance. The HCV-related modifications of HIV seropositive brain networks identified by our study suggest a synergic effect between HCV-HIV co-infection, with particular involvement of cerebellum, probably mediated by an increased SNC inflammation.

Further studies are necessary to confirm the role of RS-fcMRI and if this approach could play a role in identifying patients that can have more cognitive improvement with current HCV therapeutic options. It is important to underline that these are preliminary data, even if the statistical association is robust and further study to confirm our findings are necessary.

7. Conflicts of interest:

None

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