



Universiteit
Leiden
The Netherlands

Cerebral magnetic resonance imaging in quiescent Crohn's disease patients with fatigue

Erp, S. van; Ercan, E.; Breedveld, P.; Brakenhoff, L.; Ghariq, E.; Schmid, S.; ... ; Meulen-de Jong, A. van der

Citation

Erp, S. van, Ercan, E., Breedveld, P., Brakenhoff, L., Ghariq, E., Schmid, S., ... Meulen-de Jong, A. van der. (2017). Cerebral magnetic resonance imaging in quiescent Crohn's disease patients with fatigue. *World Journal Of Gastroenterology*, 23(6), 1018-1029. doi:10.3748/wjg.v23.i6.1018

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/117281>

Note: To cite this publication please use the final published version (if applicable).

Case Control Study

Cerebral magnetic resonance imaging in quiescent Crohn's disease patients with fatigue

Sanne van Erp, Ece Ercan, Perla Breedveld, Lianne Brakenhoff, Eidrees Ghariq, Sophie Schmid, Matthias van Osch, Mark van Buchem, Bart Emmer, Jeroen van der Grond, Ron Wolterbeek, Daniel Hommes, Herma Fidler, Nic van der Wee, Tom Huizinga, Désirée van der Heijde, Huub Middelkoop, Itamar Ronen, Andrea van der Meulen-de Jong

Sanne van Erp, Lianne Brakenhoff, Daniel Hommes, Andrea van der Meulen-de Jong, Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Ece Ercan, Perla Breedveld, Eidrees Ghariq, Sophie Schmid, Matthias van Osch, Itamar Ronen, C.J. Gorter Center, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Ece Ercan, Perla Breedveld, Eidrees Ghariq, Sophie Schmid, Matthias van Osch, Mark van Buchem, Bart Emmer, Jeroen van der Grond, Itamar Ronen, Department of Radiology, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Ron Wolterbeek, Department of Medical Statistics, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Daniel Hommes, Center for Inflammatory Bowel Diseases, University of California Los Angeles, Los Angeles, CA 90095, United States

Herma Fidler, Department of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht 3584 CX, the Netherlands

Nic van der Wee, Department of Psychiatry, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Tom Huizinga, Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Huub Middelkoop, Department of Neuropsychology, Leiden University Medical Center, Leiden, Zuid-Holland 2333 ZA, the Netherlands

Author contributions: Van Erp S and Ercan E contributed

equally to this work; Brakenhoff L, Hommes D, Middelkoop H, Ronen I and van der Meulen-de Jong A designed the research; Ercan E, Brakenhoff L, Hommes D, Fidler H, Huizinga T, van der Heijde D and van der Meulen-de Jong A treated patients and collected material and clinical data from patients; van Erp S, Ercan E, Breedveld P and Brakenhoff L performed the research; van Buchem M, Emmer B and van der Grond J contributed new analytic tools; van Erp S, Ercan E, Breedveld P, Brakenhoff L and Wolterbeek R analysed the data; van Erp S and Ercan E wrote the paper; all authors contributed to critical revision of the manuscript and final approval of the submitted manuscript.

Institutional review board statement: This study was approved by the institutional medical ethical committee of the LUMC.

Informed consent statement: All patients signed a written informed consent prior to study enrolment.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Sanne van Erp, Department of Gastroenterology and Hepatology, Leiden University Medical Center, Albinusdreef 2, Leiden 2333 ZA, the Netherlands. s.j.h.van_erp@lumc.nl

Telephone: +31-71-5262915
 Fax: +31-71-5248115

Received: August 26, 2016
 Peer-review started: August 28, 2016
 First decision: October 10, 2016
 Revised: November 14, 2016
 Accepted: December 8, 2016
 Article in press: December 8, 2016
 Published online: February 14, 2017

Abstract

AIM

To evaluate brain involvement in quiescent Crohn's disease (CD) patients with fatigue using quantitative magnetic resonance imaging (MRI).

METHODS

Multiple MRI techniques were used to assess cerebral changes in 20 quiescent CD patients with fatigue (defined with at least 6 points out of an 11-point numeric rating scale compared with 17 healthy age and gender matched controls without fatigue). Furthermore, mental status was assessed by cognitive functioning, based on the neuropsychological inventory including the different domains global cognitive functioning, memory and executive functioning and in addition mood and quality of life scores. Cognitive functioning and mood status were correlated with MRI findings in the both study groups.

RESULTS

Reduced glutamate + glutamine (Glx = Glu + Gln) concentrations ($P = 0.02$) and ratios to total creatine ($P = 0.02$) were found in CD patients compared with controls. Significant increased Cerebral Blood Flow ($P = 0.05$) was found in CD patients (53.08 ± 6.14 mL/100 g/min) compared with controls (47.60 ± 8.62 mL/100 g/min). CD patients encountered significantly more depressive symptoms ($P < 0.001$). Cognitive functioning scores related to memory ($P = 0.007$) and executive functioning ($P = 0.02$) were lower in CD patients and both scores showed correlation with depression and anxiety. No correlation was found subcortical volumes between CD patients and controls in the T_1 -weighted analysis. In addition, no correlation was found between mental status and MRI findings.

CONCLUSION

This work shows evidence for perfusion, neurochemical and mental differences in the brain of CD patients with fatigue compared with healthy controls.

Key words: Magnetic resonance imaging; Systemic inflammation; Fatigue; Crohn's disease; Cognition

© **The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The present study explores perfusion, neuro-

chemical and mental differences in the brain of Crohn's disease (CD) patients compared with healthy controls. This implies that for a gastroenterologist it is important to focus, besides gastrointestinal symptoms due to inflammation, on the effects of systemic inflammation on the brain and mental status. Knowledge and understanding of these effects in CD patients may help health professionals to set up interventions to maintain CD remission and improve mental status by *e.g.* psychosocial interventions.

Van Erp S, Ercan E, Breedveld P, Brakenhoff L, Ghariq E, Schmid S, van Osch M, van Buchem M, Emmer B, van der Grond J, Wolterbeek R, Hommes D, Fidder H, van der Wee N, Huizinga T, van der Heijde D, Middelkoop H, Ronen I, van der Meulen-de Jong A. Cerebral magnetic resonance imaging in quiescent Crohn's disease patients with fatigue. *World J Gastroenterol* 2017; 23(6): 1018-1029 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i6/1018.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i6.1018>

INTRODUCTION

Crohn's disease (CD) is a relapsing inflammatory bowel disease (IBD)^[1], characterized by segmental transmural lesions that can affect any part of the gastrointestinal tract^[2]. Besides gastrointestinal symptoms, fatigue is common in CD patients. In contrast to regular fatigue which affects nearly everyone, disease-related fatigue is more long lasting and may occur despite sufficient sleep and rest. Generally, fatigue lasting for more than 6 mo is considered chronic and is significantly more prevalent in IBD patients than in healthy controls^[3]. Although fatigue is influenced by IBD disease activity, 40% of the patients with quiescent disease report fatigue as well and contributes negatively to the patients' health-related quality of life (QoL)^[4,5].

The pathogenesis of CD is multifactorial and results from an impaired interaction between environment, commensal microbiota and the human immune system, leading to a chronic inflammatory status and eventually CD^[6,7]. Furthermore, in both quiescent and active CD patients increased levels of circulating inflammatory cytokines, such as tumour necrosis factor- α (TNF- α) are reported^[8-10]. Although quiescent CD patients report fewer clinical symptoms and score less on the clinical activity score compared with active CD patients, inflammatory cytokines are present^[9,10]. TNF- α can be secreted by a large variety of cells^[8] and can initiate a signalling stimulus to the brain parenchyma that will subsequently activate microglia. Activated microglia stimulates the production of monocyte chemo-attractant protein (MCP)-/CCP2, which recruits monocytes into the brain^[9-11]. Moreover, this cerebral infiltration of monocytes plays an important role in driving inflammation in the brain^[11-13].

Magnetic resonance imaging (MRI) is an imaging technique widely used to visualize the effect of several neurological diseases, such as Multiple Sclerosis (MS), Parkinson's Disease and Alzheimer disease, in the brain^[14]. A variety of MRI methods can be employed to identify cerebral changes due to a specific disease. These methods include T₁-weighted imaging, magnetization transfer imaging (MTI), magnetic resonance spectroscopy (MRS), arterial spin labeling (ASL) and diffusion tensor imaging (DTI). T₁-weighted imaging provides high-resolution, high-contrast anatomical images of the brain and can be used to determine the volumes of the grey matter (GM), white matter (WM), cerebral spinal fluid (CSF) and subcortical structures^[15]. Through voxel based morphometry (VBM), it is possible to visualize local changes in GM volumes^[16]. MTI is a technique sensitive to brain tissue microstructural changes, stemming from changes in macromolecules such as myelin or cell membranes^[17]. MRS measures the concentration of certain metabolites in living tissues and gives evidence for neurochemical changes^[18]. ASL is a non-invasive tool for the quantification of regional cerebral blood flow (CBF)^[19] and can reveal changes in tissue perfusion. DTI is sensitive to minute changes in tissue microstructure, such as changes in myelin integrity and axonal density in white matter fiber tracts, based on the random motion or diffusion of water molecules^[20].

Previous MRI studies have shown that systemic inflammation contributes to cognitive decline, for example in relation to aging^[21], but also to brain diseases including Alzheimer disease, MS and Parkinson's disease by promoting activation of the immune system^[22-24]. Metabolic and cerebral perfusion changes have been found in the brain of patients with Rheumatoid Arthritis (RA), Systemic Sclerosis and Systemic Lupus Erythematosus (SLE)^[11,12,25-31]. In addition, previous studies performed in patients with Chronic Fatigue Syndrome (CFS) found an association between fatigue complaints and metabolic changes in the brain as well^[32-34]. In CFS patients, the mean ratio of choline (Cho) to creatine (Cr) in the occipital cortex was significantly higher than in controls, indicating an abnormality of phospholipid metabolism in the brain in CFS^[32-33]. These findings suggest that systemic inflammation and fatigue complaints could have structural, neurochemical and functional correlates in the brain. So far, the link between systemic inflammation, disease-induced fatigue and changes in the brain have not been explored in CD patients. The aim of this exploratory study was to investigate to what extent systemic inflammation affects the brain of quiescent CD patients, by using a variety of MRI acquisition methods and neuropsychological examinations that assess cognition, mood and QoL. Furthermore, the correlation between MRI changes, clinical characteristics, including fatigue scores, and mental status was investigated.

MATERIALS AND METHODS

Study population and study design

In this case-control study 20 CD patients and 17 age and gender matched healthy controls were included. Since it is known from literature that there is an age associated decrease in brain volume, primarily caused by a decrease in neuronal size and partly due to a reduction in numbers of neurons caused by apoptosis^[35], a correction was made for this confounder by matching the subjects.

Consecutive CD patients, fulfilling the inclusion criteria, were recruited through the IBD outpatient clinic of the department of Gastroenterology and Hepatology of the Leiden University Medical Center (LUMC), the Netherlands. The patients had endoscopic proven CD for at least 3 mo before inclusion, were in clinical remission and experienced fatigue. CD patients with anemia (Hb < 7.0 mmol/L), primary sclerosing cholangitis and routine MRI-contraindications (e.g., instable metal implants or a pacemaker) were excluded. All medication deemed necessary by the gastroenterologist was allowed at study inclusion, except for anti-TNF α or corticosteroid use, since this medication could reduce systemic inflammation the most and thus influence clinical disease activity. Healthy controls were recruited *via* an advertisement in het LUMC and included in the study if they had no anamnestic brain abnormalities, nervous system disease or chronic inflammation in the body. A 1-d program was set up for all participants by the relevant medical specialists, including a gastroenterologist, radiologist, psychiatrist and neuropsychologist and all individuals were asked to complete several questionnaires at study inclusion about demographics, mental status and QoL. This study was approved by the institutional medical ethical committee of the LUMC and all patients signed a written informed consent prior to study enrolment.

Clinical characteristics

Disease activity: The clinical disease activity of the CD patients was measured with the Harvey-Bradshaw Index (HBI). The HBI consists of 12 criteria, which include general well-being, abdominal pain, daily number of liquid stools, abdominal mass and extra intestinal manifestations (arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissure, new fistula and abscess). Patients with an HBI score of 4 or less were classified as having quiescent CD disease^[36].

Fatigue: Fatigue was assessed with the Multidimensional Fatigue Index (MFI) and the Visual Analogue Scale (VAS). The MFI is a self-report measurement containing 20 questions consisting of 5 subscales covering different dimensions: general fatigue, physical fatigue, mental fatigue, reduced activity and reduced motivation. The questions are about the fatigue experienced by the subject in the 7 d prior to examination.

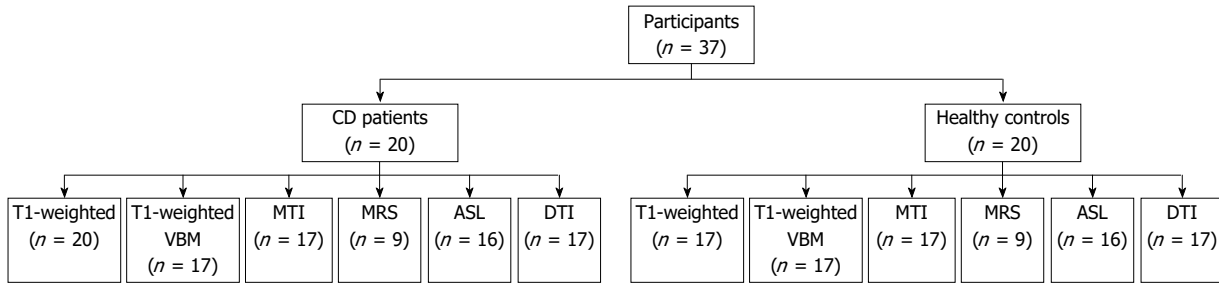


Figure 1 Flowchart of included participants in the magnetic resonance imaging analyses. CD: Crohn's disease; VBM: Voxel based morphometry; MTI: Magnetization transfer images; MRS: Magnetic resonance spectroscopy; ASL: Arterial spin labelling; DTI: Diffusion tensor imaging.

Scores range from 4 to 20, with higher scores indicating higher levels of fatigue^[37]. The VAS consists of a 10 point self-rating scale that measures subjective experiences of fatigue. The participants had to indicate on a visual line how they were currently feeling. Six points or more indicated the presence and experience of fatigue in individuals^[38].

MRI data acquisition

All study subjects underwent MRI of the brain, using a Philips Ingenia 3.0 Tesla MRI Scanner (Philips Medical Systems, Best, The Netherlands) equipped with a 12 channel head coil, and images were evaluated by an experienced neuroradiologist (MvB). The MRI protocol consisted of T₁-weighted imaging, MTI, MRS, ASL and DTI, and lasted for about 60 min. Since more CD patients were included and all patients and healthy controls were age-gender matched, in total 3 CD patients, who matched the least with the controls, got excluded from the voxel-based analysis of the T₁-weighted and DTI data. For the MRS and ASL analyses data of some CD subjects were either missing because of time limitations or excluded due to low quality, caused by subject motion. For the MRS analysis only 9 CD patients and 9 age and gender matched controls were included, and for the ASL analysis 16 CD patients and 16 age and gender matched controls were included (Figure 1). The MRI scan protocol consisted of (1) axial 3D T₁-weighted images (FOV: 224 mm × 144 mm × 182 mm, resolution: 0.88 mm × 0.88 mm × 1.20 mm, TR/TE = 9.75/4.59 ms); (2) sagittal FLAIR images (FOV: 224 mm × 144 mm × 180 mm, resolution: 0.5 mm × 0.5 mm × 3.6 mm, TR/TE/TI = 10000/120/1650 ms); (3) axial DTI (FOV: 176 mm × 144 mm × 224 mm, resolution: 1.75 mm × 1.75 mm × 3.6 mm, TR/TE = 4317/55.33 ms, one volume with $b = 0$ s/mm² and 32 diffusion-weighted volumes with $b = 800$ s/mm²); (4) axial MTI (FOV: 224 × 144 × 180, resolution: 0.88 mm × 0.88 mm × 7.2 mm, TR/TE = 100/10.95 ms, two volumes acquired one with and one without a radiofrequency saturation pulse); (5) ASL (FOV: 240 mm × 240 mm × 133 mm, resolution: 3.0 mm × 3.0 mm × 7.0 mm, TR/TE = 4000/15.19 ms, labeling duration = 1650 ms, post-labeling delay = 1525 ms, 35 label and control pairs and background

suppression inversion pulses at 50 and 1150 ms); and (6) a single volume, stimulated echo acquisition mode (STEAM) ¹H MRS scan with a volume of interest (VOI) located in the left centrum semi ovale, containing mostly white matter as shown in Figure 2 (voxel size = 30 mm × 15 mm × 15 mm, TR/TE = 2000/14 ms, mixing time = 19 ms, sample size = 2048, number of averages = 96).

Post-processing and data analysis

T₁-weighted image analysis: Brain extraction tool (BET) of FMRIB Software Library (FSL) (<http://www.fmrib.ox.ac.uk/fsl>) was used to extract the brain tissue from T₁-weighted images^[15]. FSL FMRIB's Automated Segmentation Tool (FAST)^[39] was used to segment GM, WM and CSF tissues from the brain extracted T₁-weighted images. FSL FMRIB's Integrated Registration and Segmentation Tool was used to segment subcortical structures: nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen and thalamus^[40]. Following segmentation, the volumes of GM, WM and subcortical structures were calculated using FSL Maths. The volumes were normalized to subject intracranial volume by dividing the volumes with the total brain volume of the same subject. VBM in FSL was used to assess local GM differences between CD patients and controls^[16,41].

MTI analysis: MTI were split into images with and without saturation. Both images, with and without saturation, were brain extracted with BET and the image without saturation was aligned to the image with saturation. After alignment, the magnetization transfer ratio (MTR) of the whole brain was calculated using FSL Maths. The MTR images were then registered to the T₁-weighted images from the same subject with FLIRT^[42]. Subsequently, MTR images were multiplied with the binary GM and WM masks from the same subject, to create GM and WM MTR images. Tissue-specific histograms of MTR values from the GM and WM of patients and controls were created using an in house-developed MATLAB[®] program (Mathworks, Natick, MA, United States).

MRS analysis: The MRS analysis was performed

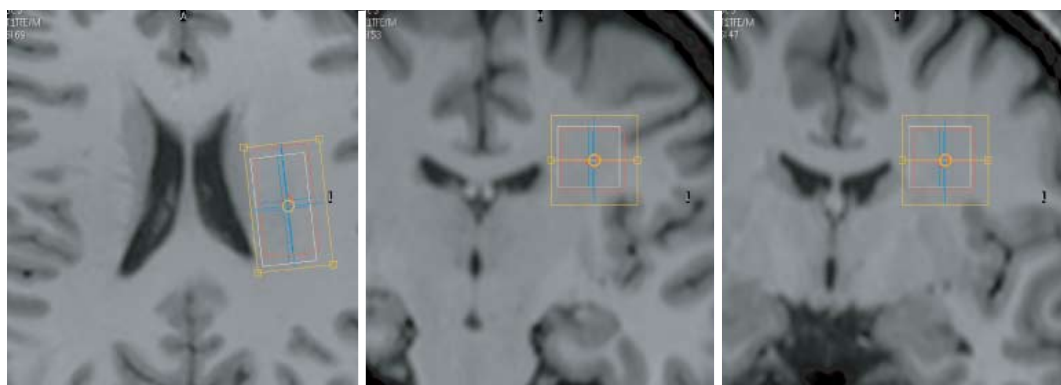


Figure 2 Planning of the ¹H-magnetic resonance spectroscopy volume of interest in the left centrum semi-ovale. Seen on axial (left) and on the coronal (right) T₁-weighted image slices. The effective volume of interest set at the tNAA frequency is shown (red rectangle) together with the shimming volume (yellow rectangle).

in MATLAB and LCmodel^[43]. An in-house developed MATLAB code was used to calculate and correct for GM, WM and CSF tissue fraction (%) within the VOI for each subject separately. LCmodel was used for the calculation of the concentration and ratio to total creatine (tCr) of the metabolites N-acetyl-aspartate (NAA), creatine (Cr), glutamate (Glu), myo-inositol (Ins), glutamine (Gln), N-acetyl-aspartyl-glutamate (NAAG) and choline (Cho). Institutional units (IU) of concentration were expressed in mmol. Among these metabolites, NAA is a neuronal marker, NAAG is suggested to be related to excitatory neurotransmission, total Creatine (tCr), the sum of phosphocreatine and creatine, is a marker of energy metabolism. Cho is related to cell membrane turnover, Glu is an excitatory neurotransmitter predominantly found in neurons, Gln is a precursor for Glu and found mostly in astrocytes, and Ins is a possible astrocytic marker^[44]. The mean ratios of NAA, Glu, Ins, Gln, NAAG and Cho to tCr were compared between the two study groups.

ASL analysis: The average GM Cerebral Blood Flow value was calculated in FSL^[45]. The ASL label and control images were motion corrected by FSL MCFLIRT^[46]. The perfusion maps were calculated for each subject by subtracting the label from the control images and averaging those images. Following that, perfusion maps from each subject were registered first linearly and then nonlinearly to GM volume segmented from the T₁-weighted image of the subject and subsequently they were first linearly and then nonlinearly registered to average brain template from the Montreal Neurological Institute (MNI). CBF of the GM is calculated by using a binary GM mask of the subject with a threshold of 60% GM probability and using the following equation:

$$CBF_{pCASL} = \frac{6000 \cdot \lambda \cdot \Delta M \cdot e^{(PLD/T_{1a})}}{SI_{PD} \cdot 2 \cdot \alpha_{pCASL} \cdot T_{1a} \cdot \alpha_{BSup} \cdot (1 - e^{-(\tau/T_{1a})})}$$

Where λ is the blood/brain partition coefficient in mL/g which was 0.9, ΔM is the signal intensity of the control image subtracted with the signal intensity of the label image, the post labelling delay was 1525

ms. T_{1a} is the longitudinal relaxation time of the blood was 1664 ms, SI_{PD} is the signal intensity of a proton density-weighted image and τ is the label duration which was 1650 ms. α_{pCASL} is the labelling efficiency, which was 0.85 and α_{BSup} was 0.83. A comparison of GM CBF was made between the patients and controls.

Diffusion tensor images analysis: ExploreDTI software^[47] was used for motion and distortion correction of the DTI images and for calculating the Fractional Anisotropy (FA) and Mean Diffusivity (MD) maps. FA and MD maps were used as an input to tract-based spatial statistics processing^[48], which was carried out in FSL. The FA maps were first linearly registered with an affine transformation, subsequently non-linearly registered to the MNI space, and a mean FA skeleton was created. For each subject, the FA map was projected on the skeleton. Following that, randomisation was used to perform *t*-test based voxel-wise comparison of the FA skeletons between patients and controls. The same procedure was repeated for MD maps.

Assessment of cognitive performance

Cognition: Several neuropsychological assessments were conducted in both healthy controls and CD patients and evaluated by an experienced clinical neuropsychologist (HM). The examination took approximately one hour and included validated test methods in a fixed order. Since the cognitive functioning of patients with IBD has not been fully previously investigated, the focus was on a wide range of neuropsychological functions. Global cognitive functioning was assessed by the Minimal Mental State Examination (MMSE). The MMSE contained 11 questions, subdivided into 5 subdomains. All questions were scored individually and added to produce a total score ranging from 0 to 30, with higher scores indicating better cognitive functioning^[49]. The memory domain was evaluated with the Digit Span Forward and Backward subtests of, respectively the revised Wechsler Adult Intelligence Scale (WAIS-R)^[50] and the revised Wechsler Memory Scale (WMS-R). Higher scores reflected better memory performance^[51].

Executive functioning was assessed by the Word Fluency Test (WFT)^[52], Stroop-Color-Word test (SCWT)^[53] given in three parts, and the Trail Making Test (TMT)^[54] subdivided into two parts, whereby part A measured attention and performance speed, and part B measured mental flexibility and ability to shift attention. The TMT involved scanning, visuomotor tracking, divided attention and cognitive flexibility. The time used for each trial was noted, with more time used indicating lower performance. The SCWT was used to measure interference sensibility. One response (reading the word) should be inhibited in order to name the colour of the ink, which leads to a delay in reaction time. The number of correct responses within 45 seconds was counted^[53]. Furthermore, the WAIS-R Digit symbol and Digit cancellation test was measured^[50].

Mental status: Cognitive performance depends on the psychiatric status of the patient^[55], and therefore the Hospital Anxiety Depression Scale (HADS) was included in the neuropsychological examination. The HADS was used to determine depressive symptoms and anxiety. HADS is a widely used measurement to identify emotional disorders in non-psychiatric patients. The scale includes 14 items, 7 items concerning anxiety and 7 concerning depression, each scored between 0 and 3. A score above 8 on each individual scale were considered as a possible case and a score above 10 as a probable case^[56].

QoL: To determine the QoL, the Short Form-36 (SF-36) was used. The SF-36 is a generic questionnaire to assess self-reported QoL. This measurement includes in total 8 subscales covering physical and mental aspects of QoL. The score ranges from 0 to 100, with higher score indicating better QoL. The Dutch translation of the SF-36 was validated in both the general population and in CD patients^[57].

Statistical analysis

Data analyses were performed using SPSS 20.0, IBM Corp, 2011, Armonk, NY, United States. Descriptive statistics were used for the patients' characteristics. All comparisons between the patient and control groups were performed with an independent *t*-test. A *P*-value ≤ 0.05 was considered statistically significant. To correct for multiple testing, the level of significance was set at $P < 0.01$ (0.05/5) and $P < 0.006$ (0.05/8) for the fatigue (five MFI subscores) and QoL (eight SF-36 subscales) scores, respectively. Based on the individual cognitive tests corrected for education, Z-scores of the different cognitive domains were created by using the UNIANOVA test with an average mean \pm SD. Correlations between the MRI outcomes, cognition and mood status were performed with the Pearson Correlation test.

RESULTS

Demographic characteristics

In this study, 20 CD patients and 17 healthy controls were age ($P = 0.46$) and gender matched ($P = 0.68$). All patients were in clinical remission at study inclusion (mean HBI = 2.16, SD = 1.12), with an average age of onset at 21.4 years and an IBD disease duration of 8.8 years. Based on the inclusion criteria, patients reported more fatigue complaints according to the MFI-20 ($P < 0.001$) and VAS fatigue score ($P < 0.001$) compared with the control subjects. Furthermore, the education level of the healthy controls was significantly higher than that of the CD patients. Since this variable might influence mental status scores, a correction was made. An overview of the clinical characteristics of the individuals is presented in Table 1.

MRI analysis

Volumetric data: The comparison of the subcortical volumes between the CD patients and controls in the analysis of the T₁-weighted images did not show significant differences between the two subject groups. The volume differences in the right amygdala ($P = 0.08$) and nucleus accumbens ($P = 0.08$) just missed significance (Table 2). VBM analysis showed a lower GM content in the superior frontal gyrus in CD patients compared with healthy controls ($P < 0.05$) (Figure 3).

MTI data: No significant differences were observed in the mean MTR values or in the MTR histogram peak heights of the CD patients compared with healthy controls.

MRS data: Lower glutamate + glutamine (Glx = Glu + Gln) concentrations (4.85 ± 0.78 mmol vs 5.96 ± 0.98 mmol, $P = 0.02$) and ratios to tCr (0.92 ± 0.13 vs 1.10 ± 0.14 , $P = 0.02$) were found in the patient population compared with control subjects (Table 3).

ASL data: Average GM CBF of the CD patients (53.1 ± 6.1 mL/100 g/min) was significantly higher than the GM CBF of the control group (47.6 ± 8.6 mL/100 g/min) ($P = 0.05$).

DTI data: No differences were observed across white matter in the FA and MD values between CD patients and controls.

Mental status

Neuropsychological examination and cognitive scores were corrected for educational level (Table 4). Generally, a difference close to significance between patients and controls was found in several individual cognitive test scores. Compared with controls, CD patients had a lower Stroop interference index ($P = 0.06$), a reduced total score of the WAIS-R Digit

Table 1 Demographic characteristics

	CD patients (n = 20)	Controls (n = 17)	P value
Age (yr) at inclusion, mean ± SD	30.1 ± 6.2	28.5 (6.7)	0.460
Female, n (%)	17 (85.0)	13 (76.5)	0.680
HBI score, mean ± SD ¹	2.2 ± 1.1	-	-
Age of IBD onset (yr), mean ± SD	21.4 ± 5.7	-	-
IBD disease duration (yr), mean ± SD	8.8 ± 7.2	-	-
Smoker, n (%)	11 (55.0)	4.0 (23.5)	0.400
VAS, mean ± SD	7.4 (1.3)	3.4 (2.3)	< 0.001
MFI, mean ± SD	66.1 (13.3)	36.4 (10.3)	< 0.001
General Fatigue	16.4 (2.8)	8.9 (3.3)	< 0.001
Physical Fatigue	14.4 (3.0)	6.2 (2.2)	< 0.001
Mental Fatigue	12.8 (4.1)	7.2 (2.9)	< 0.001
Reduced Activity	10.7 (3.5)	6.9 (2.7)	< 0.001
Reduced Motivation	12.0 (3.6)	7.1 (2.7)	< 0.001
Education level, n (%)			0.001
Low ^a	4 (20)	-	
Intermediate ^b	10 (50)	2 (11.8)	
High ^c	6 (30)	15 (88.2)	
Montreal classification			
Location CD, n (%)			
L1 ileal	3 (15.0)	-	-
L2 colonic	2 (10.0)	-	-
L3 ileocolonic	15 (75.0)	-	-
L4 upper	-	-	-
L1-3 + L4	-	-	-
Behaviour CD, n (%)			
B1 non-stricturing/penetrating	15 (75.0)	-	-
B2 stricturing	3 (15.0)	-	-
B3 penetrating	2 (10.0)	-	-
+ Perianal disease	3 (15.0)	-	-
Medication use, n (%)			
Immunosuppressive drugs (Aza/6MP)	12 (60.0)	-	-
None	8 (40.0)	-	-

¹HBI missing of 1 Crohn's disease (CD) patient. ^aLow: primary education (elementary school) and lower secondary education (preparatory secondary education); ^bIntermediate: higher secondary education (higher general continued education, pre-university secondary education) and postsecondary education (intermediate vocational education); ^cHigh: tertiary education (higher professional education, university). To correct for multiple testing, the level of significance was set at $P < 0.01$ for the MFI score. HBI: Harvey Bradshaw Index; VAS: Visual Analogue Scale; MFI: Multidimensional fatigue index.

Symbol test ($P = 0.06$) and were slower in completing trial A of the TMT test ($P = 0.08$). When the individual tests were transformed into a Z-score based on the different cognitive domains, significant reduced Z-scores of the memory domain ($P = 0.007$) and executive functioning domain ($P = 0.02$) were found in the patient population compared with the healthy controls (Table 5). CD patients experienced more depressive symptoms ($P < 0.001$), were more anxious ($P = 0.002$) and reported a significantly lower QoL.

Correlation of MRI findings with clinical characteristics and mental status

No correlations were found between mental status, including depression and anxiety, and MRI findings. Depressive symptoms were correlated with reduced scores of global cognitive functioning ($r = -0.5$, $P =$

Table 2 Group mean subcortical structure volumes as percentage of the total brain volume in Crohn's disease patients and controls

	CD patients (n = 20)	Controls (n = 17)	P value
Left Accumbens	0.04 ± 0.01	0.04 ± 0.01	0.56
Left Amygdala	0.09 ± 0.01	0.09 ± 0.01	0.61
Left Caudate	0.24 ± 0.02	0.24 ± 0.02	0.94
Left Hippocampus	0.27 ± 0.02	0.27 ± 0.03	0.94
Left Pallidus	0.13 ± 0.01	0.12 ± 0.01	0.32
Left Putamen	0.33 ± 0.02	0.32 ± 0.03	0.24
Left Thalamus	0.54 ± 0.02	0.54 ± 0.03	0.94
Right Accumbens	0.04 ± 0.00	0.03 ± 0.01	0.08
Right Amygdala	0.08 ± 0.01	0.09 ± 0.01	0.08
Right Caudate	0.25 ± 0.03	0.25 ± 0.02	0.76
Right Hippocampus	0.26 ± 0.02	0.27 ± 0.03	0.22
Right Pallidus	0.12 ± 0.01	0.13 ± 0.01	0.30
Right Putamen	0.31 ± 0.08	0.32 ± 0.02	0.56
Right Thalamus	0.53 ± 0.02	0.52 ± 0.03	0.48

Mean in % ± SD. CD: Crohn's disease.

Table 3 Mean metabolite ratio to total creatine

	CD patients (n = 9)	Controls (n = 9)	P value
Ratio Glu:tCr	0.76 ± 0.12	0.84 ± 0.10	0.19
Ratio Cho:tCr	0.29 ± 0.02	0.29 ± 0.04	0.81
Ratio Ins:tCr	0.66 ± 0.08	0.70 ± 0.10	0.38
Ratio NAA:tCr	1.31 ± 0.12	1.27 ± 0.09	0.44
Ratio NAA + NAAG:tCr	1.59 ± 0.18	1.56 ± 0.13	0.69
Ratio Glu + Gln:tCr	0.92 ± 0.13	1.10 ± 0.14	0.02

Mean metabolite ratio to tCr in mmol ± SD. CD: Crohn's disease; tCr: Total Creatine; Glu: Glutamate; Cho: Choline; Ins: Insulin; NAA: N-Acetyl Aspartate; NAAG: N-Acetyl Aspartate Glutamate; Gln: Glutamine.

0.003), memory ($r = -0.34$, $P = 0.04$) and executive functioning ($r = 0.35$, $P = 0.04$). Additionally, CD patients reported in the present study increased symptoms of anxiety and this was significantly correlated with reduced global cognitive functioning ($r = -0.36$, $P = 0.03$) and memory scores ($r = -0.32$, $P = 0.05$). No further correlations between cognitive scores, disease activity, disease duration and MRI findings were found in this study.

DISCUSSION

Several MRI techniques were used in this study in a cross-sectional manner to examine the differences in brain morphology, neurochemistry and perfusion between CD patients with fatigue and healthy controls without fatigue. The most important findings reported in this study are the significant differences in perfusion, neurochemistry and mental status (e.g., cognition, mood and QoL) between patients and controls. Lower levels of Glx concentration and their ratio to tCr were observed and an increased CBF was found in the patient population compared with control subjects. CD patients scored lower on several individual cognitive

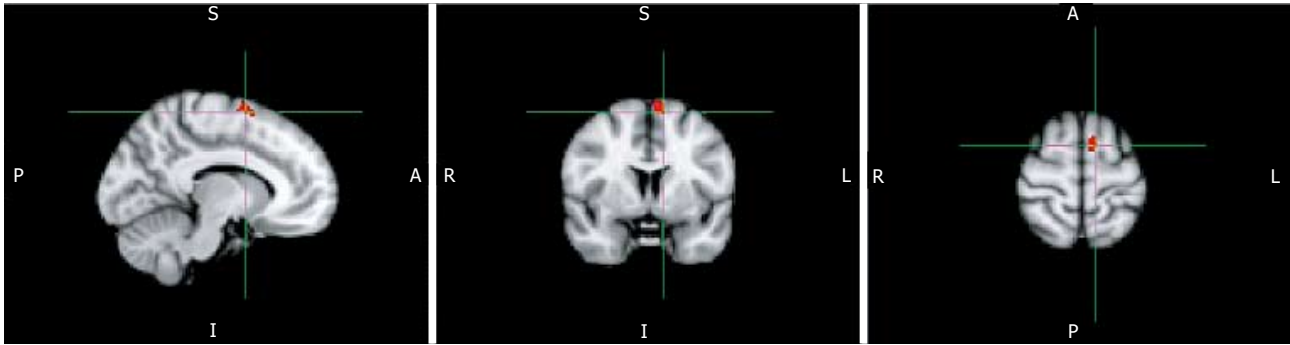


Figure 3 FMRIB Software Library voxel based morphometry analysis. Voxel based morphometry results shown on MNI152 standard space. The red colour indicates the voxels with significantly reduced grey matter volume in CD patients compared with healthy controls (with a P -value < 0.05 , corrected for multiple comparison). The red voxels correspond to the left superior frontal gyrus.

test scores, with a trend towards significance, and scored significantly lower on the memory and the executive functioning domain compared with the healthy controls. Also, the patient population had a significantly lower QoL and mood status.

The present study observed with MRS a significantly reduced Glx concentration as well as a lower ratio of Glx to tCr in the CD group. Glutamate is the predominant excitatory neurotransmitter in the brain and is involved in different brain functions including memory and mood status. Receptors are mainly present in the hippocampus^[58,59]. Glutamine is important in energy metabolism of the brain and previous studies reported that a reduced level of glutamine is associated with brain diseases such as Alzheimer^[60,61]. Increasing evidence shows that major depression disorder is associated with altered function of the major excitatory and inhibitory neurotransmitters such as glutamate and GABA^[62,63]. The present study did not find correlations between depressive symptoms and the reduced Glx concentration and ratio to tCr.

These reduced MRS results found in the present pilot study in CD patients are not in accordance with the findings of previous research performed in other inflammatory diseases such as RA and SLE^[11,64]. RA and SLE patients were shown to have increased choline and myo-inositol levels, indicating inflammation in the form of monocyte infiltration since this is a marker of cell membrane turnover^[65,66]. In addition, in SLE patients only decreased NAA signals were reported, indicating neuronal loss^[67-69], while an increased NAA ratio was found in our CD patient population. This contradiction may be due to the fact that RA and SLE are systemic inflammatory diseases, but not comparable with the systemic inflammation in IBD.

CBF values can reveal changes in tissue perfusion and are an indication for cerebral metabolism changes^[70]. In the present study, significant higher CBF values were found in the patient population. Increased CBF is thought to be a compensatory mechanism in response to ischemia or injury, which could be the case in the CD patients due to inflammation^[71,72]. Our findings are in line with the results of Wang *et al.*^[31] who

described in their cohort that SLE patients had higher CBF values compared with healthy controls.

The volumetric results in this study extend on earlier findings in IBD patients. The reduced GM content of the superior frontal gyrus demonstrated in this study is in agreement with results presented by Agostini *et al.*^[73]. The superior frontal gyrus is involved in self-awareness, and important in processing information^[74,75]. It has been suggested that the observed decrease in local GM volume could have many causes, including a decrease in cell size, neural or glial cell apoptosis or changes in blood flow^[72]. It is not clear whether this local volume reduction is directly linked to systemic inflammation, but it may represent the anatomical substrate for the development of cognitive and emotional disturbances^[73,76]. Similar significant positive correlations have been found between the GM volume in aging and measures of short-term memory^[77].

Besides MRI findings, neuropsychological findings were assessed in this study. Previously, no evidence has been obtained on the association of the intrinsic disease process and cognitive dysfunction in IBD patients. It is probable that concurrent mood disorders, in particular depression, affect the cognitive performance of IBD patients in memory and executive functioning tasks^[55]. This may be the case in the current cohort, since depressive symptoms were correlated with reduced neuropsychological scores in the three different domains: cognitive functioning, memory and executive functioning. However, Berrill *et al.*^[78] suggested that intellectual deficits existed in IBD patients compared to controls and remained significant after the correction for educational level and mood disorders.

Previous studies have shown a link between systemic inflammation and reduced brain volumes, possibly resulting in cognitive deficits. Zonis *et al.*^[79] suggested that chronic intestinal inflammation alters hippocampal neurogenesis and thus might underlie the behavioural manifestations in patients with IBD. In another study, SLE patients with cognitive deficits appeared to have reduced temporal lobe structures (hippocampus and amygdala) compared to SLE patients without cognitive

Table 4 Mental status

	CD patients (n = 20)	Controls (n = 17)	P value
Global cognitive functioning			
MMSE (total score), mean ± SD	28.9 (1.62)	29.65 (0.49)	0.87
Memory			
Verbal			
WMS memory quotient, mean ± SD ³	109.2 (10.5)	115.7 (8.7)	0.72
Non verbal			
WMS visual reproduction (total score), mean ± SD ³	11.6 (2.7)	12.9 (2.2)	0.75
WAIS-R Digit Span forward, mean ± SD	5.4 (1.0)	6.5 (1.3)	0.15
WAIS-R Digit Span backward, mean ± SD	4.5 (1.0)	5.2 (0.9)	0.15
Executive functioning			
WFT, mean ± SD ¹			
No. of good answers	42.7 (7.8)	48.2 (9.8)	0.29
No. of perseverative errors	0.28 (0.5)	0.47 (0.8)	0.58
Stroop Color-Word test, mean ± SD			
Stroop 1 time (s)	43.9 (7.2)	39.2 (8.6)	0.41
Stroop 1 No. of errors	0.2 (0.4)	0.1 (0.3)	0.98
Stroop 2 time (s)	56.6 (8.3)	53.8 (6.9)	0.62
Stroop 2 No. of errors	0.3 (0.8)	0 (0.0)	0.21
Stroop 3 time (s)	88.3 (14.7)	76.6 (8.8)	0.22
Stroop 3 No. of errors	0.6 (1.5)	0.13 (0.3)	0.20
Stroop interference index	50.1 (7.8)	56.1 (5.5)	0.06
TMT, mean ± SD			
Part A time (s)	30.3 (11.8)	22.1 (8.5)	0.08
Part A no. of errors	0.1 (0.2)	0.1 (0.2)	0.56
Part B time (s)	61.8 (29.2)	50.1 (17.4)	0.72
Part B no. of errors	0.1 (0.2)	0.2 (0.5)	0.41
WAIS-R Digit Symbol, mean ± SD ²			
Total score	59.7 (8.4)	71.0 (6.2)	0.06
No. of errors	0 (0.0)	0.1 (0.3)	0.63
Digit cancellation test, mean ± SD ⁴			
Total score	436.2 (88.1)	498.6 (82.9)	0.16
No. of good answers (%)	57.3 (29.5)	78.9 (20.9)	0.16
HADS, mean ± SD	13.1 (7.3)	4.8 (2.9)	< 0.001
Anxiety	7.5 (3.8)	3.7 (2.7)	0.002
Depression	6.1 (4.0)	0.9 (1.1)	< 0.001
SF-36			
Physical functioning	72.9 ± 20.2	96.6 ± 3.4	< 0.001
Social functioning	52.0 ± 29.0	90.7 ± 9.8	< 0.001
Role physical problem	71.3 ± 37.4	2.9 ± 8.3	< 0.001
Role emotional problem	40.4 ± 46.1	2.0 ± 8.1	0.002
Bodily pain	35.3 ± 20.7	5.6 ± 12.6	< 0.001
General health perception	65.8 ± 18.8	82.0 ± 15.2	< 0.001
Mental health	63.6 ± 16.0	80.4 ± 10.9	0.001
Vitality	30.1 ± 18.3	72.9 ± 14.3	< 0.001

¹Missing in 2 CD patients; ²Missing 1 CD patient and 1 healthy control; ³Missing in 2 healthy controls; ⁴Missing in 5 patients and 3 healthy controls. To correct for multiple testing, the level of significance was set at $P < 0.006$ for the SF-36 score. MMSE: Mini Mental State Examination; WMS: Wechsler Memory Scale; WAIS-R: Wechsler Adult Intelligence Scale-Revised; WFT: Word Fluency Test; TMT: Trial Making test; HADS: Hospital Anxiety Depression Scale; CD: Crohn's disease.

deficits^[80]. In the present study, we did not find these correlations.

Some limitations of this study need to be revealed. Although this study is an exploratory study, the population size was limited. In this pilot study we have compared the most extreme cases; quiescent CD patients with fatigue vs healthy controls without fatigue. In this

Table 5 Z-scores of the different domains of cognitive functioning

	CD patients (n = 20)	Controls (n = 17)	P value
Global cognitive functioning ¹ , mean ± SD	28.9 (1.6)	29.7 (0.5)	0.870
Memory ² , mean ± SD	1.1 (2.9)	1.3 (2.3)	0.007
Executive functioning ³ , mean ± SD	2.5 (7.7)	2.9 (4.2)	0.020

¹The global cognitive functioning domain includes the Minimal Mental State Examination; ²The memory domain includes the Wechsler Adult Intelligence Scale and the revised Wechsler Memory Scale; ³The executive functioning domain includes the Word Fluency Test, Stroop-Color-Word test and Trail Making Test. CD: Crohn's disease.

design, we have found significant differences between the groups and now further research is required. In addition, the significant difference in the fatigue score between patients and controls is not a finding of the study, but part of the design. As a consequence, it cannot be definitely concluded whether the differences in MRI measures are caused by CD per se or represent only patients with combined CD and fatigue. However, fatigue is a subjective measurement and was evaluated as such. It is hard to draw major conclusions from these questionnaires, since some healthy controls reported a high fatigue score as well due to other circumstances than IBD. In some MRI analyses, subjects got excluded due to the quality of the data. MRS data with high Cramer-Rao lower bounds, suggesting unreliable metabolite quantification, were excluded from data analysis. This could have been influenced by the patients' motion or bad shimming.

In conclusion, our findings support the hypothesis that systemic inflammation influences the brain and affects cognitive functioning and mood. This is a first step in the gathering of data and understanding of brain involvement in CD patients. This study implies that for a health professional, it is important to focus in CD patients not only on symptoms related to the gastrointestinal tract, but also on the effects of inflammation on the brain. Understanding these affects in CD patients may help health professionals to set up interventions to maintain CD remission by the use of medication and to improve mood status and QoL by e.g., psychosocial interventions.

COMMENTS

Background

Both active and quiescent Crohn's disease (CD) is a chronic inflammatory status in which levels of circulating inflammatory cytokines, such as tumour necrosis factor- α are reported in the body. These cytokines may play a role in driving inflammation in the brain by activating microglia and the recruitment of monocytes.

Research frontiers

Metabolic and cerebral perfusion changes have been found in the brain of patients with other systemic diseases including rheumatoid arthritis, systemic

sclerosis and systemic lupus erythematosus. In addition, previous studies found an association with fatigue and metabolic brain changes. Thus it is of interest, whether systemic inflammation and fatigue complaints influence the brain in CD patients as well.

Innovations and breakthroughs

The present data support the hypothesis that systemic inflammation influences the brain and affects cognitive functioning and mood status in quiescent CD patients with fatigue. This is a first step in understanding brain involvement in CD patients.

Applications

This study implies that for a health professional, it is important to focus in CD patients also on the effects of inflammation on the brain. Understanding these effects in CD patients may help health professionals to set up interventions to maintain CD remission by the use of medication and to improve mood status and QoL by e.g., psychosocial interventions.

Peer-review

Since this is an exploratory study, the authors have compared the most extreme cases; quiescent CD patients with fatigue vs healthy controls without fatigue. In this design, the authors have found significant differences between the groups and now further research is required.

REFERENCES

- Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]
- Salem M, Ammitzboell M, Nys K, Seidelin JB, Nielsen OH. ATG16L1: A multifunctional susceptibility factor in Crohn disease. *Autophagy* 2015; **11**: 585-594 [PMID: 25906181 DOI: 10.1080/15548627.2015.1017187]
- Jelsness-Jørgensen LP, Bernklev T, Henriksen M, Torp R, Moum BA. Chronic fatigue is more prevalent in patients with inflammatory bowel disease than in healthy controls. *Inflamm Bowel Dis* 2011; **17**: 1564-1572 [PMID: 21674713 DOI: 10.1002/ibd.21530]
- Romberg-Camps MJ, Bol Y, Dagnelie PC, Hesselink-van de Kruijs MA, Kester AD, Engels LG, van Deursen C, Hameeteman WH, Pierik M, Wolters F, Russel MG, Stockbrügger RW. Fatigue and health-related quality of life in inflammatory bowel disease: results from a population-based study in the Netherlands: the IBD-South Limburg cohort. *Inflamm Bowel Dis* 2010; **16**: 2137-2147 [PMID: 20848468 DOI: 10.1002/ibd.21285]
- Høivik ML, Bernklev T, Solberg IC, Cvancarova M, Lygren I, Jahnsen J, Moum B. Patients with Crohn's disease experience reduced general health and vitality in the chronic stage: ten-year results from the IBSEN study. *J Crohns Colitis* 2012; **6**: 441-453 [PMID: 22398064 DOI: 10.1016/j.crohns.2011.10.001]
- Chen ML, Sundrud MS. Cytokine Networks and T-Cell Subsets in Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 1157-1167 [PMID: 26863267 DOI: 10.1097/MIB.00000000000000714]
- Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. *Front Immunol* 2015; **6**: 551 [PMID: 26579126 DOI: 10.3389/fimmu.2015.00551]
- Nadeau S, Rivest S. Effects of circulating tumor necrosis factor on the neuronal activity and expression of the genes encoding the tumor necrosis factor receptors (p55 and p75) in the rat brain: a view from the blood-brain barrier. *Neuroscience* 1999; **93**: 1449-1464 [PMID: 10501470]
- Hagel AF, de Rossi T, Konturek PC, Albrecht H, Walker S, Hahn EG, Raithel M. Plasma histamine and tumour necrosis factor-alpha levels in Crohn's disease and ulcerative colitis at various stages of disease. *J Physiol Pharmacol* 2015; **66**: 549-556 [PMID: 26348079]
- Kader HA, Tehernev VT, Satyaraj E, Lejnine S, Kotler G, Kingsmore SF, Patel DD. Protein microarray analysis of disease activity in pediatric inflammatory bowel disease demonstrates elevated serum PLGF, IL-7, TGF-beta1, and IL-12p40 levels in Crohn's disease and ulcerative colitis patients in remission versus active disease. *Am J Gastroenterol* 2005; **100**: 414-423 [PMID: 15667502]
- Emmer BJ, van der Bijl AE, Huizinga TW, Breedveld FC, Steens SC, Th Bosma GP, van Buchem MA, van der Grond J. Brain involvement in rheumatoid arthritis: a magnetic resonance spectroscopy study. *Arthritis Rheum* 2009; **60**: 3190-3195 [PMID: 19877035 DOI: 10.1002/art.24932]
- O'Callaghan JP, Sriram K, Miller DB. Defining "neuroinflammation". *Ann N Y Acad Sci* 2008; **1139**: 318-330 [PMID: 18991877 DOI: 10.1196/annals.1432.032]
- D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. *J Neurosci* 2009; **29**: 2089-2102 [PMID: 19228962 DOI: 10.1523/JNEUROSCI.3567-08.2009]
- Hollingsworth W, Todd CJ, Bell MI, Arafat Q, Girling S, Karia KR, Dixon AK. The diagnostic and therapeutic impact of MRI: an observational multi-centre study. *Clin Radiol* 2000; **55**: 825-831 [PMID: 11069736 DOI: 10.1053/crad.2000.0546]
- Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp* 2002; **17**: 143-155 [PMID: 12391568 DOI: 10.1002/hbm.10062]
- Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 2001; **14**: 21-36 [PMID: 11525331 DOI: 10.1006/nimg.2001.0786]
- Grossman RI, Gomori JM, Ramer KN, Lexa FJ, Schnall MD. Magnetization transfer: theory and clinical applications in neuroradiology. *Radiographics* 1994; **14**: 279-290 [PMID: 8190954 DOI: 10.1148/radiographics.14.2.8190954]
- Rosen Y, Lenkinski RE. Recent advances in magnetic resonance neurospectroscopy. *Neurotherapeutics* 2007; **4**: 330-345 [PMID: 17599700 DOI: 10.1016/j.nurt.2007.04.009]
- Detre JA, Leigh JS, Williams DS, Koretsky AP. Perfusion imaging. *Magn Reson Med* 1992; **23**: 37-45 [PMID: 1734182]
- Schaefer PW, Grant PE, Gonzalez RG. Diffusion-weighted MR imaging of the brain. *Radiology* 2000; **217**: 331-345 [PMID: 11058626 DOI: 10.1148/radiology.217.2.r00nv24331]
- Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. *Ann Neurol* 2002; **52**: 168-174 [PMID: 12210786 DOI: 10.1002/ana.10265]
- Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, Culliford D, Perry VH. Systemic inflammation and disease progression in Alzheimer disease. *Neurology* 2009; **73**: 768-774 [PMID: 19738171 DOI: 10.1212/WNL.0b013e3181b6bb95]
- Moreno B, Jukes JP, Vergara-Irigaray N, Errea O, Villoslada P, Perry VH, Newman TA. Systemic inflammation induces axon injury during brain inflammation. *Ann Neurol* 2011; **70**: 932-942 [PMID: 22190366 DOI: 10.1002/ana.22550]
- Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol* 2015; **21**: 10609-10620 [PMID: 26457021 DOI: 10.3748/wjg.v21.i37.10609]
- Hamed SA, Selim ZI, Elattar AM, Elserogy YM, Ahmed EA, Mohamed HO. Assessment of biomarkers for brain involvement in female patients with rheumatoid arthritis. *Clin Rheumatol* 2012; **31**: 123-132 [PMID: 21695659 DOI: 10.1007/s10067-011-1795-1]
- Soares DP, Law M. Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. *Clin Radiol* 2009; **64**: 12-21 [PMID: 19070693 DOI: 10.1016/j.crad.2008.07.002]
- Cutolo M, Nobili F, Sulli A, Pizzorni C, Briata M, Faelli F, Vitali P, Mariani G, Copello F, Serio B, Barone C, Rodriguez G. Evidence of cerebral hypoperfusion in scleroderma patients. *Rheumatology (Oxford)* 2000; **39**: 1366-1373 [PMID: 11136880]
- Emmer BJ, Steens SC, Steup-Beekman GM, van der Grond J, Admiraal-Behloul F, Olofsen H, Bosma GP, Ouwendijk WJ, Huizinga TW, van Buchem MA. Detection of change in CNS involvement in neuropsychiatric SLE: a magnetization transfer study. *J Magn Reson Imaging* 2006; **24**: 812-816 [PMID:

- 16941632 DOI: 10.1002/jmri.20706]
- 29 **Luyendijk J**, Steens SC, Ouwendijk WJ, Steup-Beekman GM, Bollen EL, van der Grond J, Huizinga TW, Emmer BJ, van Buchem MA. Neuropsychiatric systemic lupus erythematosus: lessons learned from magnetic resonance imaging. *Arthritis Rheum* 2011; **63**: 722-732 [PMID: 21360502 DOI: 10.1002/art.30157]
 - 30 **Emmer BJ**, Veer IM, Steup-Beekman GM, Huizinga TW, van der Grond J, van Buchem MA. Tract-based spatial statistics on diffusion tensor imaging in systemic lupus erythematosus reveals localized involvement of white matter tracts. *Arthritis Rheum* 2010; **62**: 3716-3721 [PMID: 20722009 DOI: 10.1002/art.27717]
 - 31 **Wang PI**, Cagnoli PC, McCune WJ, Schmidt-Wilcke T, Lowe SE, Graft CC, Gebarski SS, Chenevert TL, Khalatbari S, Myles JD, Watcharotone K, Cronin P, Sundgren PC. Perfusion-weighted MR imaging in cerebral lupus erythematosus. *Acad Radiol* 2012; **19**: 965-970 [PMID: 22608862 DOI: 10.1016/j.acra.2012.03.023]
 - 32 **Puri BK**, Counsell SJ, Zaman R, Main J, Collins AG, Hajnal JV, Davey NJ. Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Acta Psychiatr Scand* 2002; **106**: 224-226 [PMID: 12197861]
 - 33 **Chaudhuri A**, Condon BR, Gow JW, Brennan D, Hadley DM. Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *Neuroreport* 2003; **14**: 225-228 [PMID: 12598734 DOI: 10.1097/01.wnr.0000054960.21656.64]
 - 34 **Puri BK**, Holmes J, Hamilton G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *Int J Clin Pract* 2004; **58**: 297-299 [PMID: 15117099]
 - 35 **Sandu AL**, Staff RT, McNeil CJ, Mustafa N, Ahearn T, Whalley LJ, Murray AD. Structural brain complexity and cognitive decline in late life—a longitudinal study in the Aberdeen 1936 Birth Cohort. *Neuroimage* 2014; **100**: 558-563 [PMID: 24993896 DOI: 10.1016/j.neuroimage.2014.06.054]
 - 36 **Harvey RF**, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet* 1980; **1**: 514 [PMID: 6102236]
 - 37 **Smets EM**, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res* 1995; **39**: 315-325 [PMID: 7636775]
 - 38 **Chalder T**, Berelowitz G, Pawlikowska T, Watts L, Wessely S, Wright D, Wallace EP. Development of a fatigue scale. *J Psychosom Res* 1993; **37**: 147-153 [PMID: 8463991]
 - 39 **Zhang Y**, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging* 2001; **20**: 45-57 [PMID: 11293691 DOI: 10.1109/42.906424]
 - 40 **Patenaude B**, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* 2011; **56**: 907-922 [PMID: 21352927 DOI: 10.1016/j.neuroimage.2011.02.046]
 - 41 **Smith SM**, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004; **23** Suppl 1: S208-S219 [PMID: 15501092 DOI: 10.1016/j.neuroimage.2004.07.051]
 - 42 **Jenkinson M**, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal* 2001; **5**: 143-156 [PMID: 11516708]
 - 43 **Provencher SW**. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993; **30**: 672-679 [PMID: 8139448]
 - 44 **Choi JK**, Dedeoglu A, Jenkins BG. Application of MRS to mouse models of neurodegenerative illness. *NMR Biomed* 2007; **20**: 216-237 [PMID: 17451183 DOI: 10.1002/nbm.1145]
 - 45 **Alsop DC**, Detre JA, Golay X, Günther M, Hendrikse J, Hernandez-Garcia L, Lu H, MacIntosh BJ, Parkes LM, Smits M, van Osch MJ, Wang DJ, Wong EC, Zaharchuk G. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med* 2015; **73**: 102-116 [PMID: 24715426 DOI: 10.1002/mrm.25197]
 - 46 **Jenkinson M**, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 2002; **17**: 825-841 [PMID: 12377157]
 - 47 **Leemans A**, Jeurissen B, Sijbers J, Jones DK. ExploreDTI: A graphical toolbox for processing, analyzing, and visualizing diffusion MR data. *Annual Meeting of Proc.intl.soc.mag.reson.med* 2009: 3537
 - 48 **Smith SM**, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TE. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 2006; **31**: 1487-1505 [PMID: 16624579 DOI: 10.1016/j.neuroimage.2006.02.024]
 - 49 **Folstein MF**, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189-198 [PMID: 1202204]
 - 50 **Wechsler D**. Wechsler Adult Intelligence Scale, Revised. The Psychological Corporation: 1981
 - 51 **Wechsler D**. Wechsler Memory Scale, Revised. The Psychological Corporation: 1987
 - 52 **Pendleton MG**, Heaton RK, Lehman RA, Hulihan D. Diagnostic utility of the Thurstone Word Fluency Test in neuropsychological evaluations. *J Clin Neuropsychol* 1982; **4**: 307-317 [PMID: 7174838]
 - 53 **Golden CJ**. Identification of brain disorders by the Stroop Color and Word Test. *J Clin Psychol* 1976; **32**: 654-658 [PMID: 956433]
 - 54 **Reitan RM**, Wolfson D. The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation. 2nd ed. Neuropsychology Press 1993
 - 55 **Castaneda AE**, Tuulio-Henriksson A, Aronen ET, Marttunen M, Kolho KL. Cognitive functioning and depressive symptoms in adolescents with inflammatory bowel disease. *World J Gastroenterol* 2013; **19**: 1611-1617 [PMID: 23538788 DOI: 10.3748/wjg.v19.i10.1611]
 - 56 **Brennan C**, Worrall-Davies A, McMillan D, Gilbody S, House A. The Hospital Anxiety and Depression Scale: a diagnostic meta-analysis of case-finding ability. *J Psychosom Res* 2010; **69**: 371-378 [PMID: 20846538 DOI: 10.1016/j.jpsychores.2010.04.006]
 - 57 **Ware JE**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: 1593914]
 - 58 **Riedel G**, Platt B, Micheau J. Glutamate receptor function in learning and memory. *Behav Brain Res* 2003; **140**: 1-47 [PMID: 12644276]
 - 59 **Bianchin M**, Da Silva RC, Schmitz PK, Medina JH, Izquierdo I. Memory of inhibitory avoidance in the rat is regulated by glutamate metabotropic receptors in the hippocampus. *Behav Pharmacol* 1994; **5**: 356-359 [PMID: 11224286]
 - 60 **Antuono PG**, Jones JL, Wang Y, Li SJ. Decreased glutamate + glutamine in Alzheimer's disease detected in vivo with (1)H-MRS at 0.5 T. *Neurology* 2001; **56**: 737-742 [PMID: 11274307]
 - 61 **Gunnarsen D**, Haley B. Detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer diseased patients: a potential diagnostic biochemical marker. *Proc Natl Acad Sci USA* 1992; **89**: 11949-11953 [PMID: 1361232]
 - 62 **Hasler G**, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2007; **64**: 193-200 [PMID: 17283286]
 - 63 **Bernstein HG**, Meyer-Lotz G, Dobrowolny H, Bannier J, Steiner J, Walter M, Bogerts B. Reduced density of glutamine synthetase immunoreactive astrocytes in different cortical areas in major depression but not in bipolar I disorder. *Front Cell Neurosci* 2015; **9**: 273 [PMID: 26321908 DOI: 10.3389/fncel.2015.00273]
 - 64 **Brooks WM**, Jung RE, Ford CC, Greinel EJ, Sibbitt WL. Relationship between neurometabolite derangement and neurocog-

- nitive dysfunction in systemic lupus erythematosus. *J Rheumatol* 1999; **26**: 81-85 [PMID: 9918245]
- 65 **Brenner RE**, Munro PM, Williams SC, Bell JD, Barker GJ, Hawkins CP, Landon DN, McDonald WI. The proton NMR spectrum in acute EAE: the significance of the change in the Cho: Cr ratio. *Magn Reson Med* 1993; **29**: 737-745 [PMID: 8350716]
- 66 **Bitsch A**, Bruhn H, Vougioukas V, Stringaris A, Lassmann H, Frahm J, Brück W. Inflammatory CNS demyelination: histopathologic correlation with in vivo quantitative proton MR spectroscopy. *AJNR Am J Neuroradiol* 1999; **20**: 1619-1627 [PMID: 10543631]
- 67 **Bjartmar C**, Kidd G, Mörk S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* 2000; **48**: 893-901 [PMID: 11117546]
- 68 **Demougeot C**, Garnier P, Mossiat C, Bertrand N, Giroud M, Beley A, Marie C. N-Acetylaspartate, a marker of both cellular dysfunction and neuronal loss: its relevance to studies of acute brain injury. *J Neurochem* 2001; **77**: 408-415 [PMID: 11299303]
- 69 **Adalsteinsson E**, Sullivan EV, Kleinhans N, Spielman DM, Pfefferbaum A. Longitudinal decline of the neuronal marker N-acetyl aspartate in Alzheimer's disease. *Lancet* 2000; **355**: 1696-1697 [PMID: 10905250]
- 70 **Wang Z**, Das SR, Xie SX, Arnold SE, Detre JA, Wolk DA. Arterial spin labeled MRI in prodromal Alzheimer's disease: A multi-site study. *Neuroimage Clin* 2013; **2**: 630-636 [PMID: 24179814 DOI: 10.1016/j.nicl.2013.04.014]
- 71 **Smith M**. Perioperative uses of transcranial perfusion monitoring. *Neurosurg Clin N Am* 2008; **19**: 489-502, vii [PMID: 18790384 DOI: 10.1016/j.nec.2008.07.008]
- 72 **Alsop DC**, Casement M, de Bazelaire C, Fong T, Press DZ. Hippocampal hyperperfusion in Alzheimer's disease. *Neuroimage* 2008; **42**: 1267-1274 [PMID: 18602481 DOI: 10.1016/j.neuroimage.2008.06.006]
- 73 **Agostini A**, Benuzzi F, Filippini N, Bertani A, Scarcelli A, Farinelli V, Marchetta C, Calabrese C, Rizzello F, Gionchetti P, Ercolani M, Campieri M, Nichelli P. New insights into the brain involvement in patients with Crohn's disease: a voxel-based morphometry study. *Neurogastroenterol Motil* 2013; **25**: 147-e82 [PMID: 22998431 DOI: 10.1111/nmo.12017]
- 74 **Goldberg II**, Harel M, Malach R. When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron* 2006; **50**: 329-339 [PMID: 16630842 DOI: 10.1016/j.neuron.2006.03.015]
- 75 **Miller AK**, Alston RL, Corsellis JA. Variation with age in the volumes of grey and white matter in the cerebral hemispheres of man: measurements with an image analyser. *Neuropathol Appl Neurobiol* 1980; **6**: 119-132 [PMID: 7374914]
- 76 **Zikou AK**, Kosmidou M, Astrakas LG, Tzarouchi LC, Tsianos E, Argyropoulou MI. Brain involvement in patients with inflammatory bowel disease: a voxel-based morphometry and diffusion tensor imaging study. *Eur Radiol* 2014; **24**: 2499-2506 [PMID: 25001084 DOI: 10.1007/s00330-014-3242-6]
- 77 **Taki Y**, Kinomura S, Sato K, Goto R, Wu K, Kawashima R, Fukuda H. Correlation between gray/white matter volume and cognition in healthy elderly people. *Brain Cogn* 2011; **75**: 170-176 [PMID: 21131121 DOI: 10.1016/j.bandc.2010.11.008]
- 78 **Berrill JW**, Gallacher J, Hood K, Green JT, Matthews SB, Campbell AK, Smith A. An observational study of cognitive function in patients with irritable bowel syndrome and inflammatory bowel disease. *Neurogastroenterol Motil* 2013; **25**: 918-e704 [PMID: 23981191 DOI: 10.1111/nmo.12219]
- 79 **Zonis S**, Pechnick RN, Ljubimov VA, Mahgerefteh M, Wawrowsky K, Michelsen KS, Chesnokova V. Chronic intestinal inflammation alters hippocampal neurogenesis. *J Neuroinflammation* 2015; **12**: 65 [PMID: 25889852 DOI: 10.1186/s12974-015-0281-0]
- 80 **Zimmermann N**, Corrêa DG, Kubo TA, Netto TM, Pereira DB, Fonseca RP, Gasparetto EL. Global Cognitive Impairment in Systemic Lupus Erythematosus Patients: A Structural MRI Study. *Clin Neuroradiol* 2015; Epub ahead of print [PMID: 25967601 DOI: 10.1007/s00062-015-0397-8]

P-Reviewer: Chow J, Koch TR, Lakatos PL, Prakash N

S-Editor: Qi Y **L-Editor:** A **E-Editor:** Liu WX





Published by Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>



ISSN 1007-9327

