

# Cerebrospinal fluid levels of iodothyronines and nerve growth factor in patients with multiple sclerosis and neuromyelitis optica

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## Abstract

**OBJECTIVE:** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the human central nervous system (CNS) and a major cause of neurological disability among adults in North America and Europe. Neuromyelitis optica (NMO) is a very severe disease of inflammatory demyelination located in the optic chiasm, nerves and the spinal cord. The aim of this study is to assess thyroid hormone (TH) and nerve growth factor (NGF) levels in cerebrospinal fluid (CSF) of MS, NMO patients and controls, and investigate whether there is any correlation between TH and NGF levels in the CSF. **PATIENTS AND METHODS:** 38 relapsing-remitting multiple sclerosis (RRMS), 10 NMO and 19 controls were investigated whether there was any correlation between TH and NGF levels in the CSF. **RESULTS:** MS and NMO patients exhibited significantly higher CSF NGF (respectively  $P < 0.05$ ,  $P < 0.05$ ), TT4 levels ( $P < 0.001$ ) and higher TT4/ rT3 ratio (respectively  $P < 0.01$ ,  $P < 0.01$ ) compared with the controls. Significant correlation was found between CSF NGF levels and CSF rT3 levels or TT4/ rT3 ratio in controls (respectively  $P < 0.01$ ,  $P < 0.05$ ). EDSS was significantly correlated with CSF rT3 levels and TT4/ rT3 ratio in MS patients (respectively  $P < 0.05$ ,  $P < 0.001$ ). **CONCLUSIONS:** These results indicate that an abnormal thyroid hormone may exist within the brain in the patients with MS. CSF rT3 levels and TT4/ rT3 ratio could be regarded as useful markers of underlying disease activity.

## 1. INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the human central nervous system (CNS) and a major cause of neurological disability among adults in North America and Europe. About 85% of MS is called relapsing-remitting multiple sclerosis (RRMS), which experi-

ence relapsing neurologic symptoms during their initial disease course. Major pathological changes of MS include inflammation, gliosis, demyelination, oligodendrocyte death, and axonal loss [1]. Neuromyelitis optica (NMO) is a very severe disease of inflammatory demyelination located in the

optic nerves and the spinal cord [2]. The place of NMO within the spectrum of MS has long been debated. Current evidence strongly suggests that NMO is driven by humoral immunity mechanisms, the detection of NMO immunoglobulin G (NMO-IgG), an autoantibody in the serum of patients with NMO, distinguishes NMO from other demyelinating disorders. NMO-IgG binds to aquaporin-4, which is the main channel that regulates water homeostasis in the central nervous system. These features are not seen in classical MS [3, 4]. NMO is particularly common in Japanese and Chinese patients and is found more frequently in female. Thyroid hormones (TH) play a key role in the development of brain, differentiation of oligodendrocyte progenitor cells (OPC) and myelination [5]. Previous studies have shown the alternation of serum TH levels in MS patients [6, 7], but it is unknown whether TH metabolism within the brain is also modified. CSF concentrations of iodothyronines reflect the TH metabolism in the brain, so analysis of CSF TH levels might provide better clues for investigating the status of TH metabolism and the role of TH in the brain of patients with MS or NMO.

Degradation of thyroxine (T4) in the central nervous system (CNS) occurs through deiodination by two separate enzymes, Type II deiodinase (D2) and type III deiodinase (D3) respectively [8]. In the brain, D2 is predominantly expressed in astrocytes and tanocytes [9, 10], and D3 is expressed in neurons [11, 12]. D2 converts T4 into the biologically active form, triiodothyronine (T3), for neuronal use, whereas D3 degrades T3 and T4 to the inactive metabolites, 3,3'-diiodothyronine (T2) and 3,3',5'-L-T3 (reverse T3, rT3) respectively, which do not activate the TH receptor [8]. Thus, D2 is believed to play a critical role in the maintenance of TH function in the CNS [13]. Eighty percent of T3 in the brain is derived from the local conversion of T4, and most of the rT3 present in the CNS is locally generated from T4 [8].

TH can interact with various neurotrophic factors by regulating their receptor expression [14], and most of them are NGF [15, 16]. TH enhances NGF expression in both neonate and adult mice [17, 18], also modulates NGF expression in the cerebellum of perinatal rats [16]. In experimental allergic encephalomyelitis (EAE), a widely used animal model for MS of autoimmune pathogenesis [19], NGF was found to protect against immune mediated demyelination [20], and TH could increase NGF content in the spinal cord during EAE [21]. Levels of NGF in CSF were significantly increased in MS [22] and RRMS patients during relapses were significantly high [23].

Up to now, studies about thyroid hormone of CSF in patients with MS are rare [24], and the relationship between CSF TH and NGF in human beings is unknown. In the present study we assessed TH and NGF levels in CSF in MS, NMO patients and controls, and investigated whether there was any correlation between TH and NGF levels in the CSF.

## 2. MATERIALS AND METHODS

### 2.1. Patients

Thirty eight patients with RRMS, 10 patients with NMO and 19 controls were included in this study. All the patients were admitted to the Department of Neurology, the third affiliated hospital of Sun Yat-sen University, from August 2006 to July 2007.

RRMS patients comprised of 27 women (71%) and 11 men (29%), the mean age was  $37.4 \pm 13.4$  years and mean EDSS levels was  $3.8 \pm 1.6$ . All the MS patients in this study matched McDonald diagnostic criteria for MS [25]. NMO patients comprised of 8 women and 2 men, the mean age was  $34.3 \pm 14.2$  years and mean EDSS levels was  $2.4 \pm 0.2$ . All the NMO patients met the criteria proposed by Wingerchuck [26]. None of the patients had thyroid autoimmunity disease or other diseases with hormone disturbance, and neither were treated with corticosteroids or other immunosuppressives in the lately two months. All CSF and serum samplings were performed when the patients were experiencing attacks of MS or NMO.

Nineteen person (12 women and 7 men) were served as controls during the same period, including 4 cervical spondylosis, 4 calculus of urinary system, 3 knee joint injury, 3 femoral head fracture, 1 spasmodic torticollis and 4 cholecystitis. These control subjects matched the RRMS group for age, sex and matched the NMO group for age. We obtain CSF samples when they were carried out epidural anesthesia. All clinical detections had been approved by the local ethics committee and all patients had learned about our examination and agreed with it..

### 2.2. Thyroid hormonal analysis in CSF and serum

All the patients with MS and NMO, as well as controls, were subject to physical examinations and their medical history were taken. Blood for thyroid hormonal analysis was collected in the morning (07:00– 08:00h) on empty stomach. 10 ml blood was collected from vena basilica following skin disinfection with 70% ethanol. After clotting, the blood was subject to centrifugation for 20 minutes (2000 g/min.). Serum was stored at  $-20^{\circ}\text{C}$  until the time when thyroid hormonal analysis was conducted.

After obtained informed consent, approximately 5 ml CSF were taken by lumbar puncture, and centrifugated for 10 minutes (4000 g/min). An aliquot of each serum and CSF sample was used for routine analysis, and the remained (1.5 ml) was frozen within 20 min from sampling and stored at  $-80^{\circ}\text{C}$  until thyroid hormone was detected. All CSF thyroid hormone measurements were conducted at the same time within the same assay.

For assessment of thyroid function, total T4 (TT4), total T3 (TT3), free T4 (fT4), free T3 (fT3) levels in serum samples and TT4, fT4 and fT3 levels in CSF samples were determined by highly sensitive MAIA (magnetic antibody enzyme linking immunoassay) following the standard procedures. Reverse T3(rT3) levels

were determined in serum and CSF samples by highly sensitive RIAs following the manufacturer's instructions. The sensitive limits of the assays were 5.0 ng/ml (6.435 nmol/l), 0.1pg/ml (0.129 pmol/l), 0.3pg/ml (0.462 pmol/l) and 3.0 ng/dl (0.05 nmol/l) for TT4, fT4, fT3 and rT3, respectively. Because TT4 concentrations in individual CSF samples were below the limit of determination (6.435 nmol/l) of the assay, 1ml CSF aliquots were concentrated to 50ul by vacuum concentration in room temperature (Eppendorf concentrator 5301, 1400rpm), TT4 determinations were carried out in concentrated pools of CSF samples.

### 2.3. Determination of NGF in CSF

NGF concentration was determined in undiluted CSF by commercially available sandwich-type enzyme-linked immunosorbent assays (NGF E<sub>max</sub><sup>®</sup> Immuno-Assay System, Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. The detection limit was 3.9 pg/mL for the assays.

## 3. STATISTICAL ANALYSIS

Statistical analysis (means  $\pm$  standard error of mean, SEM) and significance of group differences were evaluated using the SPSS 13.0 statistical package. Statistical hypothesis testing as well as the assessment of inter-group statistical significance were based on t-Student significance test. The relationship between variables was analyzed using Pearson's correlation coefficient. Statistical significance was assumed at  $P \leq 0.05$ .

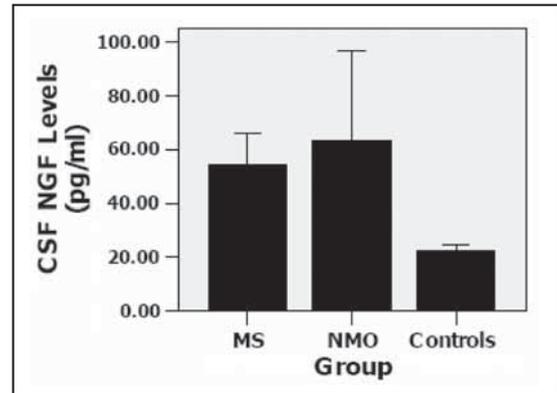
## 4. RESULTS

### 4.1 Serum levels of total and free iodothyronines

Serum TT4, TT3, rT3 and fT4 concentrations in MS patients were markedly lower than those in controls (respectively  $P < 0.001$ ,  $P < 0.05$ ,  $P < 0.05$ ,  $P < 0.01$ ). MS patients also had lower TT4/ rT3 ( $P < 0.01$ ), TT4/ TT3 ( $P < 0.05$ ) than controls. NMO patients had lower serum rT3 concentrations than controls ( $P = 0.05$ ). However, no difference was found in levels of serum iodothyronines, TT4/ rT3 and fT4/ fT3 between NMO patients and controls. In addition, serum concentrations of iodothyronines and TT4/ rT3 and fT4/ fT3 in MS patients were not significantly different from those in the NMO patients.

### 4.2. CSF levels of total and free iodothyronines

TT4 levels and the TT4/ rT3 molar ratios in concentrated CSF were significantly higher in MS patients than in controls (respectively  $P < 0.001$ ,  $P < 0.01$ ), and TT4 levels, fT4 levels and TT4/ rT3 molar ratios were significantly enhanced in NMO patients when compared with controls (respectively  $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.01$ ). However, there was no correlation between CSF rT3 and CSF TT4 concentrations in three groups.



**Fig 1.** The CSF NGF levels in different group.  $P = 0.01$ , significant increase compared MS patients with controls.  $P < 0.05$ , significant increase compared NMO patients with controls.

### 4.3. Relationship between thyroid hormones and NGF levels in CSF

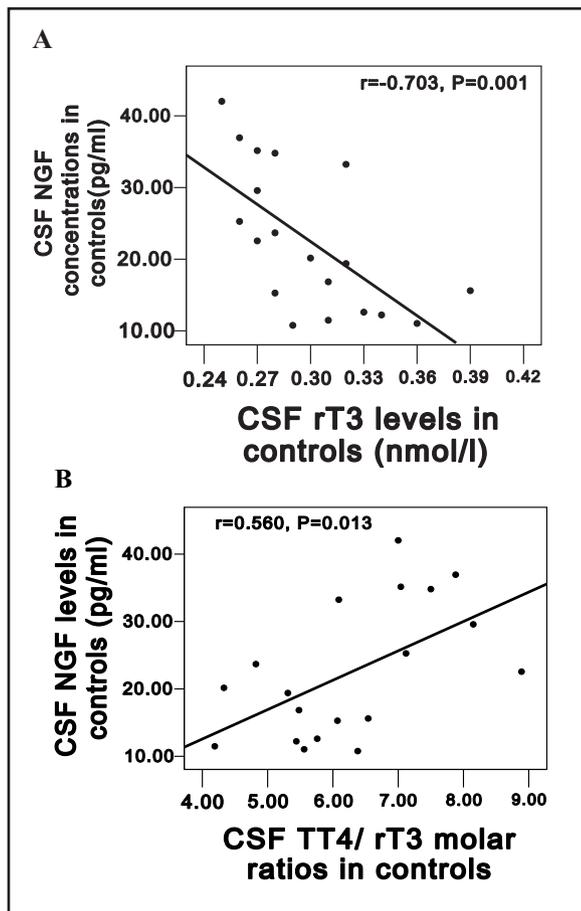
The CSF NGF levels were significantly higher in MS patients ( $54.3 \pm 11.60$  pg/ml,  $P < 0.05$ ) and NMO patients ( $63.4 \pm 33.45$  pg/ml,  $P < 0.05$ ) than in controls ( $22.6 \pm 2.30$  pg/ml) (Fig 1), but there was no difference in CSF NGF levels between MS and NMO patients. In controls group, CSF NGF levels had significant correlations with CSF rT3 concentrations ( $r = -0.703$ ,  $P < 0.01$ ) (Fig 2A), as well as CSF TT4/ rT3 molar ratios ( $r = 0.560$ ,  $P < 0.05$ ) (Fig 2B), whereas in MS and NMO patients, CSF NGF levels did not correlate with CSF thyroid hormonal levels.

### 4.4. EDSS levels and thyroid hormones

Severity of the MS and NMO was evaluated on EDSS (Kurtzke's Expanded Disability Status Scale score) [27]. The EDSS levels were  $4.0 \pm 0.30$  in MS patients and  $2.4 \pm 0.07$  in NMO patients. Significant positive correlation was found in MS patients between the mean EDSS levels and CSF rT3 concentrations ( $r = 0.378$ ,  $P < 0.05$ ) (Fig 3A), and significant negative correlation between the mean EDSS levels and CSF TT4/ rT3 molar ratios ( $r = -0.695$ ,  $P < 0.001$ ) (Fig 3B). However, there was no significant correlation between EDSS levels and CSF or serum thyroid hormones levels in NMO patients.

## 5. DISCUSSION

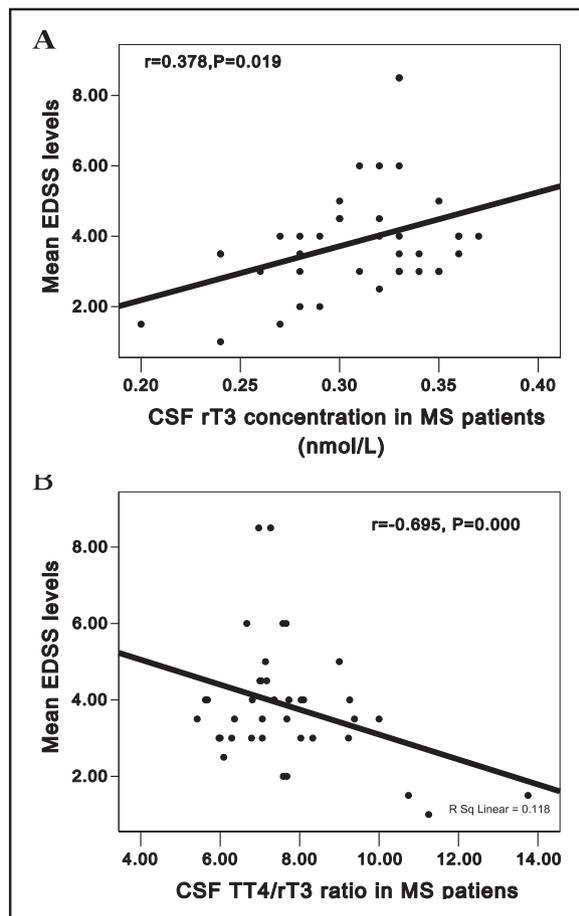
In this study, we found that MS and NMO patients had lower serum TH levels, higher CSF TT4 levels, lower CSF TT4 to rT3 ratio and higher CSF NGF levels when compared to controls. Also, significant correlation was found between EDSS and CSF rT3 levels, TT4/ rT3 ratio in MS patients. These results indicate that an abnormal thyroid hormone may exist in the patients with MS, and CSF rT3 levels and TT4/ rT3 ratio are useful indicators to appraise the clinical severity of this disease.



**Fig. 2.** The relationship between CSF NGF concentrations and CSF rT3 concentrations, CSF TT4/ rT3 molar ratios in controls.

Previous studies have shown that the MS patients had higher T4 and lower T3 levels in serum than controls, suggesting the existence of thyroid dysfunction in MS. However in our study, serum TT4, TT3 and rT3 levels were significantly lower in MS subjects when compared to controls (see Table 1). Reduced T3 and rT3 levels may indicate a change in peripheral conversion of thyroid hormones in MS patients. In addition, the ratio of TT4/ rT3 and TT4/ TT3 were significantly lower than those in controls (see Table 1), which might be related to markedly lower serum TT4 levels in MS. In numerous acute and chronic diseases, low T3 syndrome often occurs, and these diseases are not directly related to pathologies of thyroid gland [28, 29]. Low T3 syndrome is characterised by low total T3 and fT3 levels and elevated rT3 concentration as well as either low, normal or even increased (rarely) fT4 levels. In the present study we found serum TT3 levels were decreased, and the serum rT3 levels were not elevated. And the mechanism which leads to our results is unknown, at least not sufficiently explained by low T3 syndrome.

T4 is produced entirely by the thyroid gland [30]. Two possible mechanisms are responsible for the presence of thyroid hormone in CSF: 1) T4 may cross the BBB from serum into CSF, and 2) T3 and rT3 can be



**Fig. 3.** The relationship between EDSS levels and CSF rT3 concentrations, CSF TT4/ rT3 ratios in MS patients.

derived from thyroidal secretion of T4, which undergo peripheral conversion and is then transferred across BBB, and/or both T3 and rT3 may derive from local conversion of T4 within the CNS [31].

In present study, an increase in the CSF TT4 concentration has been found in MS and NMO. This finding could be linked to a relatively unspecific passage of TT4 from the serum into the CSF across an eventually damaged blood-CSF barrier. The CSF fT4 and fT3 concentrations determined in our study were lower than those found in previous reports [31–34]. In previous studies, RIA protocols were used for free iodothyronine determinations. In our study free iodothyronine was determined by MAIA, which may display variable antigen selectivity. We found that the levels of fT4 in NMO were significantly higher than those in MS and controls, indicating TH metabolism was different between MS and NMO patients. The CSF TT4/ rT3 molar ratio was found significantly higher in the CSF of MS and NMO patients than controls, suggesting a change in D3 activity in MS and NMO. Axonal injury begins at disease onset in MS, and there is extensive axonal loss in long-term MS and NMO [1, 35], which leads to a decrease of D3 activity in these diseases. TT4/rT3 molar ratio may be related with the degree of neuronal injury,

**Table 1.** Serum levels of iodothyronines (mean±SEM)

	RRMS(n=38)	NMO(n=10)	Controls (n=19)
TT3(nmol/l)	<b>2.2±0.07 a</b>	2.2±0.08	2.4±0.07
TT4(nmol/l)	108.3±2.83 b	119.8±6.45	133.6±3.50
rT3 (nmol/l)	<b>0.7±0.02 a</b>	0.7±0.03a	0.8±0.01
ft3(pmol/l)	4.1±0.10	4.0±0.22	4.4±0.19
ft4(pmol/l)	<b>13.5±0.34b</b>	14.1±0.68	15.4±0.41
TT4/ rT3	<b>153.2±4.48 b</b>	178.2±14.62	173.1±4.34
TT4/TT3	<b>51.7±1.85a</b>	56.1±3.49	56.3±1.35
ft4/ ft3	3.4±0.13	3.6±0.24	3.6±0.17

a) p≤0.05 compared with controls

b) p≤0.01 compared with controls

which leads to disability in MS. We also found TT4/rT3 molar ratio was correlated with EDSS levels, which was applied for evaluating the degree of disability in MS patients, the higher EDSS level indicated more severe disability [27]. Gliosis occurs in brain of MS patients, thus, an enhancement of astrocytic D2 activity might be the primary event affecting TH metabolism in MS brain. This could, in turn, explain the lower T4 to rT3 conversion rate as observed in our MS patients.

TH is required for the normal timing of OPC differentiation and maturation [3] and can increase the endogenous synthesis of NGF in the CNS [17]. T4 administration can activate oligodendrocyte precursors and increase a myelin-forming protein and NGF content in the spinal cord during EAE [21]. We have known that NGF plays an important role in the maintenance of adult CNS homeostasis and in the response to brain tissue damage [36], but we have no idea of the relationship between NGF levels and TH levels in human CSF, in this study we attempt to investigate the relationship.

During acute attacks, the RRMS patients exhibit a significant increase of NGF content in CSF when compared to controls, in contrast during remission, the mean NGF levels in CSF markedly decrease [22]. Another report confirmed that levels of NGF in RRMS patients during relapses were significantly higher than in the other groups (such as SPMS, CIS or health controls) [23]. Our study also found the levels of NGF in RRMS patients during their relapses were significantly higher than controls, meanwhile the levels of NGF in NMO patients also higher than controls, but there was no difference in NGF levels between RRMS and NMO. We consider that NGF is produced to protect CNS tissue against inflammation as previous report [22]. During CNS damage, NGF plays a role in recovering brain homeostasis [23].

In our study, we firstly found that both CSF rT3 concentrations and the ratio of T4/rT3 were significantly correlated with CSF NGF levels in controls, thus indicating that a homeostasis between T4 and NGF, and the amount of rT3 in CSF and T4 metabolism could be linked to NGF production. Recent years, some studies confirmed effective T4 to T3 conversion in CSF

**Table 2.** CSF levels of iodothyronines and NGF (mean±SEM)

	RRMS(n=38)	NMO(n=10)	Controls (n=19)
TT4(nmol/l)	<b>2.3±0.04a</b>	<b>2.4±0.06a</b>	1.9±0.08
rT3 (nmol/l)	0.31±0.006	0.32±0.009	0.30±0.009
ft3(pmol/l)	2.4±0.06	2.4±0.06	2.3±0.08
ft4(pmol/l)	<b>4.4±0.07b</b>	<b>4.8±0.14a</b>	4.3±0.07
TT4/ rT3	<b>7.7±0.27a</b>	<b>7.6±0.24a</b>	6.3±0.29
ft4/ ft3	1.9±0.06	2.1±0.08	1.9±0.07
NGF(pg/ml)	<b>54.3±11.60a</b>	<b>63.4±33.45a</b>	22.6±2.30

a) p≤0.01 compared with controls

b) p≤0.05 compared with NMO

(decreased rT3 levels and increased ratio of T4/rT3) could promote NGF gene expression due in part to a significantly enhanced rate of NGF gene transcription [40], NGF is complementary to thyroid hormone, and the T4 action is partly mediated and regulated by NGF. Thyroid hormone appears to have a long-term permissive role, while NGF may be a local and short-term limiting neurotrophic factor. In short, the results show us that presenting the correlation of CSF NGF with TT4/rT3 in controls but not patients is essential for ensuring a normal time course of cerebellar histogenesis in healthy controls as previous study [37].

But in MS and NMO patients, there was no correlation between T4/rT3 and NGF, as well as rT3 and NGF, suggesting the homeostasis between T4 metabolism and NGF could be destroyed in status of diseases, and the imbalance of the homeostasis between TH levels and NGF levels might be caused by gliosis, oligodendrocyte apoptosis, axonal degeneration and proinflammatory cytokine production in MS or NMO.

Generally, EDSS level is used to evaluate the degree of disability in MS patients, much higher EDSS levels indicate more severe disability [27]. In this study, we firstly found the MS patients with more severe disability (higher EDSS levels) had the higher CSF rT3 concentrations and the lower CSF TT4/ rT3 molar ratios, which reflected that CSF rT3 levels and CSF TT4/ rT3 were related with clinical functional impairment in MS patients. T3 is an active form, which is an important factor in oligodendrocyte differentiation, particularly regarding the distribution of myelin proteins [38], however rT3 is a inactive metabolite, which do not activate the TH receptor [8] and has no influence on oligodendrocyte differentiation, which can contribute to improvement of disability of MS [39], therefore CSF rT3 levels and CSF TT4/ rT3 molar ratios were useful markers of underlying disease activity, but because the levels of normals and patients have a very broad overlap, we could not consider that the assay would be useful in terms of diagnosis or management of individual patients. meanwhile, no correlation between EDSS levels and thyroid hormones was found in NMO patients, and that might suggest TH metabolism plays a

different role in clinical functional impairment between MS and NMO patients.

In conclusion, we consider that there is different TH metabolism between MS and NMO patients. In MS patients, the change of CSF rT3 levels and TT4/ rT3 ratio could be regarded as useful markers of underlying disease activity. CSF T4 metabolism was related with clinical functional impairment in MS patients and the homeostasis between T4 metabolism and NGF could be destroyed in status of diseases.

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