

# Chapter 5

## Brain Involvement in Rheumatoid Arthritis: a Magnetic Resonance Spectroscopy Study

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## ABSTRACT

### Background

Tumor necrosis factor  $\alpha$  was recently implicated as an important mediator of communication between the peripheral and cerebral immune systems in an animal model of chronic inflammation. The purpose of this study was to examine by proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) the influence of inflammation on cerebral metabolism in patients with rheumatoid arthritis (RA).

### Methods

Single-voxel  $^1\text{H}$ -MRS of the centrum semiovale was performed on 35 RA patients (6 men and 29 women; mean  $\pm$  SD age  $51.8 \pm 14.6$  years) and 28 healthy age- and sex-matched control subjects (9 men and 19 women; mean  $\pm$  SD age  $50.2 \pm 10.4$  years). None of the study subjects had any neurologic signs or symptoms. Clinical markers of disease activity were correlated with the  $^1\text{H}$ -MRS findings.

### Results

Patients with active RA, as reflected by an elevated erythrocyte sedimentation rate (ESR), had a significantly higher ratio of choline to creatine and a significantly lower ratio of *N*-acetylaspartate to choline than did patients with inactive RA, as reflected by a normal ESR. Moreover, the ratios of choline to creatine and NAA to choline were significantly correlated with the ESR after correction for age, sex, smoking status, handedness, alcohol consumption, medication use, and disease duration. Medication use had no additional effect on these associations.

### Conclusion

Our data show that systemic inflammation in RA is associated with metabolic changes in the brain.

## INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic inflammatory autoimmune disease without a pervasive direct autoimmune attack on the central nervous system (CNS), as has been implicated in multiple sclerosis and to a lesser degree in systemic lupus erythematosus (SLE). Nonetheless, nonspecific neurologic symptoms, such as mood disorders, sleep disturbances, and fatigue, play an important role in RA patients' perception of their disease. (1) RA is predominantly characterized by synovial inflammation and increased levels of circulating proinflammatory cytokines, in particular, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ). (2) In a recent study of an animal model of systemic inflammation, high systemic levels of TNF $\alpha$  were shown to attract monocytes across the blood-brain barrier by activation of microglia. This was subsequently associated with behavioral changes (i.e., sickness behavior). (3)

Until recently, the influence of systemic inflammation on the CNS was unclear. Local inflammation is known to play a role in numerous neurologic conditions and processes, such as stroke/ischemia, trauma, epilepsy, multiple sclerosis, Alzheimer's disease, and Parkinson's disease. (4) In contrast, systemic inflammation, characterized by increased circulating levels of proinflammatory cytokines, has been implicated in cognitive decline and dementia (i.e., Alzheimer's disease) in more than 1 study. (5-8) In their analysis of an *in vivo* animal model of autoimmune inflammation of the CNS, Brenner et al (9) found that choline, a marker of cell membrane turnover, was associated with monocyte infiltration in the brain.

We hypothesized that elevated levels of choline are present in patients with RA, depending on the level of disease activity. We used proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) of the periventricular normal-appearing white matter to compare cerebral metabolism in RA patients with that in healthy controls and to study the influence of RA disease activity on the brain.

## METHODS

### Patients

RA patients who were included in earlier studies performed at our institution (10,11) were analyzed retrospectively for the present study. Both the scientific and the ethical review boards of our institution approved this study. All patients gave their written informed consent.

Thirty-five RA patients (6 men and 29 women) treated at the Outpatient Clinic of the Department of Rheumatology underwent  $^1\text{H}$ -MRS and magnetic resonance imaging (MRI) to examine the effects of medications given for RA. The mean  $\pm$  SD age of the RA

patients was  $51.8 \pm 14.6$  years. All patients were diagnosed independently as having RA by an experienced rheumatologist (AEvdB or TWJH), based on the 1987 criteria of the American College of Rheumatology (formerly, the American Rheumatism Association). (12) Patients with neurologic involvement were excluded from the study. None of the RA patients demonstrated brain abnormalities on MRI. The characteristics of the study patients are shown in Table 1.

**Table 1.** \* RA = rheumatoid arthritis; DAS28 = Disease Activity Score 28-joint assessment; DMARDs = disease-modifying antirheumatic drugs; ESR = erythrocyte sedimentation rate; RF = rheumatoid factor.

Characteristics of the 35 RA patients*	
Age, mean $\pm$ SD years	51.8 $\pm$ 14.6
Disease duration, mean $\pm$ SD years	7.7 $\pm$ 9.0
Weekly alcohol consumption, mean $\pm$ SD units	4.1 $\pm$ 6.8
DAS28, mean $\pm$ SD	4.4 $\pm$ 1.1
No. of DMARDs taken, mean $\pm$ SD	2.8 $\pm$ 1.7
ESR, mean $\pm$ SD mm/hour	23.8 $\pm$ 18.3
% with elevated ESR <sup>†</sup>	43
% female	79
% smokers	20
% left handed	8.6
% taking prednisone	71
% RF positive	74
% with erosive joint disease	71

<sup>†</sup>According to the cut-off values of our institution (11)

The median number of disease-modifying antirheumatic drugs (DMARDs) taken at the time of the study and previously taken was 3 (range 1-6). DMARD treatment at the time of the study was as follows: infliximab in 1 patient, methotrexate in 22, sulfasalazine in 8, leflunomide in 4, aurothiomalate in 1, azathioprine in 1, and antimalarial drugs in 4. Thirty-four patients were taking DMARDs; 26 of them took a combination of DMARDs.

A total of 28 age- and sex-matched control subjects (9 men and 19 women) also underwent <sup>1</sup>H-MRS. The mean  $\pm$  SD age of the control subjects was  $50.2 \pm 10.4$  years. Control subjects with a history of neurologic involvement or with any brain abnormalities seen on conventional MRI were excluded.

#### Markers of Inflammation and disease activity

The erythrocyte sedimentation rate (ESR) was determined upon collection of blood samples by the hematology laboratory at our institution. Active and inactive RA was distinguished according to the previously described reference ESR values at our institution. (13) The following ESR values were used as the normal range, based on age and sex: for patients 13-51 years old, 0-15 mm/hour in males and 0-25 mm/hour in females; for patients 51-66 years old, 0-20 mm/hour in males and 0-33 mm/hour in females; and for

patients  $\geq 66$  years old, 0-40 mm/hour in males and females. Patients with an ESR that was lower than or equal to the upper limit of normal were classified as having inactive disease ( $n = 20$ ), and patients with an ESR that was higher than the upper limit of normal were classified as having active disease ( $n = 15$ ).

In a subset of patients, the Disease Activity Score 28-joint assessment (DAS28) was calculated as described previously (14) in order to incorporate disease activity markers other than hematologic ones in the analysis. DAS28 scores are composed of 4 components: the visual analog scale score, as a measure of subjective complaints of disease activity such as fatigue, pain, and morning stiffness; the number of tender joints (of 28 assessed) at the time of physical examination; the number of swollen joints (of 28 assessed) at the time of physical examination; and the ESR, as a marker of systemic inflammation. In RA patients, scores on the DAS28 typically range between 2 and 6. Average values for the ESR, the DAS28, and the percentage of patients with an elevated ESR are shown in Table 1.

#### **MRI and 1H-MRS parameters**

All study subjects underwent MRI of the brain, using a scanner with an operating field strength of 1.5T (Philips Medical Systems, Best, The Netherlands). For the T2-weighted sequences, the parameters were echo time (TE) 120 msec, repetition time (TR) 2,500 msec, slice thickness 6 mm, interslice gap 0.6 mm, number of slices 22. For the fluid-attenuated inversion recovery (FLAIR) sequences, the parameters were TE 120 msec, TR 8,000 msec, inversion recovery delay 2,000 msec, slice thickness 6 mm, interslice gap 0.6 mm, number of slices 22.

Single-voxel point-resolved spectroscopy (PRESS)  $^1\text{H}$ -MRS was performed with a double spin-echo sequence. The volume of interest was selected in the left centrum semiovale of each study subject. Grey matter was excluded to ensure homogeneity of the selected tissue and comparability of the selected voxel between different subjects. The dimensions of the selected volumes of interest were typically 40 mm in the anteroposterior direction, 15 mm in the left-right direction, and 10 mm in the caudocranial direction. Special care was taken to exclude gray matter and cerebrospinal fluid, and the dimensions were adapted to the subject's head size, if needed. Measurement parameters were as follows: TR 2,000 msec, TE 136 msec, 2,048 time-domain data points, spectral width 2,000 Hz, and number of signals acquired 128. After zero-filling to 4,096 data points, exponential multiplication of 2 Hz, Fourier transformation, and linear baseline correction, *N*-acetylaspartate (NAA) (referenced at 2.0 parts per million), the integrated area under the curve of choline peaks, and the total creatine peaks were quantified using integration software routines, which were provided by the manufacturer of the MRI scanner. The following ratios were calculated from the obtained metabolites: NAA to creatine, choline to creatine, and NAA to choline.

### Statistical analysis

Statistical analysis was performed using commercially available software (SPSS version 12.0.1 for Windows, release date November 11, 2003; SPSS, Chicago, IL). Due to differences in sample size between the study groups, nonparametric tests were used. *P* values less than 0.05 were considered significant.

### Tests for associations between cerebral metabolite ratios and disease activity

To study whether there was a statistical association between metabolite ratios and the ESR value or the DAS28 score, nonparametric regression analysis was performed. In addition to the uncorrected analyses, we performed similar analyses in which we controlled for age, sex, smoking status, handedness, alcohol consumption, medication use, and disease duration. (15-18)

### Comparisons between RA patients and controls

Nonparametric independent-samples Mann-Whitney U tests were used to compare metabolite ratios in RA patients versus those in healthy controls. Mann-Whitney U tests were also used to compare metabolite ratios in patients with active RA, as determined according to the ESR values, with those in patients with inactive RA as well as with those in healthy controls.

## RESULTS

### Cerebral metabolite ratios in RA patients versus healthy controls

The mean ratios of NAA to creatine, choline to creatine, and NAA to choline and the respective ranges for the RA patients and the healthy control subjects are shown in Table 2. No significant difference in any of the metabolite ratios between the RA patients and the healthy controls was found. In none of the patients or control subjects was lactate found.

**Table 2.** Cerebral metabolite ratios in RA patients and controls\*

	RA patients (n = 35)	Controls (n = 28)
NAA:creatine	2.15 ± 0.23	2.20 ± 0.25
Choline:creatine	1.07 ± 0.26	1.09 ± 0.11
NAA:choline	2.09 ± 0.42	2.03 ± 0.23

\* Healthy age- and sex-matched subjects served as controls. Values are the mean ± SD. *P* values were not significant, as determined by Mann-Whitney U test. RA = rheumatoid arthritis; NAA = N-acetylaspartate.

### Cerebral metabolite ratios in patients with active versus inactive RA.

Table 3 shows that patients with active RA ( $n = 15$ ) had significantly higher ratios of choline to creatine ( $P = 0.016$ ) and significantly lower ratios of NAA to choline ( $P = 0.028$ ) than did patients with inactive RA ( $n = 20$ ). The NAA-to-creatine ratio was not significantly different between these 2 groups of patients. Examples of  $^1\text{H}$ -MRS spectra in an RA patient with a high ESR, an RA patient with a low ESR, and a healthy control subject are shown in Figure 1.

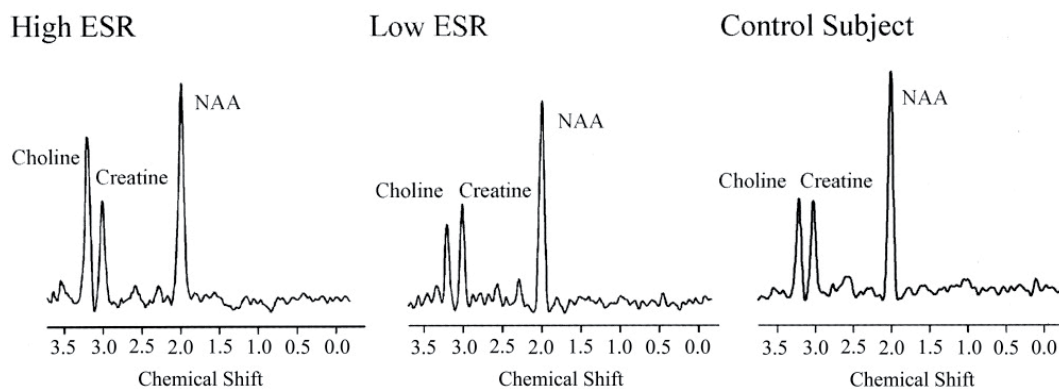
**Table 3.** Cerebral metabolite ratios in patients with active and inactive RA\*

	Patients with inactive RA ( $n = 20$ )	Patients with active RA ( $n = 15$ )
NAA:creatine	$2.12 \pm 0.16$	$2.19 \pm 0.30$
Choline:creatine	$0.99 \pm 0.23$	$1.17 \pm 0.26^\dagger$
NAA:choline	$2.23 \pm 0.42$	$1.93 \pm 0.37^\ddagger$

\* Values are the mean  $\pm$  SD. RA = rheumatoid arthritis; NAA = N-acetylaspartate.

$^\dagger P = 0.016$  by Mann-Whitney U test.

$^\ddagger P = 0.028$  by Mann-Whitney U test.



**Figure 1.** Proton magnetic resonance spectroscopy spectra of a patient with active rheumatoid arthritis (RA), as determined by a high erythrocyte sedimentation rate (ESR) (left), a patient with inactive RA, as determined by a low ESR (middle), and a healthy control subject (right). The choline signal is markedly increased in the patient with active RA. NAA = N-acetylaspartate.

### Association between cerebral metabolite ratios and disease activity

Associations between cerebral metabolite ratios and the ESR, the DAS28, and disease duration in RA patients are summarized in Table 4. The ESR was statistically significantly associated ( $P < 0.05$ ) with both the choline-to-creatine ratio and the NAA-to-choline ratio. These associations remained significant after correction for age, sex, smoking status, handedness, alcohol consumption, medication use, and disease duration. The association between the DAS28 and the choline-to-creatine ratio was statistically significant ( $P < 0.05$ ) only when it was not corrected for age, sex, smoking status, handedness, alcohol consumption, medication use, and disease duration.

**Table 4.** Association between cerebral metabolite ratios and disease activity in RA patients\*

	ESR (n = 35)				DAS28 (n = 16)			
	Uncorrected		Corrected		Uncorrected		Corrected	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>
NAA:creatinine	-	NS	-	NS	-	NS	-	NS
Choline:creatinine	0.43	0.009	0.43	0.037	0.59	0.017	-	NS
NAA:choline	-0.42	0.013	-0.46	0.044	-0.55	0.029	-	NS

*P* values were calculated using nonparametric regression analysis; both uncorrected values and values corrected for age, sex, and duration of rheumatoid arthritis (RA) are presented. ESR = erythrocyte sedimentation rate; DAS28 = Disease Activity Score 28-joint assessment; NAA = N-acetylaspartate; NS = not significant.

No association was found between disease duration and any of the metabolite ratios. Moreover, in our patient group, we could not determine any additional effect of medication on the association between the ESR and the choline-to-creatinine ratio or the NAA-to-choline ratio. The values in RA patients who were taking prednisone ( $n = 14$ ) and those who were not ( $n = 21$ ) were not significantly different ( $P = 0.893$  by Mann-Whitney U test).

## DISCUSSION

The most important finding of our study is that significant neurochemical changes are observed in the brain of RA patients with active systemic inflammation but without cerebral abnormalities on MRI. The choline-to-creatinine ratio correlated positively with the ESR and with the overall disease activity, as determined by the DAS28, in RA patients, whereas the NAA-to-choline ratio was negatively correlated with the ESR. Together with the finding that the NAA-to-creatinine ratio was not correlated with these disease activity measures and was not significantly different between patients with active and inactive RA, our results suggest that choline signals in the brain of patients with active RA are likely to be increased.

We did not find a significant difference between the combined group of patients with active and inactive RA and the group of age- and sex-matched healthy control subjects. This is probably because patients with inactive RA have lower choline ratios than do patients with active RA, as well as healthy controls. One explanation for these abnormally low choline levels in patients with inactive RA could be the previously described effect of the medications taken. (16,17) Nonetheless, our linear regression analyses showed that the influence of systemic inflammation on the neurochemical changes in RA patients persisted after correction for medication use.

Our finding of increased choline signals could be caused by monocyte infiltration during increased disease activity, consistent with a recent study in an animal model of



chronic inflammation. This suggests an influence of the immune system on the brain during increased disease activity. (3) Studies in patients with multiple sclerosis and in animal models of autoimmune inflammation of the CNS have shown that increased choline signals, as a marker of cell membrane turnover, are associated with inflammation in the form of monocyte infiltration, rather than demyelination. (9,19) D'Mello and colleagues (3) recently found that microglial cells are activated by TNF $\alpha$  to produce monocyte chemoattractant protein 1 (MCP-1) and the chemokine (C-C motif) CCL2, attracting circulating systemic monocytes across the blood-brain barrier in a mouse model of chronic systemic inflammation. Microglial cells represent the brain's innate immune system and are highly active immune cells that constantly survey the brain. (20) Unfortunately, in our study, the echo time was too long to reliably detect myoinositol. However, a longer echo time provides a less ambiguous baseline and less signal overlap with neighboring resonances, each of which contributes to easier and more reliable quantification. Furthermore, it is still unclear whether the temporal changes in microglial activation coincide with, or possibly precede, the infiltration of monocytes into the brain.

Systemic inflammation, characterized by increased circulating levels of proinflammatory cytokines, such as TNF $\alpha$ , has been implicated in cognitive decline and dementia (i.e., Alzheimer's disease) in more than 1 study. (5-8) One population study found an association between elevated levels of markers of inflammation during middle age and the development of dementia during old age. (7) Local inflammation plays a role in numerous neurologic conditions and processes, such as stroke/ischemia, trauma, epilepsy, multiple sclerosis, and Alzheimer's disease. (4) Elevated cerebral levels of choline are a common finding of <sup>1</sup>H-MRS in patients with these conditions. (9, 21-23) However, the studies mentioned above were not aimed at finding an association between levels of inflammation and neurochemical abnormalities.

Increased choline signals in the brain have been found in SLE and Behçet's disease. In Behçet's disease, increased choline signals have also been found in the periventricular normal-appearing white matter. (22) SLE patients showed elevated choline signals and myoinositol signals, indicating inflammation, and decreased NAA signals, indicating neuronal loss. (23-25) A recent <sup>1</sup>H-MRS study of SLE patients showed increased choline signals in the normal-appearing brain tissue in areas where lesions had appeared 4-6 years later on followup scans. (26) However, none of these studies reported an association between choline signals and serologic evidence of disease activity.

The functional consequences of abnormal choline levels in humans are not well known. As mentioned above, nonspecific neurologic symptoms, such as mood disorders, sleep disturbances, and fatigue, play an important role in RA patients' perception of their disease. (1) In other MRS studies, elevated levels of choline have been associated with fatigue in patients with chronic fatigue syndrome (CFS). Compared with healthy control subjects, choline levels were found to be increased in patients with CFS. (27, 28) We

found no significant difference in the NAA ratios between the study groups; this finding seems to be consistent with the lack of overt neurologic symptoms. NAA ratios, used as a marker of neuronal function, have previously been described as being associated with neurologic functioning. (23,24).

A limitation of our retrospective study is the fact that more-specific markers of disease activity, such as the TNF $\alpha$  level and measures of fatigue, were not available in all patients. The ESR is a nonspecific marker of inflammation. (13) More extensive quantitative neuropsychological investigation might reveal subclinical neurologic symptoms. Prospective studies are needed to more precisely determine the relationship between clinical measures of disease activity, neurochemical abnormalities, and clinical symptoms such as fatigue.

In conclusion, elevated ratios of choline to creatine and lowered ratios of NAA to choline in RA patients are associated with systemic inflammatory disease activity in the absence of abnormalities on conventional MRI sequences. Our data suggest that the presence of (subclinical) systemic inflammation may affect the neurochemical status of the CNS. Therefore, when studying changes in cerebral metabolism, one should take inflammatory diseases and their activities into account. Further study into the association between more-specific markers of systemic inflammation, cerebral metabolism, and clinical symptoms such as fatigue is necessary to more precisely determine their relationship.

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