Original Article

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Clinical Significance of AQP4 Antibody Detection in Chinese Patients with Neuromyelitis Optica (NMO) and High-Risk NMO: A Meta-Analysis

Nagam Varshithreddy*, Yin Zhou*, Gu Yanzheng, Dong Wan-Li*, Fang Qi*, Xue Qun*,****

Abstract

Aim: To determine the prognostic value of AQP4 (aqua protein 4) antibody in the cohort of Chinese patients, and evaluate the diagnosis of AQP4 antibody detection inneuromyelitisoptica (NMO) and HR-NMO (high-risk NMO), and explore the treatment. *Methods:* China national Knowledge Infrastructure, Wanfang Data, China Biology Medicine disc, Chinesescience and technology journal database, PubMed, Embase and the Cochrane Library weresearched up to November 2014 for randomized control trails of related key words. The quality ofstudies was assessed according to the inclusion and exclusion criteria as recommended by the Cochrane Handbook for Systematic Reviews. Meta-analysis was conducted using STATA V.12. *Results:* Total 13 trials of 11 studies having 472 and 362 cases in NMO and HR-NMO groups were included and the numbers of AQP4 antibody positive were 350 and 168 casesrespectively. The meta-analysis showed: The total AQP4 antibody positive ratein NMO group was higher than HR-NMO group (RR=0.66y95%Cl (0.53, 0.83); after stratification, inthe cell based assay group (RR = 0.636, 95%CI (0.461,0.878), the AQP4-IgG positive rate in NMOgroup was higher than HR-NMO. In indirect immunofluorescence assay method group (RR =0.681, 95%CI (0.460,1.009), there were no significant differences in AQP4 antibody positive rate between two groups. The Egger linear regression and sensitivity analysis of these studies suggested no evidence of publication bias. *Conclusion:* The AQP4 antibody positive rate in NMO group was higher than in HR-NMO group of Chinese population; with a significant difference between the two groups.

Keywords: Demyelinating Disease; Neuromyelitisoptica; High-Risk NMO; Aquaporin-4 Antibody; Meta-Analysis.

Introduction

AQP4 antibodies (AQP4-Ab) have a clear clinical value in the diagnosis and differential diagnosisof demyelinating diseases [1]. There is extensive research related to AQP4 indemyelinating diseases in China. There are inconsistencies in methods of detection, equipment condition and other factors, that influence the results of AQP4-Ab in the NMO and HR-NMO patients, and also there is a major difference between the treatment and prognosis in the antibodies of HR-NMO patients [2,3]. Therefore, in this present systematic review, we collected studies published at home and abroad about the AQP4-Ab in Chinese population, the diagnosis and differential diagnosis of AQP4-Ab in NMO and HR-NMO groups. The purpose of this meta-analysis was to makean objective and reliable assessment for clinical differential diagnosis for NMO and HR-NMO serum, based on the AQP4-Ab positivity as treatment and prognosis vary for the two conditions.

Aim of the Study

To determine the prognostic value of AQP4 (aqua protein 4) antibody in the cohort of Chinese patients, and evaluate the diagnosis of AQP4 antibody detection in neuromyelitisoptica (NMO) and HR-NMO (high-risk NMO), and explore the treatment.

Author's Affiliation: *Department of Neurology, First Affiliated Hospital of Soochow University, Suzhou Jiangsu Province, 215006, P.R.China. **Clinical Immunology Key Lab of Jiangsu Province, First Affiliated Hospital of Soochow University, and Institute of Medical Biotechnology, Soochow University, Suzhou, Jiangsu, 215006, P.R. China.

Reprint Request: Nagam Varshithreddy, Department of Neurology, First Affiliated Hospital of Soochow University, Suzhou Jiangsu Province, 215006, P.R. China.
E-mail: nagamvarshith@hotmail.com

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Materials and Methods

Information Retrieval

We searched China National Knowledge Infrastructure (CNKI), Wanfang Data, China BiologyMedicine disc (CBM-disc), Chinese science and technology journal database (CSTJ), ChineseJournal of Neurology, Journal of Clinical Neurology, PubMed, Embase and the Cochrane Library, till Nov. 2014. Chinese and English search words included were: AQP4, longitudinally extensivetransverse myelitis (LETM), neuromyelitis-optica spectrum of disease (NMOSD), high-risk NMO, recurrent optic neuritis (RON), optico spinal multiple sclerosis (OSMS), transverse myelitis (TM)and optic neuritis (ON).

Inclusion and Exclusion Criteria

Document design types were limited mainly for randomized controlled trials (RCT's); theresearch literature that was retrieved were full text documents, the study population was limitedto Chinese population, language was limited only to Chinese and English.

Inclusion Criteria

1. NMO group: as referred by Wingerchuk et al [2] revised diagnostic criteria for NMO in 2006.
2. High-risk neuromyelitisoptica (LETM, RON, OSMS, ON or TM along with other autoimmune-diseases): a) long segmental myelitis, spinal MRI showing lesions of length > 3, with or withoutbrain lesions, and do not meet the diagnostic criteria for MS. b) recurrent optic neuritis withoutbrain lesions. c) optic neuritis with myelitis (segmental lesions <3), which does not meet thetypical MS brain lesions radiological diagnostic criteria. d) ON or TM patients accompanied withother autoimmune diseases, such as systemic lupus erythematosus (SLE), systemic siccativesyndrome (SSS). The patient's sex, age, race are not limited; determination of AQP4 methodwas not limited.

Exclusion Criteria

1. Case reports, case series, and cohort trials.
2. Animal experimental trials.
3. Trials in which the number of participants, duration of trial, and study location were not mentioned.
4. Trials lacking sufficient data.
5. Trials that were not RCTs.

Evaluation of the Literature

Two reviewers selected literature by Cochrane System Manual version 4.2.2, using the Jadadscore [4] of 7 points scoring system (1 to 3 points considered low quality, 4 to 7 points regardedas high quality) evaluation of all documents included in the quality score in randomized, diagnosticcriteria, the number of cases and so on. If the literature did not provide the required information,then the author was contacted for information on the research. Quality evaluation tests wereconducted independently by the two evaluators.

Data Synthesis and Analysis

Heterogeneity was assessed using the I^2 measurement, with I^2>0.3 indicating significan the terogeneity. We combined data from all trials using the Mantel-Haenszel model whenheterogeneity did not exist between aggregated trials. For dichotomous outcomes a risk ratio (RR)and the corresponding 95% confidence interval (CI) were calculated. A RR and 95% CI <1 indicate asignificant lower incidence in the intervention group, whereas, an RR and 95% CI >1 favour thecontrol group. There is no statistical significance when RR equals 1 or its 95% CI crosses 1between the groups. Whenever heterogeneity was found, the sources were analysed and trialswere considered for stratified analyses. For continuous data, the mean difference (MD) wascalculated, and P <0.05 was considered to indicate a statistically significant difference.

Assessment of Reporting Biases

To detect reporting bias, a funnel plot was constructed. However, if there were too few trials topermit proper evaluation of reporting bias, we used Begg and Egger tests to evaluate the potentialasymmetry of the funnel plot.

Statistical Analysis

All statistical analysis was performed using STATA V.12 (StataCorp LP, Texas, USA) for windows.According to the size of heterogeneity, different models were used to merge the effect value (fixedeffect model or random effect model). When there was no heterogeneity between the studies, afixed effects model was used. When there was heterogeneity between the studies, we analyzed theheterogeneous

source, and if necessary, large heterogeneous data was removed, even then ifheterogeneity still existed, the random effects model or subgroup analysis was used.

Results

Documents Incorporated Status

Our search returned 96 AQP4 clinical research literatures in demyelinating diseases. Aftersearching the title, reading the full text according to inclusion criteria, 85 studies did not meetthe inclusion criteria after the initial screening. The remaining 11studies were RCT's [4-15]. Two randomized studies used different methods [7,13] and they were also included in the Meta-analysis.

Meta-Analysis

The study included 13 trials of 11 studies in the NMO and HR-NMO group with a total of472 and 362 cases respectively. The positive AQP4 numbers were 350 and 168 cases respectivelyin both groups. The overall incidence ratio was higher in women than men.

T:total of the patients, +:the number of cases for AQP4-Ab positive, W:women, M:men.

Meta-analysis showed that the test for heterogeneity between studies of (I^2 = 72.6%, Z = 3.52, P <0.001), so theuse of the combined effect of the amount of random-effects model RR = 0.66, 95% CI (0.53, 0.83), the positive rate of two groups was statistically significant.

Table 1: The specific studies included and basic information

NMO+/T	NOMW/M	NOM Age	HR-NOM+/T	HR-NOMW/M	HR-NOM Age	Method	Sensitivity	Specificity
36/47	4.9:1	42±13	7/23	_		CBA	76.6	69.6
31/35	4:1	29.8	6/14	1.3:1	41.5(11-75)	CBA	88.6	57.1
52/72	_	_	6/22	_		CBA	72.2	72.7
12/14	6:1	35.2+10.1	15/22	1.4:1	41(14-79)	CBA	85.7	31.8
17/19	_	_	21/30	_		CBA	89.5	30
10/15	4:1	36±6	11/17	7.5:1	37±6	CBA	66.7	35.3
22/24	7:1	36(14-50)	19/22	2.5:1	33(9-35)	CBA	91.6	13.6
78/106	7.8:1	35(11-71)	28/84	3.2:1	43(11-76)	CBA	73.6	66.7
39/53	9.6:1	36(12-60)	12/24	2.5:1	38(12-76)	IIFA	86.8	71.4
20/34	2.1:1	38	11/24	_		IIFA	58.8	54.2
15/24	7:1	36	13/22	2.5:1	33(9-53)	IIFA	62.5	40.9
12/19	_	_	14/30	_		IIFA	63.2	53.3
6/10	9:1	45.8±9.7	5/10	2.3:1	47.4+13.6	ELISA	60	50

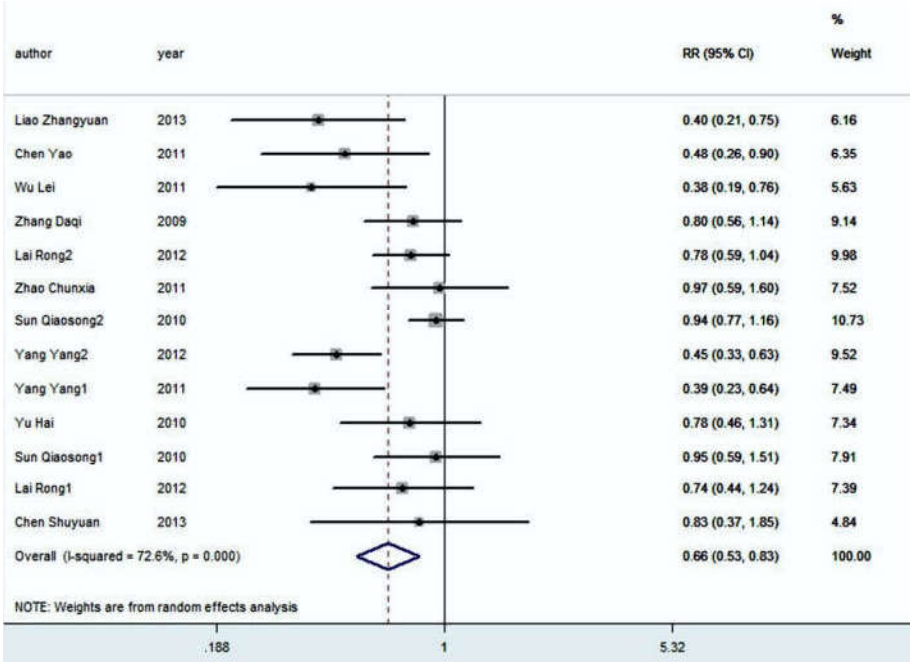

Fig. 1: Meta-analysis of trials comparing the effect of the positive rate of two groups

After the overall analysis using a random effects model, large heterogeneity was observed (I^2 = 72.6%, P <0.001), for further analysis of heterogeneous sources between the different methods of analysis, a subgroup analysis was performed.

The combined effect of the random effects model volume was grouped according to the measurement method. Meta-analysis: AQP4-Ab was higher in NMO group than HR-NMO group, which was statistically significant as measured by cell based assay (CBA) (I^2 = 82.0%, RR = 0.636, 95% CI (0.461, 0.878).

The positive rate of AQP4-Ab measured by Indirect immunofluorescence method (IIFA) and ELISA method was not statistically significant between the two groups (I2 = 59.5%, RR = 0.681, 95% CI (0.460, 1.009), (RR = 0.883, 95% CI (0.374, 1.855) respectively (Figure 2).

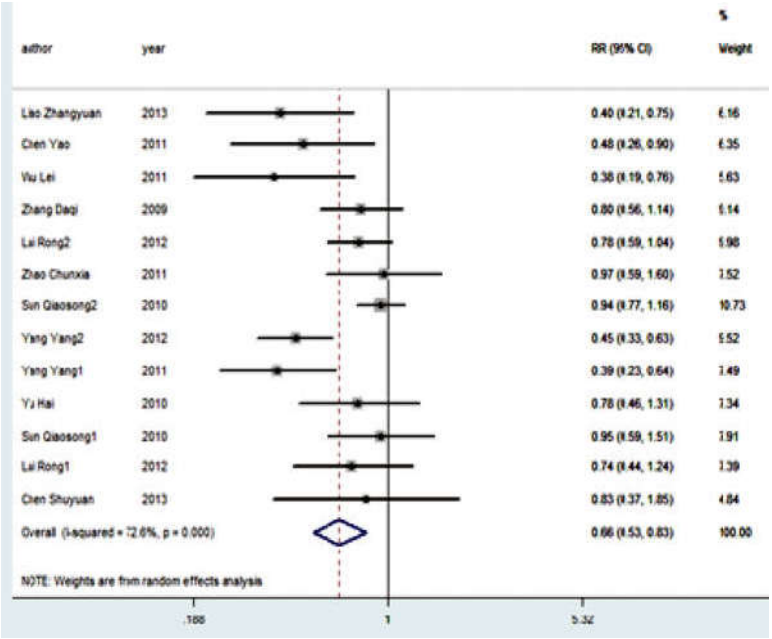

Fig. 2: Meta-analysis of trials comparing the effect of the positive rate of two groups with different methods

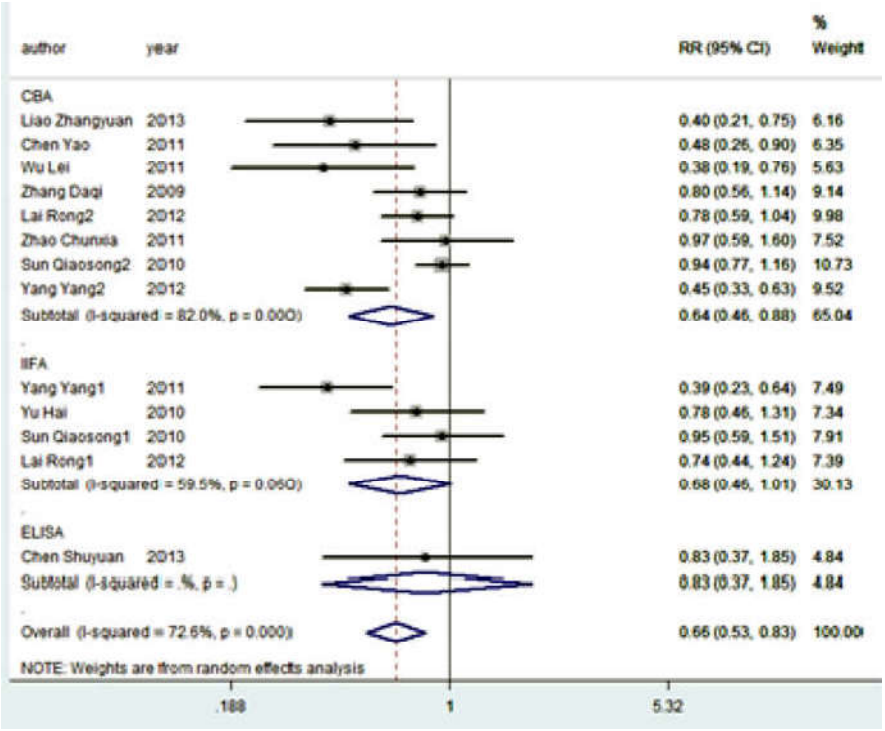

Fig. 3: Metatrim Funnel Plot for detection of publication bias

Egger Regression and Sensitivity Analysis

Detection of publication bias was analyzed by Egger law, where it intercepts a correspondingt-value, p-value, and 95% CI contains '0' to judge the existence of publication bias. If it intercepted acorresponding p <0.05 or 95% CI contains 0, suggesting that there may be some publication bias. This may be difficult to research and publish negative results; the methodological quality ofrelated literatures that were included in this study were not high. In this present study, t = -1.80, p =0.099, 95% CI = -4.2894 ~ 0.4298, therefore there was no existence of publication bias in theincluded studies.

After inclusion of studies, we conducted sensitivity analysis andestimated the overall average value of E = 0.264, 95% CI (0.192, 0.362), showing no significantgreater heterogeneity bias, indicating that positive AQP4-Ab rate clearly differentiates NMO andHR-NMO group respectively.

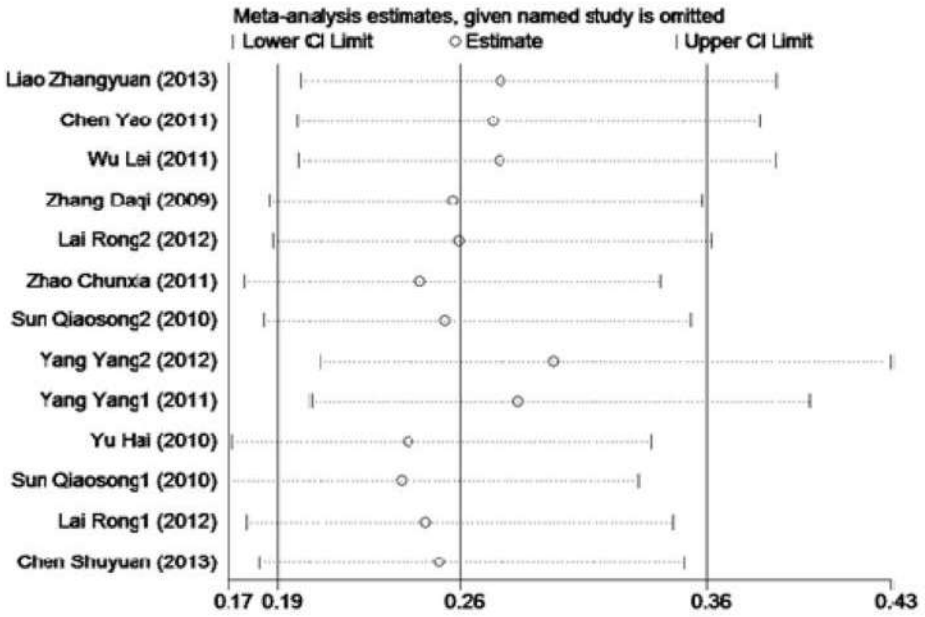

Fig. 4: Heterogeneity bias research with sensitivity analyzes for all studies included

Discussion

Neuromyelitisoptica (NMO) is a heterogeneous condition of the central nervous systemconsisting of recurrent and simultaneous inflammation and demyelination of the optic nerve andthe spinal cord [16,17]. NMO was once thought to be a subtype of multiple sclerosis (MS) [18], since the target antigen, aquaporin 4 (AQP4) and its specific antibody NMO-IgG was found,researchers gradually realized that NMO is distinct from of MS, and is an autoantibody-mediatedautoimmune disease [19]. In a subsequent study, it was found that NMOSD not only meets thediagnostic criteria for NMO, but also of recurrent optic neuritis (RON), accompanied by alongitudinally extensive transverse myelitis (LETM), optico spinal MS (OSMS), Sjogren's syndromeor systemic lupus erythematosus (SLE) and other autoimmune diseases, inflammation or opticneuritis, which is often called high-risk NMO (HR-NMO) [20]. 2010 European Federation of Neurology guidelines on diagnosis and treatment of NMOSD clearly defined and statedimmunopathogenesis of NMO lesions and clinical manifestations were similar, but not exactly in line with NMO, and made the detection of AQP-4 antibodies in serum orcerebrospinal fluid (CSF) in NMOSD diagnosis as the main support for its diagnostic standard [21]. To date, it is still a point of debate between East and West in the clinical diagnosis of NMO, hence, it is often difficult to classify OSMS, RON and LETM patients. Such kinds of patients are diagnosedas MS in Japan, but in North America and Europe, they are diagnosed as NMO [22]. For AQP4-Abpositive demyelinating disease of the central nervous system of this class, the current preferencefor the general diagnosis is neuro-myelitisoptica spectrum of disease [23].

However, in a study of HR-NMO patients, changes were observed in the serum antibody titers ofAQP4. AQP4-Ab positivity in serum or CSF may indicate worsening of the symptoms orconversion to NMO. In

a prospective study by Weinshenker BG et al [2], of 29 patients with LETM, 23 patients were followed-up for 1 year after the first attack; nine patients exhibited AQP4-Abpositive, of the 9 AQP4-Ab positive patients, the condition relapsed in five patients, and one patientdeveloped ON. In the remaining 14 AQP4-Ab positive cases, there was no recurrence or developmentof ON [2]. In a retrospective study by Matiello M et al [3], on recurrent optic neuritis (RON), AQP4-Abwas positive in 6 of 12 patients and experienced an episode of myelitis there by fulfilling the criteria for NMO, on the other hand, 1 of 15 AQP4-Ab negative patients experienced subsequent myelitis fulfilling the criteria for MS, and RON AQP4-Ab positive patients exhibited severe visualimpairment. Therefore, HR-NMO lesions may eventually progress after the onset of disease. Research on HR-NMO is of greater significance as it has bearing on the treatment and prognosis of patients [24]. Thus, in the demyelinating diseases of central nervous system, if the diagnosis of MS by MRI is not satisfactory, and also not in accordancewith the NMO criteria such as bilateral or recurrent ON, LETM, and other autoimmune diseases accompanying AQP4-Ab positive, the presence of AQP4 antibodies may help classify thesediseases, thus facilitating further treatment and prognosis [2,3].

Another data showed, in OSMS patients, those with AQP4-Ab positive and when the central graymatter of thoracic spinal cord was injured, associated with severe swelling in enhanced scan, itwas interpreted as NMO; whereas in AQP4-Ab negative OSMS patients, with cervical spinal cordinjury, the lesions were located in the surrounding white matter are more inclined to MS. However, in OSMS patients the presence of spinal cord injury in MRI, cannot be solely attributed for thediagnosis of OSMS NMO or MS [15]. Also it was found that some patients with LETM and RON withelevated serum NMO-IgG-positive, have a high proportion of the development of NMO after follow-up, a significant portion of these patients NMO spectrum may belong to the group ofdiseases, as variation of NMO or incomplete NMO [25]. At the same time a large number ofstudies have shown that, AQP4-Ab seropositive patients have developed serious ON or TM; therisk of recurrent disabling; and AQP4-Ab-positive patients need immunosuppressive therapy [26]. It is reasonable to assume that AQP4-Ab-positivity in patients with HR-NMO may havesome predictive value. It is suggested that HR-NMO patients, should be detected for AQP4 antibodies as part of routine clinical work, so that early diagnosis and appropriate treatment can be made.

In addition, AQP4-IgG NMO and NMOSD pathogenic role has been confirmed by in vitro passive transfer experiments, but studies have shown that in AQP4 gene, functional areas were detected in 15 SNP loci, of which two loci (rs3763043, rsl4393) were significantly higher in NMO patientsthan NMOSD patients. In the 33 cases of NMOSD patients, mutation in AQP4 exon was not found,which suggests that AQP4 gene may be associated with the pathogenesis of NMO, regardless ofthe NMOSD [27], however, there may be differences in the genetic background of patients. Simone Mader [28], studied the serum of 290 patients, including 30 patients diagnosed with NMO, 26patients with high-risk NMO, 101 patients with multiple sclerosis, 27 with clinically isolatedsyndrome (CIS), 30 with SLE, and 29 patients with other diseases of the central nervous system.

The sensitivity of the M-23 AQP4 IgG assay was 97% for NMO, 65% for HR-NMO, and thesensitivity with M-1 AQP4 transfected cells for NMO was 70% and 39% for HR-NMO, suggesting that different subtypes of AQP4 in NMO and HR-NMO group have some differences.

There may be essential differences between the HR-NMO andNMO, especially with regard to subtype of AQP4-Abpositive cases.

In recent years, AQP4-Ab detection achieved great progress and the main detection methods include:indirect immunofluorescence assay (IIFA) method, cell based assay (CBA) method, fluoro immunoprecipitation assay (FIPA), radioimmuno-precipitation assay (RIPA), enzyme-linked immunosorbent assays (ELISA) but inconsistencies may exist indifferent detection methods [29]. The sensitivity and specificity between the various methodsremains controversial and a gold standard remains to be elucidated. Additionally, NMO or HR-NMO patients from different geographical areas in clinical phenotypes have different characteristics [30], and the diagnosis and determination of antibodies will differ. The different method of determination may also be one of the sources for the local heterogeneity inthis study.

With the finding of AQP4-Ab, there is a new understanding to the pathogenesis, differential diagnosis and treatment methods in NMO. But as the AQP4-Ab detection time is relativelyshort in China, there is no long-term clinical follow-up data.

Thesource of heterogeneity between HR-NMO and NMO could not be explained fully due to the limited size of the study sample. But there are definitely differences between the rate of positivity for the antibody and more attention should be paid to the HR-NMO group as far as treatment and prognosis are concerned.

Conclusion

The AQP4 antibody positive rate in NMO group was higher than in HR-NMO group of Chinese population with a significant difference between the two groups.

Multi-centre, large sample comparative studies are required to clarify the types and pathogenetic mechanisms of HR-NMO and to address the role and status of AQP4-Ab needs to be studied further as it influences the outcome and prognosis of HR-NMO. The results will behelpful in improving the diagnostic standards and provide a basis for individualized treatment.

Acknowledgments

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Conflicts of Interest

The authors report no conflicts of interest.

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