Characteristics of cerebrospinal fluid cytology in anti-N-methyl-D-aspartate receptor encephalitis

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Abstract

Background: The aim of the current study was to explore the characteristics of cerebrospinal fluid (CSF) cytology in anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis. Methods: CSF was collected from patients with anti-NMDAR encephalitis at Peking Union Medical College Hospital and Henan Provincial People's Hospital from 01 January 2015 to 31 December 2018. Cytological characteristics and other parameters of the CSF were analyzed. Results: CSF cytological data were obtained from 164 patients with anti-NMDAR encephalitis. Visible signs of inflammation were identified in cytological analyses of 112 patients' CSF, including 46 cases of mild inflammation, 58 of moderate inflammation, and 8 of severe inflammation. With regard to inflammation type, 89 cases were classified as lymphocytic inflammation, 22 as mixed inflammation with both lymphocytes and neutrophils, and 1 case was classified as mixed inflammation with lymphocytes, neutrophils, and eosinophils. Activated lymphocytes were detected in 51 patients, and plasma cells were detected in 16. Oligoclonal bands were detected in 111/164 patients, and 51 patients were positive for specific oligoclonal bands in CSF. The positivity rate was 45.9%. In non-pleocytosis subgroup based on routine CSF examination, mRS score was positively correlated with white cell count and degree of inflammation determined via CSF cytology (rho = 0.259, p = 0.047; rho = 0.264, p = 0.043, respectively), and patients with CSF inflammation detected by cytology were likely to have more serious disease severity (mRS>3) ($\chi 2 = 5.544$, p = 0.019) in comparison to patients with negative cytological findings. Conclusions: The superiority of evaluating cell morphology and the ability of detecting inflammatory state that has not been exhibited in routine examination render CSF cytology a candidate for identifying anti-NMDAR encephalitis and investigating its pathogenesis.

Background

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a treatable autoimmune disorder characterized by antibody-mediated loss of N-methyl-D-aspartate glutamate receptors, resulting in progressive mental deterioration with symptoms such as psychosis, memory deficits, seizures, dyskinesia, involuntary movements, reduced consciousness, and autonomic dysfunction [1, 2]. To date reports pertaining to anti-NMDAR encephalitis have been largely clinically oriented, including descriptions of the clinical presentation and course, diagnostic methods utilized, and potential clinical treatments. Notably however, the underlying molecular mechanisms contributing to the complex immunological cellular transformation that is associated with the progression of anti-NMDAR encephalitis remain to be adequately explored. A few studies have investigated cerebrospinal fluid (CSF) changes in patients with anti-NMDAR encephalitis, and abnormal alterations including mild to moderate lymphocytic pleocytosis, mild increases in protein concentration, and high rates of positivity for specific oligoclonal bands (OCBs) have been reported[1, 3]. Notably however, CSF cytology—which is a reliable and rapid technique for the initial diagnosis of central nervous system inflammation—has not been investigated in anti-NMDAR encephalitis. In the present study CSF cytology was examined in patients with anti-NMDAR encephalitis from two medical centers in China, in order to investigate CSF cytology characteristics associated with anti-NMDAR encephalitis.

Methods

Objective

The current study included patients with anti-NMDAR encephalitis from two clinical centers in China, Peking Union Medical College Hospital and Henan Provincial People's Hospital, recruited from 01 January 2015 to 31 December 2018. The inclusion criteria were (1) encephalitic signs with psychiatric symptoms, seizures, or focal neurological signs, (2) detection of anti-NMDAR antibodies in CSF. Exclusion criteria were human immunodeficiency virus infection, meningitis, brain abscess, prion disease, cerebral malaria, brain tumor, diagnosis of a noninfectious central nervous system disease such as acute demyelinating encephalomyelitis, or laboratory evidence of infectious encephalitis, *e.g.*, viral, bacterial, mycobacterium tuberculosis-associated, parasitic, or fungal[3]. General patient data and the results of routine CSF examination, biochemical tests, cytological investigations, and OCB tests were collated. All CSF data were obtained from patients who had acute or recurrent anti-NMDAR encephalitis. If single individuals had underwent lumber puncture multiple times, only the data at the first time were collected. The modified Rankin Scale (mRS) was used at admission to evaluate disease severity. The Institutional Review Board of the Peking Union Medical College Hospital and the Henan Provincial People's Hospital approved this study. All patients or patient guardians provided written informed consent for the clinical assessment and registration according to the registration project for encephalitis and paraneoplastic neurologic syndrome (medical ethics committee approval number JS-891).

Determination of antibodies to NMDAR

Lumbar punctures of all patients were performed within 3 days after they were admitted to the hospital, and serum and CSF samples were obtained simultaneously. Anti-NMDAR antibodies were evaluated using the indirect immunofluorescence test kit autoimmune encephalitis mosaic 1 (catalog number FA 112d-1, Euroimmun Ag, Germany) and used according to the manufacturer's instructions. Antibody titers in the CSF were recorded into four grades: mild positive(1:1 or 1:10), moderate positive(1:32), strong positive(1:100) and extensive positive(≥1:320).

CSF cytology

For cytological examination slides were prepared using a spontaneous sedimentation chamber (Sayk's sedimentation technique) with 0.5 mL CSF. First, 0.5 mL CSF was put into the spontaneous sedimentation chamber and stored overnight at 4℃, during which time the cells precipitated onto the slide. The slide was then air-dried and subjected to May-Grunwald-Giemsa staining. The morphology, classification, and numbers of white cells were determined under a light microscope[4, 5]. On the basis of cell counts determined via microscopy, degree of inflammation was divided into four levels: normal, ≤ 200 cells/0.5 mL; mild, 201–500 cells/0.5 mL; moderate, 501–2000 cells/0.5 mL; and severe, > 2000

cells/0.5 mL. The cell collection rate via natural sedimentation is approximately 15%. The proportions of various cell types present were calculated, and based on these proportions inflammation was classified as lymphocytic, lymphocytic and neutrophilic, or lymphocytic, neutrophilic, and eosinophilic. Levels of activated lymphocytes and plasma cells were also evaluated.

Other CSF examinations

Intracranial pressure was evaluated using a CSF pressure gauge, and pressure > 180 mmH_2O was considered “increased”. Integrated analyses included routine CSF examinations, biochemical CSF tests, and OCBs in both CSF and serum. Abnormally elevated cell counts were defined as total cell counts > 5×10^6/L without erythrocytosis, and CSF protein > 0.45 g/L was considered abnormally elevated. OCBs that were present in CSF but not in serum were considered specific OCBs, which are markers of autochthone intrathecal IgG production.

Statistical analyses

SPSS 21 statistical software was used to analyze the general patient data and the characteristics of CSF. Continuous values are expressed as means ± the standard deviation (SD) or medians with 25_{th} and 75_{th} percentiles, and dichotomous values are expressed as numbers and percentages. Correlation analyses were conducted to evaluate associations between mRS score and CSF parameters such as white blood cell count determined via routine CSF examination, degree of inflammation determined via routine CSF examination, white cell count determined via CSF cytology, degree of inflammation determined via CSF cytology and NMDAR antibody titer. Correlations between routine CSF examination results and CSF cytology results were also investigated, as were associations between plasma cells and specific OCBs. Spearman’s rank correlational analysis was used to assess relationships between two continuous variables in the case of non-normally distributed data or ranked data, the $\chi 2$ test was used to analyze associations between two categorical variables, and the kappa test was used for consistency analysis. Statistical significance was set at $p < 0.05$.

Results

General data

One hundred and sixty-four patients with anti-NMDAR encephalitis were enrolled in the study. Of them, 95 were from Peking Union Medical College Hospital and 69 were from Henan Provincial People’s Hospital. Ninety-four (57.3%) were female and 70 (42.7%) were male, yielding a female:male ratio of 1.34:1.00. The mean age was 25.85 ± 12.62 years (range 2–65 years). 18 women (19.1% of female) presented ovarian, while 1 woman thymoma, 1 man carcinoma. 43 patients (26.2% of all patients) have received

immunotherapy before lumbar puncture. The median mRS score was 4 (range 1–5). The general data of the enrolled patients were presented in table 1.

Table 1: The general data of the enrolled 164 patients with anti-NMDAR encephalitis

Variable	Value
Female (No, percentage)	94 (57.3%)
Age (year, median (25th, 75th percentile))	23 (17, 30.75)
Time since symptom onset (day)	30□15, 60□
Presence of tumor (No, percentage)	20 (12.2%)
Tumor types	
Ovarian teratoma (No, percentage of female)	18 (19.1%)
Thymoma (No, percentage)	1 (0.6%)
Carcinoma (No, percentage)	1 (0.6%)
First episode (No, percentage)	139 (84.8%)
Recurrence episode (No, percentage)	25 (15.2%)
Immunotherapy before lumbar puncture (No, percentage)	43 (26.2%)
mRS score (median (25th, 75th percentile))	4□3, 5□

mRS, modified Rankin Scale; NMDAR, N-methyl D-aspartate receptor

CSF cytology

All 164 patients underwent CSF cytology examination (Table 2), and lymphocytes and monocytes were detected in all samples (100%). Neutrophils were detected in 23/164 (14.0%) samples, and eosinophils were detected in 1/164 (0.6%). Activated lymphocytes were detected in 51/164 (31.1%) samples and plasma cells were detected in 16/164 (9.8%). Inflammatory reactions were evident in samples from 112/164 (68.3%) patients, of which 46 were deemed mild, 58 moderate, and 8 severe. With regard to inflammation type, 89 patients exhibited lymphocytic inflammation, 22 exhibited lymphocytic and neutrophilic mixed inflammation, and 1 exhibited lymphocytic, neutrophilic, and eosinophilic mixed inflammation. The proportion of neutrophils in the total number of leukocytes ranged from 1% to 80%. In 11 patients the proportion of neutrophils was < 10%, in 8 patients it was between 10% and 50%, and in 3 patients it exceeded 50%. In the 1 patient with lymphocytic, neutrophilic, and eosinophilic mixed inflammation there was 3% neutrophils and 2% eosinophils. Representative images of CSF cytology in patients with anti-NMDAR encephalitis are shown in Figure 1.

Table 1 The CSF results of 164 patients with anti-NMDAR encephalitis

Variables	Units	Value
CSF cytology (164 cases)		
Abnormal	Samples	112/164 (68.3%)
Inflammation degree in CSF cytology		
Mild inflammatory reaction	Samples	46/164 (28.0%)
Moderate inflammatory reaction	Samples	58/164 (35.4%)
Severe inflammatory reaction	Samples	8/164 (4.9%)
Inflammatory type		
Lymphocytic inflammation	Samples	89/164 (54.3%)
Lymphocytic and neutrophilic mixed inflammation	Samples	22/164 (13.4%)
Lymphocytic, neutrophilic and eosinophilic mixed inflammation	Samples	1/164 (0.6%)
Activated lymphocyte	Samples	51/164 (31.1%)
Plasma cell	Samples	16/164 (9.8%)
NMDAR antibody titers (142 cases)		
Mild positive	Samples	24/142 (16.9%)
Moderate positive	Samples	59/142 (41.5%)
Strong positive	Samples	54/142 (38.0%)
Extensive positive	Samples	5/142 (3.5%)
CSF routine examination (164 cases)		
White cell counts (range)	Cells ×10 6/L	0-242
White cell counts (median (25th, 75th percentile))	Cells ×10 6/L	10 (2, 34)
White cell counts (0-5)×10^6/L	Samples	59/164 (36.0%)
White cell counts (6-10)×10^6/L	Samples	26/164 (15.9%)
White cell counts (11-100)×10^6/L	Samples	71/164 (43.3%)
White cell counts > 100×10^6/L	Samples	8/164 (4.9%)
CSF protein (164 cases)		
CSF protein (range)	g/L	0.083 -1.93
CSF protein (median (25th, 75th percentile))	g/L	0.36 (0.26, 0.52)
CSF protein < 0.45g/L	Samples	110/164 (67.1%)
CSF protein (0.45-1)g/L	Samples	43/164 (26.2%)
CSF protein (1-1.5)g/L	Samples	8/164 (4.9%)
CSF protein > 1.5g/L	Samples	3/164 (1.8%)
CSF glucose (164 cases)		
CSF glucose (range)	mmol/L	1.38-7.0
CSF glucose (mean±SD)	mmol/L	3.46 ± 0.88
CSF glucose < 2.5mmol/L	Samples	9/164 (5.5%)
CSF chlorine (164 cases)		
CSF chlorine (range)	mmol/L	109 - 135
CSF chlorine (mean±SD)	mmol/L	123.46 ± 4.49
CSF chlorine <120mmol/L	Samples	23/164 (14.0%)
Intracranial pressure (146 cases)		
Intracranial pressure (range)	mmH2O	20 - 350
Intracranial pressure (median (25th, 75th percentile))	mmH2O	160 (130, 220)
Intracranial pressure >180mmH2O	Samples	54/146 (37.0%)
OCBs (111 cases)		
Specific OCBs positive	samples	51/111 (45.9%)

NMDAR, N-methyl-D-aspartate receptor; CSF, cerebrospinal fluid; OCBs, oligoclonal bands

Routine CSF examination

Intracranial pressure data were obtained from all 146 patients. High intracranial pressure was detected in 54 (37.0%), low intracranial pressure was detected in 5, and in 87 patients intracranial pressure was normal. Increased white cell counts in CSF were detected in 105/164 (64.0%) samples via routine CSF examination, with a median of 10 x 10^6 cells/L (range 0–242 x 10^6 cells). Severe pleocytosis, defined as CSF white blood cell counts ≥ 100 x 10^6/L, was only present in 8/164 (4.9%) samples (median 130 x

10^6/L, 25_{th} and 75_{th} percentiles 115.5 x 10^6/L and 210 x 10^6/L, range 101 x 10^6/L–242 x 10^6/L). All the above results are presented in Table 2.

CSF biochemical tests

As shown in Table 2, total protein levels in the CSF were elevated in 54/164 (32.9%) patients (range 0.46–1.93 g/L). In 43/164 (26.2%) patients elevated total protein levels of > 0.45 and < 1.0 g/L were detected, in 8/164 (4.9%) patients elevated total protein levels between 1.0 and 1.5 g/L were detected, and in 3/164 (1.8%) patients elevated total protein levels exceeded 1.5 g/L. CSF glucose levels were decreased in 9/164 (5.5%) patients (median 2.30, 25_{th} and 75_{th} percentiles 1.57 and 2.35 mmol/L, range 1.38–2.43 mmol/L). Chlorine was decreased in the CSF of 23/164 (14.0%) patients, with a median of 117 mmol/L (range 109–119 mmol/L).

Specific OCBs

Of the 164 patients in the study 111 underwent OCB examination, and in 51 patients specific OCBs were detected in the CSF (Table 2). The positivity rate in the 111 patients examined was 45.9%.

NMDAR antibody titers in CSF

As at the beginning of our work, some patients' NMDAR antibody titers were not been fully evaluated, finally 142 patients' NMDAR antibody titers in CSF were obtained, of which 24 were deemed mild positive, 59 moderate positive, 54 severe positive and 5 extensive positive (Table 2).

Correlations between mRS score and CSF parameters, and associations between plasma cells and specific OCBs

Spearman's rank correlations between mRS score and CSF parameters showed that mRS score was significantly correlated with NMDAR antibody titer in CSF (rho = 0.357, $p < 0.001$); however, mRS score was not significantly correlated with white cell count determined via routine CSF examination (rho = 0.130, $p = 0.096$), degree of inflammation determined via routine CSF examination (rho = 0.147, $p = 0.060$), white cell count determined via CSF cytology (rho = 0.120, $p = 0.125$), or degree of inflammation determined via CSF cytology (rho = 0.101, $p = 0.198$)(Figure 2 and Table 3). The χ2 test was used to assess the association between plasma cells and specific OCBs (Table 4), and the two parameters were not significantly associated ($\chi^2 = 0.831$, r = 0.086, $p = 0.362$).

Table 3: Correlations between mRS score and CSF parameters in patients with anti-NMDAR encephalitis[a]

	mRS score (n=164)	
	rho	*p* value
White cell count determined via routine CSF examination	0.130	0.096
Degree of inflammation determined via routine CSF examination	0.147	0.060
White cell count determined via CSF cytology	0.120	0.125
Degree of inflammation determined via CSF cytology	0.101	0.198
NMDAR antibody titer in CSF	0.357	<0.001*

[a] Spearman's rank correlational test

*p <0.05

mRS, modified Rankin Scale; NMDAR, N-methyl D-aspartate receptor; CSF cerebrospinal fluid

Table 4: Association[a] between plasma cells and specific OCBs in patients with anti-NMDAR encephalitis.

		Specific OCBs			Value
		Negative	Positive	Total	
Plasma cells	Negative	55	44	99	$\chi 2 = 0.831$
	Positive	5	7	12	r = 0.086
	Total	60	51	111	$p = 0.362$

[a]$\chi 2$ test

*$p < 0.05$

OCBs, oligoclonal bands; NMDAR, N-methyl-D-aspartate receptor

Correlations and comparisons between routine CSF examination and CSF cytology

White cell count determined via routine CSF examination was significantly positively correlated with white cell count determined via cytology (rho = 0.630, $p < 0.001$), and degree of inflammation determined via

routine CSF examination was significantly positively correlated with degree of inflammation determined via cytology (rho = 0.592, $p < 0.001$)(Table 5). In comparison of the detective ability of routine CSF examination at a positivity threshold of 5×10^6 white blood cells/L with that of CSF cytology, the difference was statistically significant ($p < 0.001$). The associated kappa value was 0.388 ($p < 0.001$), thus the consistency between the two methods was poor (Table 6).

Table 5: Correlations[a] between routine CSF examination and CSF cytology in patients with anti-NMDAR encephalitis.

	White cell count determined via cytology (n=164)			Degree of inflammation determined via cytology (n=164)	
	rho	*p* value		rho	*p* value
White cell count determined via routine CSF examination	0.630	*<0.001**	Degree of inflammation determined via routine CSF examination	0.592	*<0.001**

[a]Spearman's rank correlational analysis

*$p < 0.05$

CSF; cerebrospinal fluid; NMDAR N-methyl-D-aspartate receptor

Table 6: Analysis of the comparison and consistency between CSF cytology and routine examination in 164 patients with anti-NMDAR encephalitis.

	CSF cytology		χ2 test	Kappa test
	Positive	Negative		
CSF routine examination <=5×10^6/L	26	33	*χ2 = 24.974*	*Kappa* = 0.388
CSF routine examination >5×10^6/L	86	19	*p<0.001**	*p<0.001**

*$p < 0.05$

CSF; cerebrospinal fluid; NMDAR N-methyl-D-aspartate receptor

Correlations between mRS score and CSF cytology parameters in non-pleocytosis subgroup based on routine diagnostics

Correlations between CSF cytology results and disease severity in the non-pleocytosis subgroup based on routine diagnostics were also conducted(Table 7). In the total 59 patients those presented non-pleocytosis based on CSF routine examination, 26 samples showed inflammation in CSF cytology while 33 were negative. In this subgroup, mRS score was positively correlated with white cell count and degree of inflammation determined via CSF cytology (rho = 0.259, $p = 0.047$; rho = 0.264, $p = 0.043$, respectively), and patients with CSF inflammation detected by cytology were likely to have more serious disease severity (mRS>3) ($\chi 2 = 5.544$, $p = 0.019$) in comparison to patients with negative cytological findings (Figure 3).

Table 7: Correlations between mRS score and CSF cytology parameters in non-pleocytosis subgroup based on routine diagnostics[a]

	mRS score(n=59)	
	rho	*p* value
White cell counts determined via cytology	0.259	0.047*
Degree of inflammation determined via cytology	0.264	0.043*

[a] Spearman's rank correlational analysis

* $p < 0.05$

mRS, modified Rankin Scale; CSF cerebrospinal fluid

Discussion

Cytological inflammatory reactions were detected in approximately two thirds of the patients with anti-NMDAR encephalitis in the present study. The inflammation was most commonly mild to moderate, and a few patients exhibited severe leukocytosis. The most frequent type of inflammation was lymphocytic, but neutrophils were also seen in a few cases. Eosinophils were rare. Activated lymphocytes and plasma cells were detected in some patients. The positivity rate of specific OCBs in patients with anti-NMDAR encephalitis was 45.9%. In non-pleocytosis subgroup based on routine diagnostics, patients detected inflammation via CSF cytology were likely to have more serious disease severity (mRS>3) in comparison to patients with negative cytological findings. These CSF characteristics may contribute to the diagnosis of anti-NMDAR encephalitis, and future investigation of its pathogenesis.

Routine CSF examination and biochemical tests are typical initial investigations in patients suspected of encephalitis. Consistent with previously reported results[1, 6], many of the patients in the present study exhibited mild to moderate pleocytosis, but few exhibited severe pleocytosis via routine examination. Mild to moderate elevations in protein concentration were relatively common, and a few patients exhibited

mild decreased glucose and/or chlorine levels. The median number of white blood cells (10×10^6/L) and the median protein level (0.36 g/L) in the present study are significantly lower than those reportedly associated with infectious etiologies[7, 8]. The observation that in most patients glucose and chlorine levels were within normal ranges is also useful with regard to differentiating the diagnosis of anti-NMDAR encephalitis from infectious etiologies.

In evaluations of the correlation between CSF cytology and routine CSF examination, the current study showed that cytologically determined inflammation was positively correlated with pleocytosis determined via routine CSF examination. However, the consistency between the two methods was poor (kappa = 0.338). This indicates that though CSF cytology is a nice candidate for CSF routine diagnostics, abnormal CSF cytology is not inherently associated with pleocytosis determined via routine CSF examination. This lack of consistent parallel detection may be because the amount of CSF used in CSF cytology (500 μl) is far greater than that used in routine CSF examination (5 μL). It can be inferred from this that CSF cytology can provide more information on cell morphology and cell type proportions than routine CSF examination. In addition, in the present study more than a third of patients with anti-NMDAR encephalitis did not exhibit pleocytosis via routine CSF examination. This result is concordant with a previously reported case series in which initial non-inflammatory CSF varied widely in 15%–96% of patients at the first evaluation[9]. It is notable that initial non-inflammatory CSF evidently does not exclude a diagnosis of anti-NMDAR encephalitis, and in the past some patients may have been misdiagnosed leading to delays in the initiation of immunotherapy. The current study showed that in non-pleocytosis subgroup based on routine diagnostics, CSF cytology could detect some patients' inflammatory stage, and the disease severity of those patients was likely to be more serious. These results suggest that CSF cytology is a candidate for the detection of an inflammatory state that has not been detected via routine CSF examination.

CSF cytological characteristics suggest that lymphocytes and plasma cells play vital roles in immune processes involved in anti-NMDAR encephalitis. Lymphocytes include B cells and T cells. Following activation by B cell-activating factor[10], undifferentiated B cells can become fully differentiated B cells known as plasma cells, which secrete antibody[11-13]. In patients with anti-NMDAR encephalitis memory B cells reach the brain then undergo clonal expansion triggered by antigen stimulation, and differentiation into anti-NMDAR antibody-producing plasma cells[14]. The role of T cells in the immunopathogenesis of anti-NMDAR encephalitis has not been fully elucidated, but recent studies suggest that they are also involved[15, 16]. It has been reported that Th17 cells appear to support B cell responses outside germinal centers and promote antibody production more efficiently than their Th1 counterparts in animal models[16].

In the current study neutrophils were detected in patients with anti-NMDAR encephalitis via CSF cytology, and in a few patients the proportion of neutrophils was higher than the proportion of lymphocytes. Similar CSF changes have only been described previously in one case report[17] . The effects of neutrophils on the immune processes involved in anti-NMDAR encephalitis remain unclear. It has been reported that neutrophil depletion after subarachnoid hemorrhage improves memory via NMDARs[18],

and that in neuromyelitis optica spectrum disorders the presence of neutrophils is a very common characteristic of the associated lesions[19]. It has also been reported that complement activation by aquaporin-4-IgG results in a marked rise in the amount of circulating neutrophils, which can then enter the central nervous system and participate in early neuromyelitis optica lesion development via a neutrophil elastase-dependent mechanism[20]. Based on this, we speculate that neutrophils may also participate at least some of the immunopathology processes involved in anti-NMDAR encephalitis.

The relatively high rate of specific OCB positivity and the presence of plasma cells determined via CSF cytology in patients with anti-NMDAR encephalitis in the current study constitutes strong evidence supporting the concept that anti-NMDAR antibody was generated via intrathecal synthesis. The intrathecal source of pathogenic antibodies in anti-NMDAR encephalitis has been suggested to originate from $CD138^+$ plasma cells identified in perivascular and interstitial spaces in biopsy and autopsy studies[21, 22]. It has previously been reported that expanded intrathecal plasma cells may be the main source of intrathecal immunoglobulin, often detectable as OCBs[23, 24]. Malviya et al. [25] reported that $CD19^+$ B cells and $CD138^+$ $CD19^+$ plasma cells were significantly elevated in the CSF of patients with anti-NMDAR encephalitis, but not in their serum. These data suggest intrathecal B cell and plasma cell accumulation as a likely intrathecal source of antibody production in patients with anti-NMDAR encephalitis.

In some previous studies CSF titers of anti-NMDAR antibody were significantly correlated with outcome, and decline in CSF titer was positively correlated with relapse[1, 26]. The current study supported this view and showed that anti-NMDAR antibody titer in CSF was statistically correlated with disease severity. Therefore, we speculate that there may be a correlation between disease severity and the degree of inflammation evident in the CSF. Notably however, we only found significant relationship between mRS score and inflammatory parameters of CSF cytology in non-pleocytosis subgroup based on routine diagnostics. These results supported that CSF cytology is a helpful tool in detecting inflammatory state that has not been exhibited in routine CSF examination.

The present study had some limitations. There was no control group, and the data were derived from two centers. Furthermore, while the doctors who conducted CSF cytology analysis at Henan Provincial People's Hospital underwent training for at least 6 months at Peking Union Medical College, there may still have been subtle differences between the different evaluators from the two clinical centers.

Conclusions

In the present study investigating the cytological characteristics of CSF in patients with anti-NMDAR encephalitis, inflammatory reactions were detected in two thirds of patients. The most common type of inflammation was lymphocytic, but neutrophils were also seen in a few patients. A substantial number of eosinophils was only detected in 1 of the 164 patients in the entire study. Activated lymphocytes, plasma cells, and specific OCBs were detected in some CSF samples. CSF cytology could detect inflammatory state that has not been exhibited in routine diagnostics, and in this subgroup, the inflammatory

parameters determined via CSF cytology was positively correlated with disease severity. Such findings may constitute initial evidence of potential anti-NMDAR encephalitis, and may also facilitate better understanding of the immune mechanisms involved in the condition. The superiority for assessing cell morphology and the ability of detecting inflammatory state that presents non-pleocytosis in routine CSF examination render CSF cytology to be a candidate for the evaluation of anti-NMDAR encephalitis.

Abbreviations

NMDAR: anti-N-methyl D-aspartate receptor; CSF: cerebrospinal fluid; OCBs: Oligoclonal bands; mRS: modified Rankin Scale; SD: standard deviation.

Declarations

Consent

Written informed consent were obtained from all the patients or their guardians for publication of this work and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Study concept and design (HZ-G and HQ-L); evaluating of CSF cytology (HZ-G and XY-G); acquisition of data (HT-R, YY-S and JZ); analysis and interpretation of data (HQ-L and SY-F); drafting of the manuscript (HQ-L); Critical revision of the manuscript for important intellectual content (HZ-G, HT-R, WL and JW-Z); administrative, technical, and material support (HT-R, YH-Z, YY-S and XY-G). All authors read and approved the final manuscript.

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Figures

Figure 1

Representative images of cerebrospinal fluid cytology in patients with anti-N-methyl-D-aspartate receptor encephalitis (May-Grunwald-Giemsa stain, ×200). (A) and (B): Lymphocytic inflammation. (C) and (D): Lymphocytic and neutrophilic mixed inflammation. The red arrow indicates an activated lymphocyte, the yellow arrows indicate plasma cells, and the black arrows indicate neutrophils. The percentage of neutrophils in (C) is higher than that in (D).

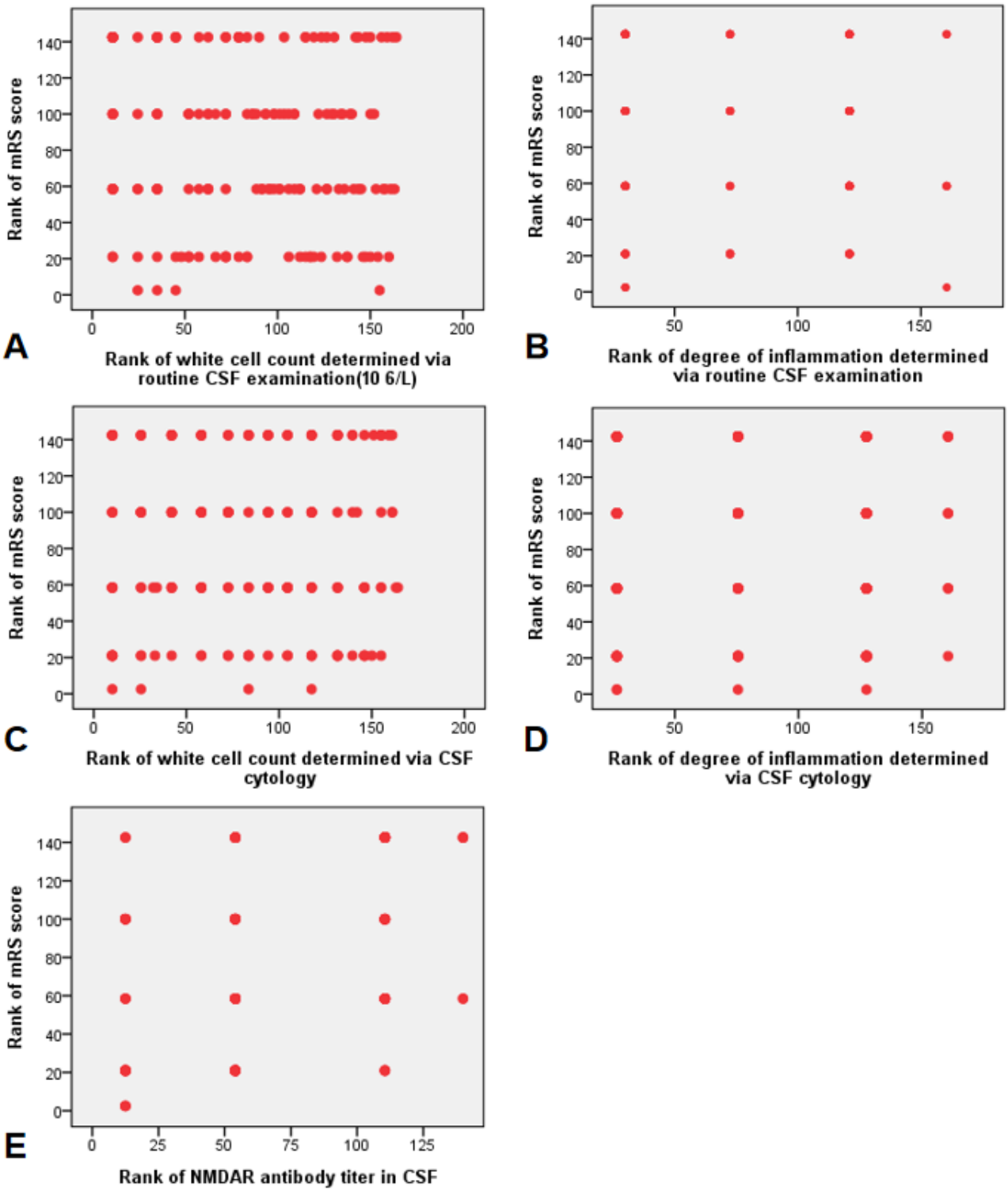

Figure 2

Correlations between mRS score and CSF parameters in patients with anti-NMDAR encephalitis. (A): Correlation between mRS score and white cell count determined via routine CSF examination. (B): Correlation between mRS score and degree of inflammation determined via routine CSF examination. (C): Correlation between mRS score and white cell count determined via CSF cytology. (D): Correlation

between mRS score and degree of inflammation determined via CSF cytology. (E): Correlation between mRS score and NMDAR antibody titer in CSF.

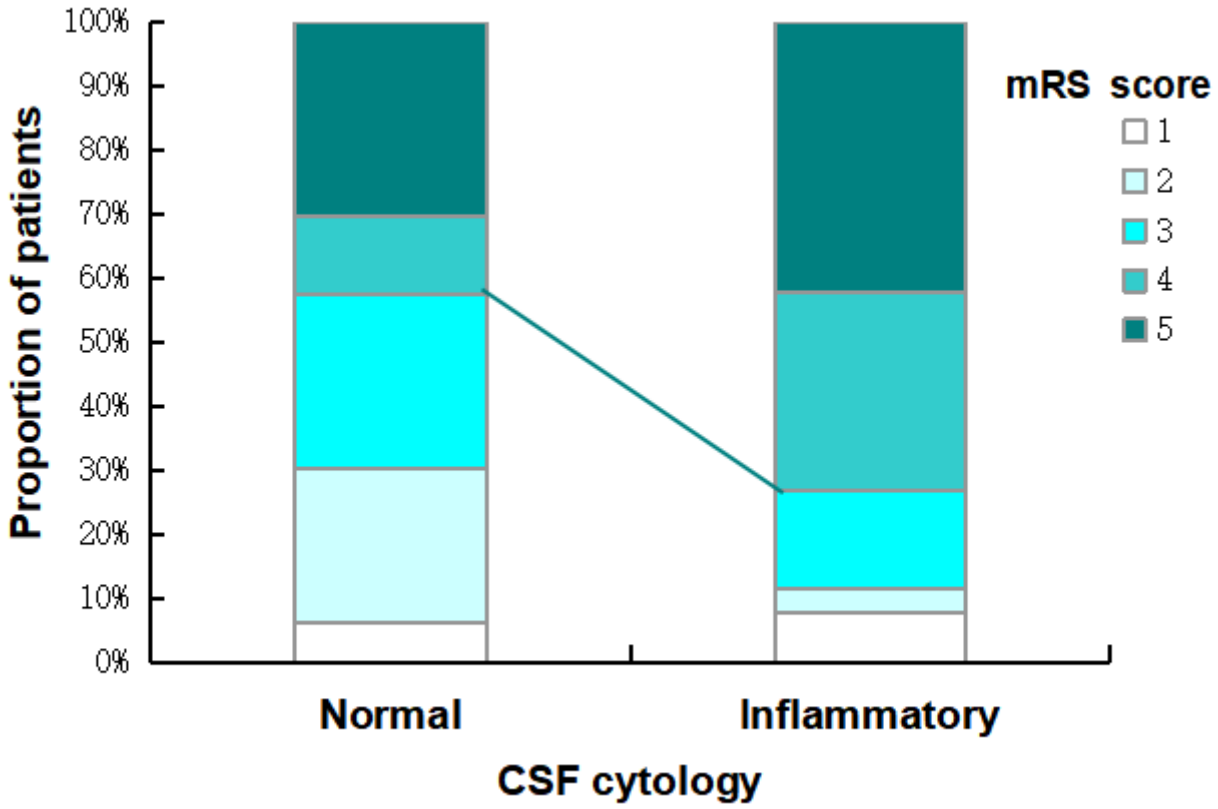

Figure 3

Distribution of mRS score of patients with normal or inflammatory CSF cytology in the non-pleocytosis subgroup based on routine CSF examination. mRS, modified Rankin Scale.