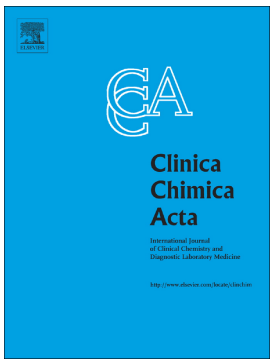

Elevated neuron-specific enolase and S100 calcium-binding protein B concentrations in cerebrospinal fluid of patients with anti-N-methyl-d-aspartate receptor encephalitis

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Elevated Neuron-specific enolase and S100 calcium-binding protein B concentrations in cerebrospinal fluid of patients with anti-N-methyl-D-aspartate receptor encephalitis

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Abstract

Background: Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a relatively common autoimmune neurological disease of the central nervous system (CNS). Neuron-specific enolase (NSE) and S100 calcium-binding protein B (S100B) are structural proteins of the central nervous system (CNS). In patients with CNS injury accompanied by nervous tissue and cellular damage, these structural proteins are released from cells; their extracellular concentrations, including those in cerebrospinal fluid (CSF) and blood, subsequently increase.

Methods: Thirty-six patients with anti-NMDAR encephalitis were prospectively recruited. The CSF NSE and S100B concentrations were measured in 19 and 17 patients, respectively. The CSF NSE and S100B concentrations were measured in 21 patients with noninflammatory neurological disease as controls. All measurements were performed using enzyme-linked immunosorbent assays.

Results: The CSF NSE and S100B concentrations were remarkably higher in the patients with anti-NMDAR encephalitis than in the controls. The early NSE or S100B concentrations in CSF were associated with the mRS.

Conclusion: CSF NSE and S100B concentration in patients with anti-NMDAR encephalitis is higher than in patients with non-inflammatory neurological disease. The early NSE or S100B concentrations in CSF were associated with the mRS and we can use it to determine the prognosis of the disease.

Keywords: anti-N-methyl-D-aspartate receptor encephalitis, S100B, neuron-specific enolase, modified Rankin scale

1. Introduction

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a relatively common autoimmune neurological disease of the central nervous system (CNS). The typical clinical symptoms of anti-NMDAR encephalitis are hallucinations, catatonia, altered levels of consciousness, seizures, hypoventilation, dyskinesia, and autonomic dysfunction [1]. Anti-NMDAR encephalitis has become one of the most commonly identified causes of encephalitis since its discovery in 2007 [1]. Magnetic resonance imaging(MRI) findings are unremarkable in 50% of patients with anti-NMDAR encephalitis[2]. Indirect immunofluorescence, as Josephe [3] reported, has been the most commonly employed technique by diagnostic laboratories for the detection of anti-NMDAR. While identification of the characteristic indirect immunofluorescence patterns may be a good screening test for distinguishing positive from negative sera, the specificity of indirect immunofluorescence is less than ideal [2].

Neuron-specific enolase (NSE) is a dimer formed in neurons and neuroendocrine cells with subordinate units α-γ or γ-γ and belongs to the group of hydrolytic enzymes[4, 5]. Many studies have shown that an elevated serum concentration of NSE is commonly found in a variety of conditions associated with CNS damage, such as stroke, traumatic brain injury, multiple sclerosis, and Alzheimer's disease [6-9]. S100 calcium-binding protein B (S100B) belongs to a family of calcium-binding proteins. It is mostly expressed in astrocytes and, to a certain extent, in a small subset of oligodendrocytes [10, 11]. Similarly, many reports have described a significantly increased serum concentration of S100B in various CNS disorders, including traumatic brain injury, Creutzfeldt–Jakob disease, schizophrenia, and stroke [12]. Additionally,

the astrocytic marker S100B in the cerebrospinal fluid (CSF) is substantially elevated in the acute phase of neuromyelitis optica(NMO), indicating its clinical usefulness as biomarker for this condition [12].

However, no reports have described the relationship between the CSF NSE or S100B concentration and anti-NMDAR encephalitis. Thus, we measured the concentrations of NSE and S100B in CSF obtained in the acute phase of anti-NMDAR encephalitis to clarify the variations in the CSF concentration of NSE and S100B in these patients when compared with control patients with non-inflammatory disease. The modified Rankin scale (mRS), which is often used to evaluate neurological recovery and measure outcomes of disease, was used to determine whether the NSE or S100B concentration in the CSF can be used to assess the prognosis of anti-NMDAR encephalitis. Additionally, the relationship between the CSF NSE or S100B concentration and the mRS score was clarified.

2. Materials and Methods

2.1 Patients and controls

Nineteen patients diagnosed with anti-NMDAR encephalitis were prospectively recruited into this study from December 2014 to November 2016, and their CSF NSE concentrations were measured. Due to the limitation of sample size, seventeen other patients with anti-NMDAR encephalitis were simultaneously enrolled, and their CSF S100B concentrations were measured. The control group comprised 21 patients with non-inflammatory neurological disease. The anti-NMDAR1 (anti-NR1) antibody in

CSF was measured to assess the severity of anti-NMDAR encephalitis. The mRS was used at the most critical time and 3 months after discharge to evaluate neurological recovery and assess the prognosis of the disease, and the outcome was dichotomized into a favorable prognosis (mRS score of 0–2) and poor prognosis (mRS score of 3–6 or death). Considered that the concentration of NSE and S100B in CSF varied during different stages of the disease, we divided the sampling time into early(≤3 days) and late(>3days). The detailed clinical data of all patients and controls are summarized in Table 1. There were no statistically significant differences in sex or age between the patients with anti-NMDAR encephalitis and the controls. All patients received standard therapy at Nangfang Hospital of Southern Medical University. The study was performed in accordance with the ethical standards of the Helsinki Declaration of 1975, and written informed consent was obtained from each participant.

2.2 Preparation of CSF samples

Lumbar puncture was performed before treatment to collect 3 to 5 mL of CSF. The first 2 mL was used for clinical purposes, while the remaining 1 to 3 mL was kept refrigerated until aliquoted into polypropylene tubes and stored at −80°C within 2 h of collection.

2.3 Enzyme-linked immunosorbent assay

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure the concentrations of NSE (R&D Systems, Inc., Minneapolis, MN,

USA) and S100B (Cusabio Biotech Co., Ltd., Baltimore, MD, USA) in CSF according to the manufacturers' instructions. Samples in which the NSE or S100B concentration was higher than the highest standard were serially diluted in sample diluent until they reached the dynamic range of the assay. The minimum detectable dose of NSE ranged from 0.013 to 0.038 ng/mL, while the minimum detectable dose of S100B was <19.5 pg/mL according to the manufacturers. A standard curve was created by reducing the data using computer software capable of performing four-parameter logistic curve fitting, and the best fit line was determined by regression analysis.

2.4 Statistical analysis

Data are presented as mean ± standard deviation. Differences in the concentrations of CSF NSE and S100B between different subgroups were analyzed using independent sample t test . Correlations between CSF NSE or S100B and the mRS score or anti-NR1 antibody titer were assessed by Spearman's rank analysis or Pearson's correlation analysis, as appropriate. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, release 20.0 (IBM Corp., Armonk, NY, USA). A P value of <0.05 was considered statistically significant.

3. Results

3.1 CSF NSE concentration in patients with anti-NMDAR encephalitis and in controls

The concentration of NSE in CSF was determined in patients with anti-NMDAR encephalitis (n=19) and in controls (n=21) using ELISA. The mean NSE concentration in patients with anti-NMDAR encephalitis was 4.53 ± 1.60 ng/mL, which was significantly higher than that in controls (1.98 ± 0.80 ng/mL, P < 0.001) (**Figure 1**).

3.2 CSF S100B concentration in patients with anti-NMDAR encephalitis and in controls

The concentration of S100B in CSF was determined in patients with anti-NMDAR encephalitis (n = 17) and in controls (n = 21) using ELISA. The goodness of fit for the representative standard curve was r^2 =0.963. The mean S100B concentration in patients with anti-NMDAR encephalitis was significantly higher than that in controls (375.53 ± 167.80 and 142.99 ± 33.45 pg/mL, respectively; P < 0.001) (**Figure 1**).

3.3 Relationship between CSF NSE or S100B concentration and mRS score

The mRS score was obtained at the most critical time and 3 months after discharge in patients with anti-NMDAR encephalitis (**Table 1**). The correlation between the CSF NSE concentration and mRS score was not statistically significant at either the most critical time or 3 months after discharge P=0.353 and 0.553, respectively) (**Figure. 2A**). Likewise, the correlation between the CSF S100B concentration and mRS score at these time points were not statistically significant (P=0.403 and 0.760, respectively) (**Figure. 2B**). At the 90-day follow-up, the functional outcome was favorable in 6 and poor in 13 patients who underwent NSE

measurement, while the functional outcome was favorable in 4 and poor in 13 patients who underwent S100B measurement. In both groups, the CSF NSE and S100B concentrations in patients with a poor functional outcome were not significantly higher than those in patients with a favorable functional outcome (NSE: 4.38 ± 1.07 vs. 4.87 ± 2.23 ng/mL, P = 0.551; S100B: 382.48 ± 156.73 vs. 352.94 ± 179.25 pg/mL, P = 0.769) **(Figure. 3**).

The sampling time has been divided into early(≤3 days) and late(>3days). Our result suggest that the concentration of CSF NSE and S100B was significantly higher in early stage (**Figure. 4**). Moreover, it was significantly correlated with prognosis (**Figure. 5**).

3.4 Relationship between CSF NSE or S100B concentration and anti-NR1 antibody titer

Among the patients with anti-NMDAR encephalitis, the correlation between the CSF NSE concentration and anti-NR1 antibody titer was not statistically significant (P=0.150) (**Figure. 6A**). Likewise, the correlation between the CSF S100B concentration and anti-NR1 antibody titer was also not statistically significant (P=0.307) (**Figure. 6B**).

4. Discussion

In the present study, we examined the concentrations of NSE and S100B in the CSF of patients with anti-NMDAR encephalitis. To our knowledge, this is the first

report on this. The results show that CSF NSE and S100B concentrations were remarkably higher in the patients with anti-NMDAR encephalitis than in controls. Futhermore, significant correlation was detected between the early CSF NSE or S100B concentration and the mRS score in these patients, indicating that early NSE and S100B concentration in CSF is associated with the prognosis of the disease.

Studies have documented that the structural proteins of the CNS, including NSE and S100B, are released from cells when CNS injury accompanied by nervous tissue and cellular damage occurs, and their extracellular concentrations increase, including those in the CSF and blood [13-15]. Which is consistent with our finding.

Additionally, Ikonomidou et al. [16] found that blockade of the NMDAR led to apoptosis and neurodegeneration in the fetal and neonatal rat brain. They stated that the excitatory neurotransmitter glutamate, acting at the NMDAR, controls neuronal survival [16]. The fact that anti-NMDAR encephalitis has great relevance with respect to the humoral immune response has been concluded from the beneficial effect of plasma exchange, which is widely considered as first-line immunotherapy [17]. Antibodies to the NR1 subunit of the NMDAR are thought to be pathogenic. Based on all of these conclusions, we hypothesized that when the NMDAR is blocked by the antibody, NSE and S100B are released and neuronal damage occurs.

S100B and NSE was also found to be associated with the mRS score in some patients, such as those with ischemic stroke and intracerebral hemorrhage, and patients with a high blood S100B concentration were more likely to have a poor functional outcome [18]. As described by Foerch et al. [19], the S100B and NSE concentration

at 48 and 72 h after symptom onset was correlated with the mRS score in patients with ischemic stroke. In consideration of the long time between symptoms onset and admission, we divided the sampling time into early(≤3 days) and late(>3days), which were determined by the half-life of NSE and S1000B [19], and detected the relationship between the concentration of early or late CSF NSE or S100B and mRS. The results show that CSF NSE and S100B concentration in early stage was significantly elevated compared to those in late stage. At the same time, we found that the concentration of CSF NSE and S100B in the early stage was significantly correlated with mRS, which mean in the early stage of the disease, measuring NSE and S100B concentration can help us determine prognosis.

Another recent study showed that binding of human monoclonal NR1 antibodies to NMDARs is sufficient to cause morphological and electrophysiological changes in neurons by NMDAR downregulation [20]. The clinical symptoms of hallucinations, catatonia, altered levels of consciousness, seizures, hypoventilation, dyskinesia, and autonomic dysfunction may be associated with this.

5. Conclusion

In summary, this is the first report of higher CSF NSE and S100B concentration in patients with anti-NMDAR encephalitis than in patients with non-inflammatory neurological disease. The early NSE or S100B concentrations in CSF were associated with the mRS and we can use it to determine the prognosis of the disease. However, the mechanism of the increases in the CSF NSE and S100B concentrations remain

unclear. One hypothesis is that the increases are related to neuronal damage, which should be further elucidated with more studies.

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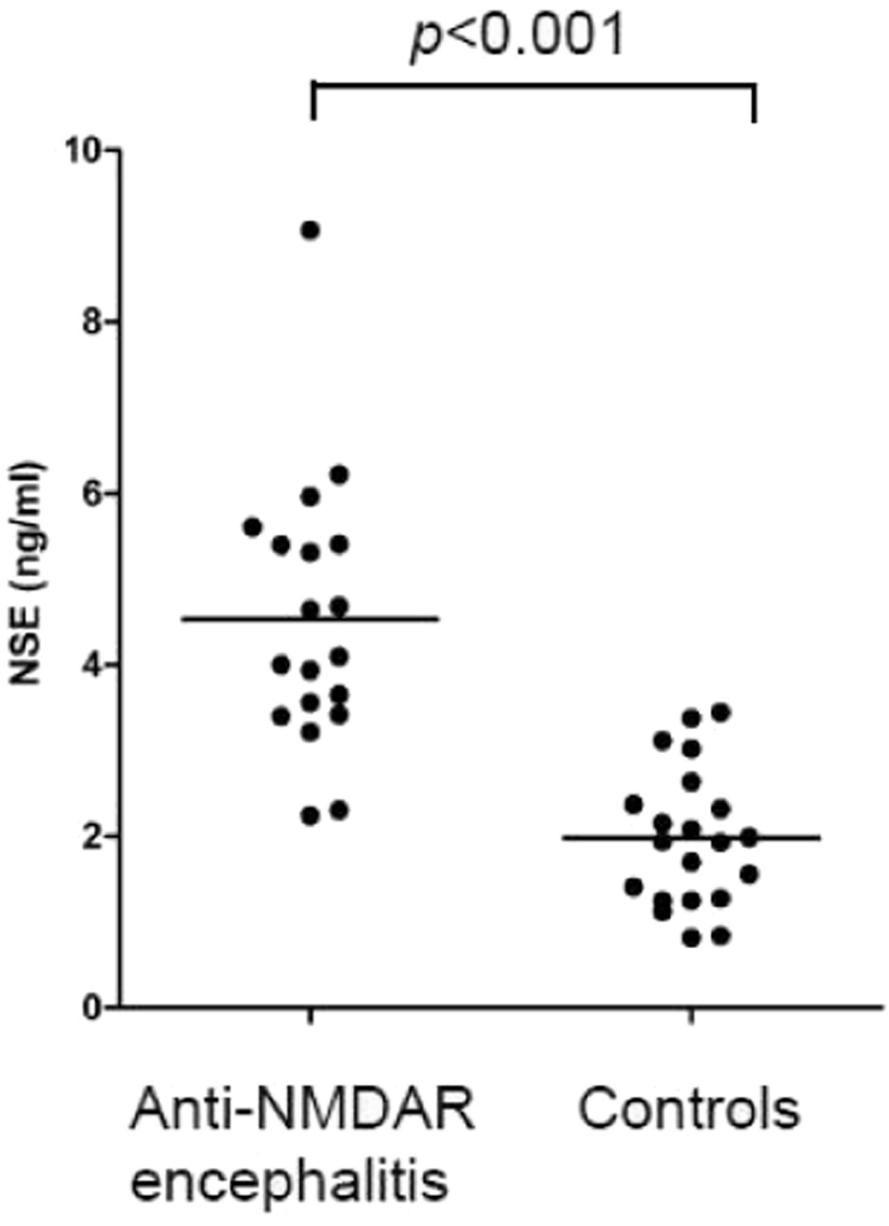
Table 1 Clinical data of patients with anti-NMDA receptor（NMDAR）encephalitis and non-inflammatory neurological disorder controls

	Characteristic	anti-NMDAR encephalitis	Control
NSE	Gender(famale/male)	12/7	14/7
	Age(years)	34.72±17.61	33.14±7.93
	Time between symptoms onset and admission	19.82±14.44	---
	Max MRS	4.15±0.87	---
	Favorable（MRS 0-2）	1	---
	Poor（MRS 3-5 or death）	18	---
	3 Month MRS	2.87±0.97	---
	Favorable（MRS 0-2）	6	---
	Poor（MRS 3-5 or death）	13	---
	NMDAR antibody	Positive	Negative
S100B	Gender(famale/male)	10/7	14/7
	Age(years)	36.00±17.34	33.13±7.94
	Time between symptoms onset and admission	18.58±13.48	---
	Max MRS	4.12±0.84	---
	Favorable（MRS 0-2）	1	---
	Poor（MRS 3-5 or death）	16	---
	3 Month MRS	3.23±0.87	---
	Favorable（MRS 0-2）	4	---
	Poor（MRS 3-5 or death）	13	---
	NMDAR antibody	Positive	Negative

Highlights

- CSF NSE and S100B levels were remarkably higher in anti-NMDAR encephalitis groups when compared with controls.
- No significant correlation was detected between the CSF NSE or S100B concentration and Modified Rankin Scale (MRS) in anti-NMDAR encephalitis patients.
- Levels of NSE or S100B in CSF have nothing to do with prognosis of the disease.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NSE (ng/ml)
10
8
6
4
2
0
Anti-NMDAR encephalitis
Controls

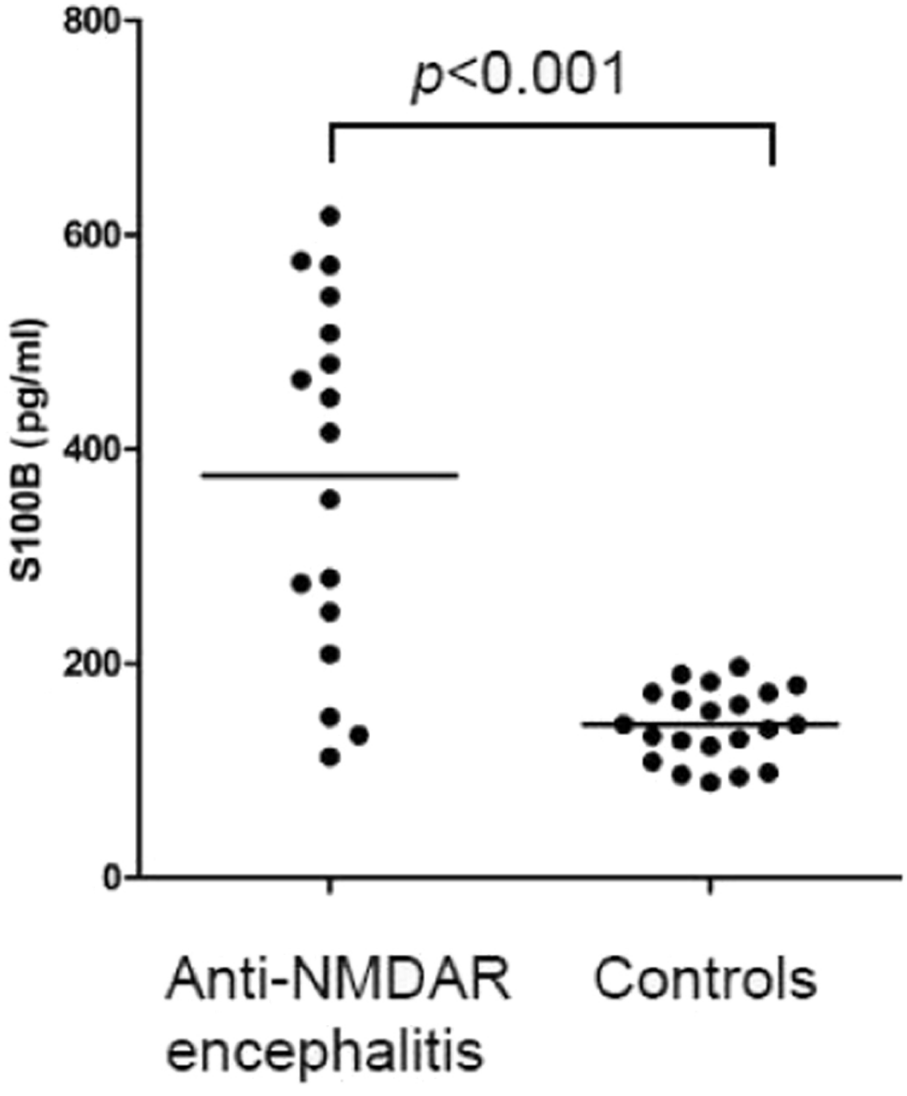
p<0.001
S100B (pg/ml)
800
600
400
200
0
Anti-NMDAR encephalitis
Controls

Figure 1

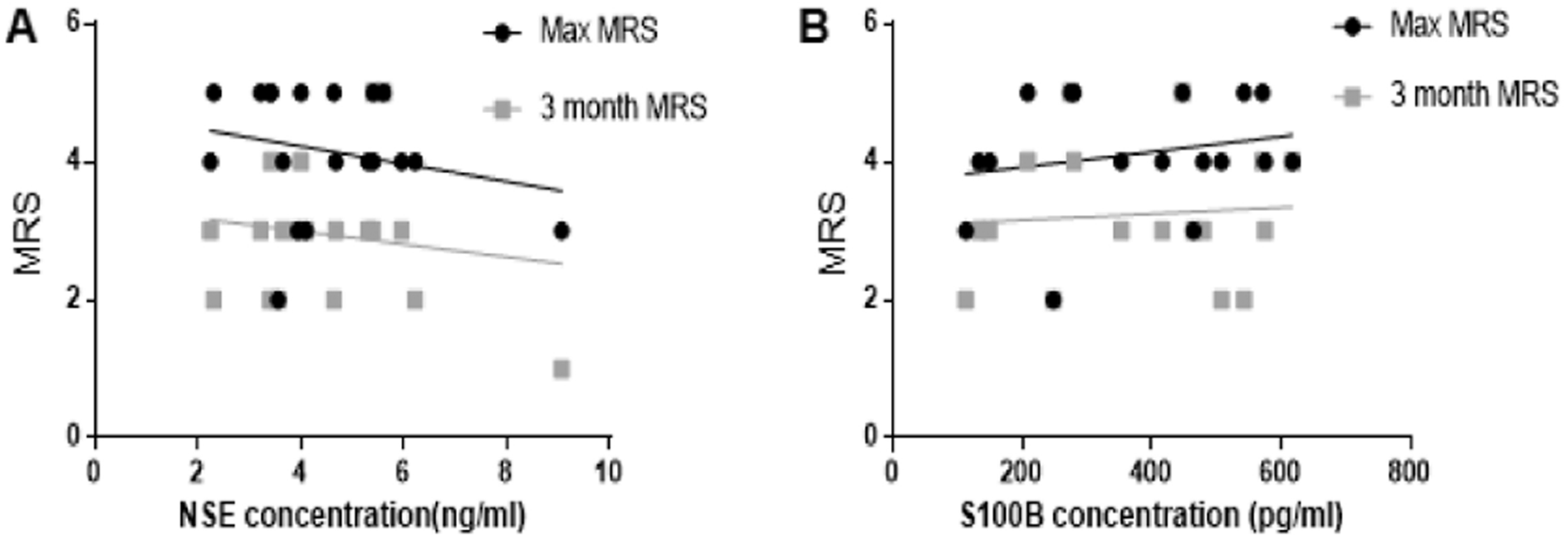
A
Max MRS
3 month MRS
MRS
NSE concentration(ng/ml)
B
Max MRS
3 month MRS
MRS
S100B concentration (pg/ml)

Figure 2

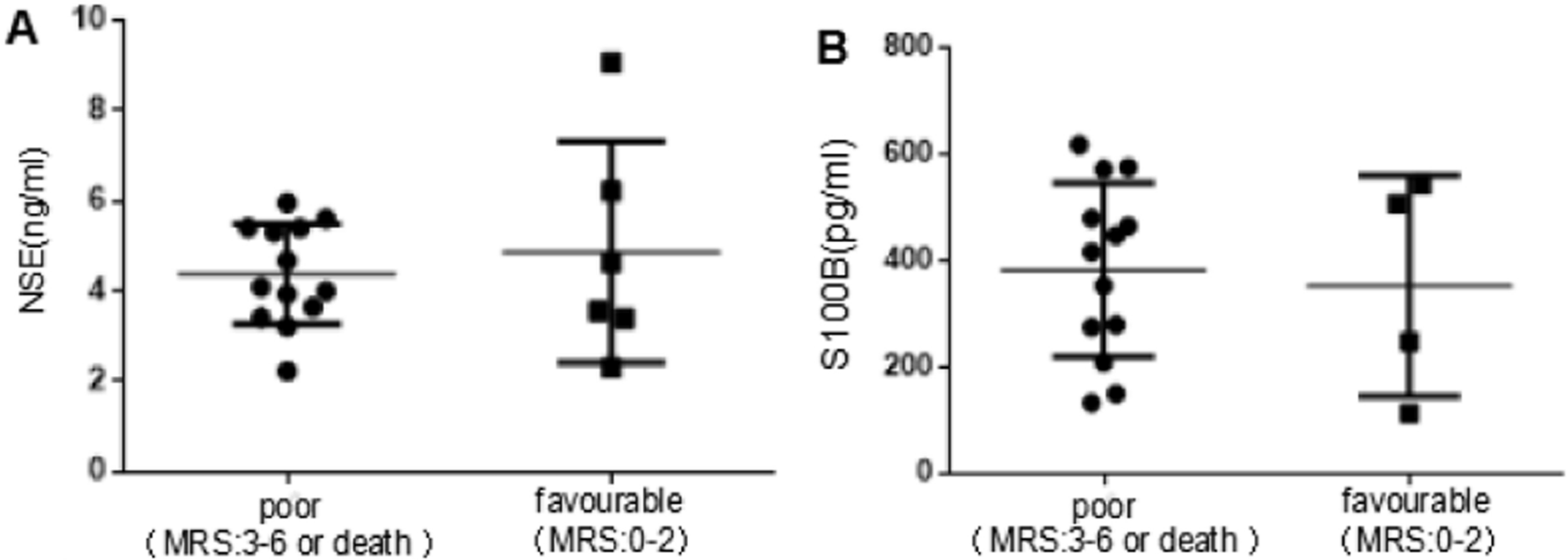
A
NSE(ng/ml)
10
8
6
4
2
0
poor
(MRS:3-6 or death)
favourable
(MRS:0-2)
B
S100B(pg/ml)
800
600
400
200
0
poor
(MRS:3-6 or death)
favourable
(MRS:0-2)

Figure 3

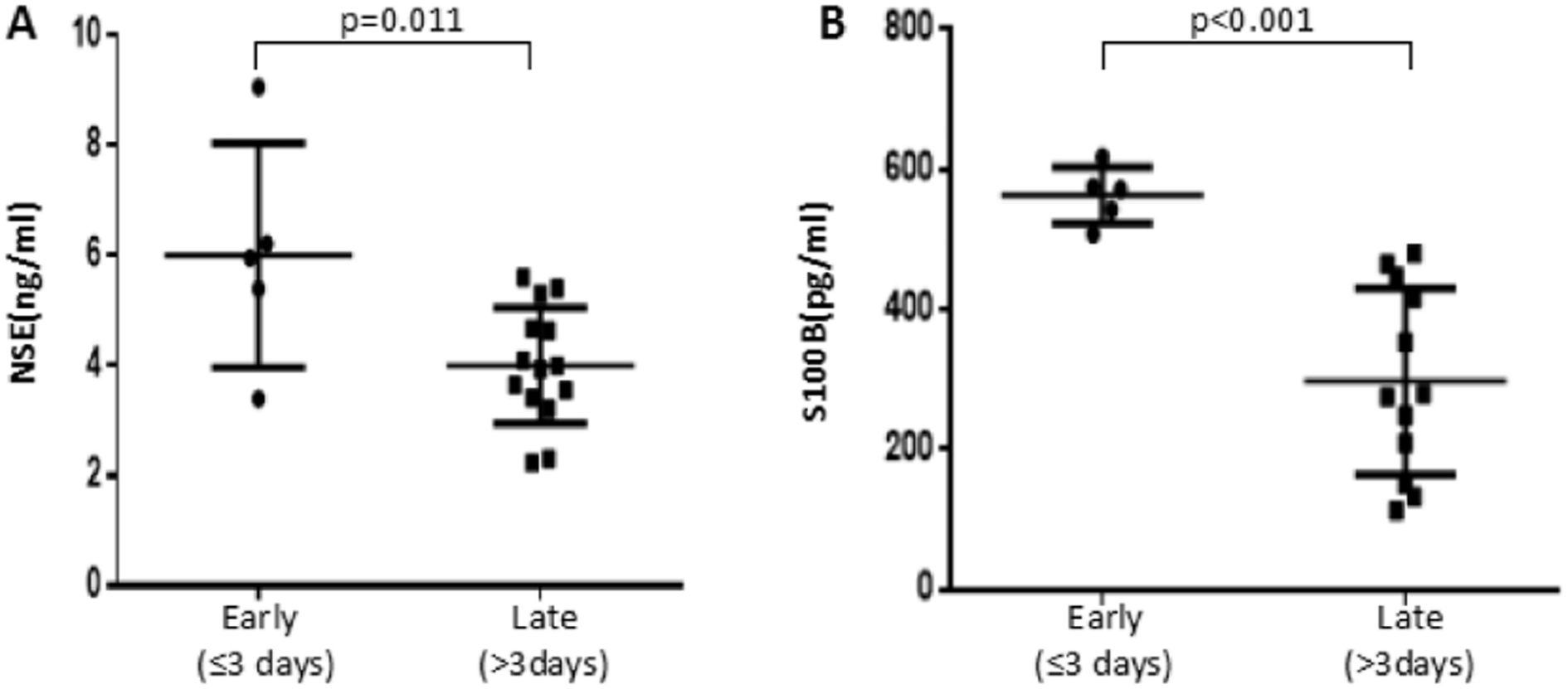
A
p=0.011
NSE(ng/ml)
10
8
6
4
2
0
Early
(≤3 days)
Late
(>3days)
B
p<0.001
S100B(pg/ml)
800
600
400
200
0
Early
(≤3 days)
Late
(>3days)

Figure 4

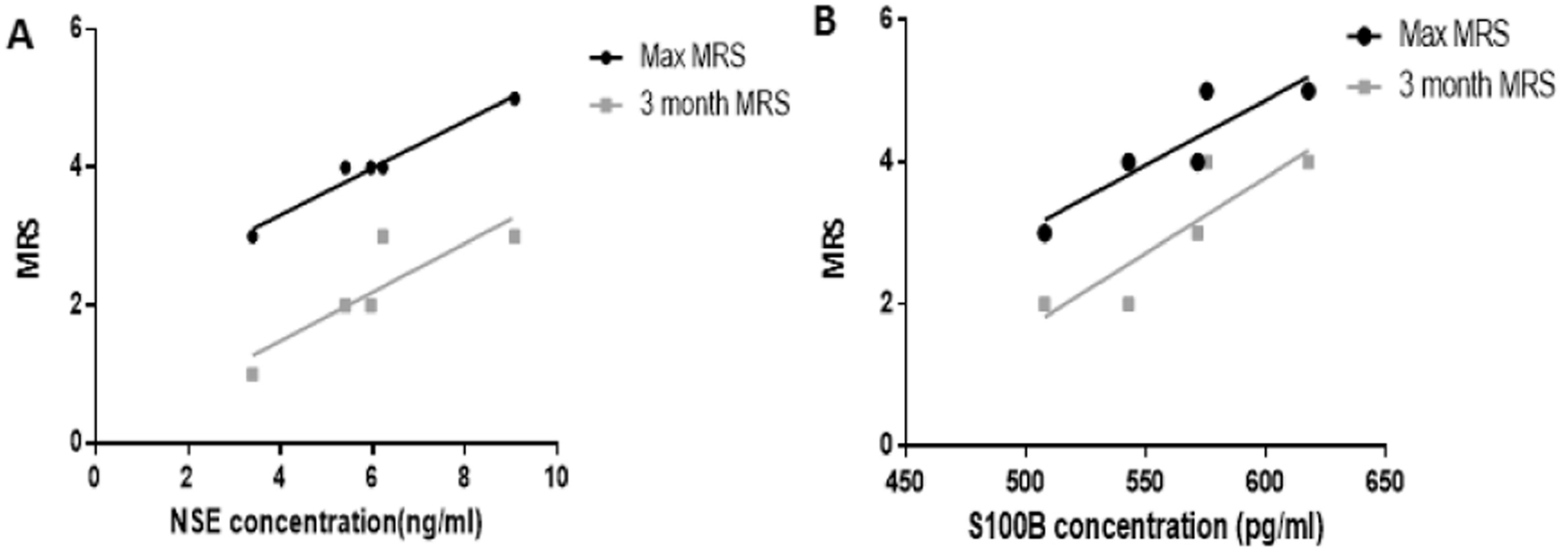
A
MRS
NSE concentration(ng/ml)
Max MRS
3 month MRS
B
MRS
S100B concentration (pg/ml)
Max MRS
3 month MRS

Figure 5

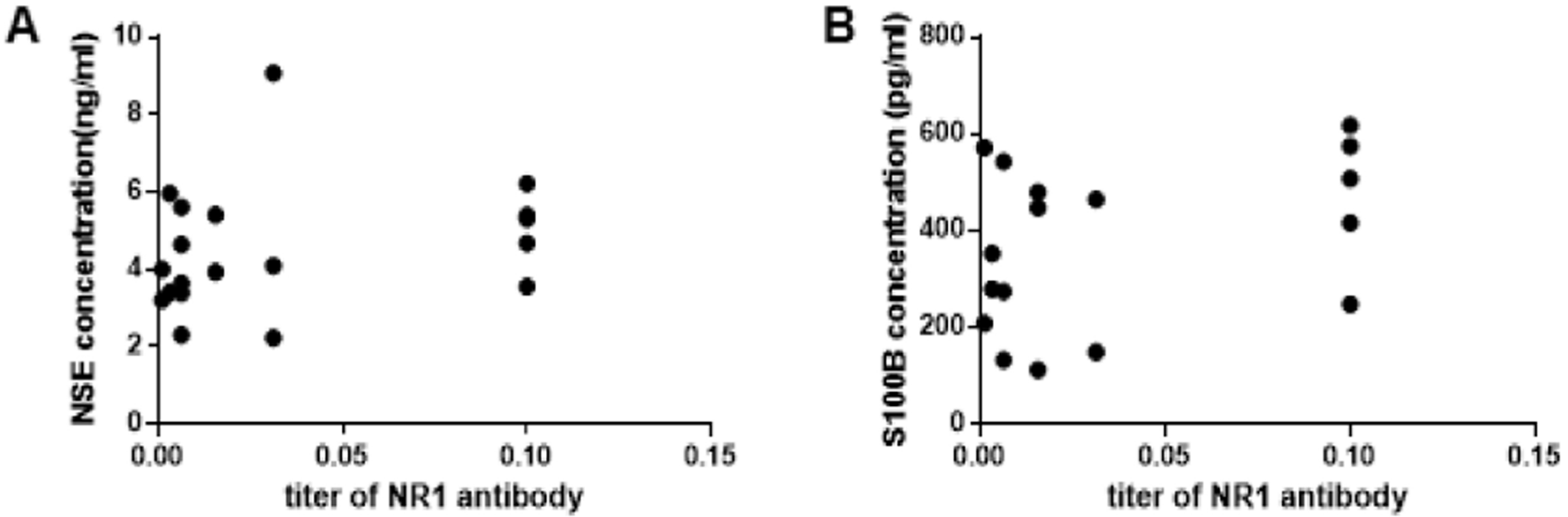
A
NSE concentration(ng/ml)
10
8
6
4
2
0
0.00
0.05
0.10
0.15
titer of NR1 antibody
B
S100B concentration (pg/ml)
800
600
400
200
0
0.00
0.05
0.10
0.15
titer of NR1 antibody

Figure 6