Neutralising antibodies to interferon-beta therapy in relapsing multiple sclerosis: a pilot study

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KEY MESSAGES

1. In a cohort of Chinese patients with multiple sclerosis who received interferon-beta for ≥9 months, binding antibodies to interferon-beta was found in 78% of patients, whereas neutralising antibodies (NAB) were found in 28% of patients, based on an ELISA-based MxA protein induction assay.
2. Patients with NAB are six times more likely to respond poorly to interferon-beta, as evidenced by multiple relapses and extensive activity on magnetic resonance imaging.
3. MxA gene induction and protein induction assays are reliable screening and confirmatory tests for NAB.
4. Routine testing for NAB should be implemented for Chinese patients with multiple sclerosis to identify poor responders early.

Hong Kong Med J 2019;25(Suppl 5):S22-5
HMRF project number: 01120486

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Introduction

Multiple sclerosis (MS) is a disabling neurological disease affecting the central nervous system of young adults, and is the most common autoimmune inflammatory demyelinating disease worldwide.[1] Its prevalence in Hong Kong has risen by 40% from 4.8 per 100,000 persons in 2008 to 6.8 per 100,000 persons in 2015. Relapsing-remitting MS is the commonest presentation accounting for 80% of patients. For these patients, interferon-beta (IFN-beta) therapy remains the efficacious first-line treatment by reducing relapses, disability, and magnetic resonance imaging (MRI) disease burden. Up to 30% of patients with MS treated with IFN-beta may develop neutralising antibodies (NABs) to IFN-beta, which are associated with reduced treatment efficacy, disease relapse and progression.[2] Patients with persistent NABs and poor clinical response are recommended to switch to other disease-modifying treatment in most practice guidelines.[2]

To date there is no study on anti-IFN-beta and NAB in Chinese. We performed a cross-sectional study with an aim to develop and validate in-house assays to investigate the prevalence of anti-IFN-beta and neutralising antibodies among Chinese patients, and to study the association between NAB and clinical-radiological treatment response.

Methods

We recruited Chinese patients (aged ≥18 years) with relapsing multiple sclerosis who received IFN-beta (1a or 1b) for ≥9 months and evaluated the clinical response in terms of relapse and lesion progressions on MRI. All patients gave written informed consent. A standard proforma was used to collect data of demographics, relapse, functions (assessed by the Expanded Disability Status Scale by neurologists), type, dosage, route of administration, duration of IFN-beta and other disease-modifying treatments, diagnosis, and relapses. The presence of new T2-hyperintense lesions, contrast-enhancing lesions, and enlarging lesions was assessed using MRI by radiologists. The clinical status of patients was assessed by neurologists in terms of overall response to IFN-beta as: (1) doing well: no relapses, no or limited MRI activity, (2) intermediate disease activity: one relapse during therapy, no or limited MRI activity, and (3) doing poorly: one or multiple relapses and extensive MRI activity.

The sample size was estimated to be 80 by convenience sampling. Blood was collected for isolation of serum and total RNA extraction. RNA was extracted from collected blood samples for relative gene expression of myxovirus resistance protein (MxA). Total RNA extracted from PBMC or PAXgene blood were converted to cDNA and then by qRT-PCR assay for MxA protein relative gene expression analysis. Sera were tested for IFN-beta binding antibodies (BAB), and NABs by MxA gene induction assay and protein induction assay. Luciferase reporter gene assay and cytopathic effect

assay were used as internal and external reference, respectively. Assay performances were evaluated by receiver operating characteristic curve (ROC) analysis. Statistical analyses were performed using SPSS (version 22.0, IBM) and GraphPad Prism 5.03. A two-tailed P value of <0.05 was considered statistically significant.

Results

We evaluated 78 patients (62 female) with MS using IFN-beta for ≥9 months from 10 hospitals in Hong Kong (Table 1). Their median age was 35 years (interquartile range [IQR], 27-45 years). The mean disease duration was 5.2±4.2 years. The mean neurological disability (measured by the Expanded Disability Status Scale) was 2.0 (range, 1.0-3.0), indicating mild disability. The mean duration of IFN-beta use was 3.7±2.9 years. The most commonly used IFN-beta was IFN-beta-1a SC (Rebif, n=52, 67%), followed by IFN-beta-1b SC (Betaferon, n=15, 19%), and IFN-beta-1a IM (Avonex, n=11, 14%). The most common clinical status was doing well (n=48, 62%), followed by doing poorly (n=21, 27%) and intermediate disease activity (n=9, 11%).

TABLE 1. Characteristics of 78 patients with relapsing multiple sclerosis receiving interferon-beta

Characteristic	Value
Age, y	36.0±9.9; 35 (27-45)
Female	62 (80)
Disease duration, y	5.2±4.2
Interferon use duration, y	3.7±2.9
Expanded Disability Status Scale score	2.0 (1-3)
Type of interferon used	
Interferon-beta-1a SC (Rebif)	52 (67)
Interferon-beta 1a IM (Avonex)	11 (14)
Interferon-beta-1b SC (Betaferon)	15 (19)
Clinical status	
Doing well	48 (62)
Intermediate disease activity	9 (11)
Doing poorly	21 (27)
Presence of binding antibodies	61 (78)
Presence of neutralising antibodies	
Luciferase assay	10 (12.8)
20-100 TRU/mL	1 (1.3)
>100 TRU/mL	9 (11.5)
ELISA-based MxA protein induction assay	22 (28.2)
20-100 TRU/mL	12 (15.4)
>100 TRU/mL	10 (12.8)

* Data are presented as mean ± standard deviation, median (interquartile range), or No. (%) of patients

BAB was found in 61 (79%) patients who tended to be older (36.7 vs. 31.6 years, P=0.07), had higher Expanded Disability Status Scale score (2.1 vs. 1.3, P=0.06), and used IFN-beta for a longer duration (4.0 vs. 2.6 years, P=0.07). NAB was present in 22 (28.2%) patients. The titre was between 20 and 100, with tenfold reduction unit (TRU)/mL in 12 (15.4%) patients and >100 TRU/mL in 10 (12.8%) patients. Among various types of IFN-beta, NAB was present in 16/52 (31%) patients using Rebif (IFN-beta-1a SC), 2/11(18%) patients using Avonex (IFN-beta 1a IM), and 4/15 (27%) patients using Betaferon (IFN-beta-1b SC). The presence of NAB was not associated with age, gender, disease duration, types or duration of IFN-beta use (Table 2). However, the presence of NAB was associated with a higher number of clinical relapses after IFN-beta use (1.9 vs. 0.6, P=0.01) and poor clinical response (46% vs. 20%, P=0.03). In univariate analysis, patients with high titre NAB (>100 TRU/mL) were six times more likely to have poor outcome (odds ratio=6.3, 95% confidence interval=1.5-26.1, P=0.012). Nonetheless, no significant prediction was noted for intermediate NAB titre (TRU 20-100/mL) [odds ratio=2.4, 95% confidence interval=0.51-9.9, P=0.24). Relative MxA gene expression was significantly lower (0.13 vs. 0.40, P<0.01) in NAB-positive patients than in NAB-negative patients (P<0.01) using high titre cut-off of 100 TRU/mL.

Twelve (15%) samples were sent to Mayo clinic for external validation by cytopathic effect assay. For MxA protein induction assay, the concordance was 67% (8/12) for a lower titre cut-off (>20 TRU/mL) and 100% when using a high titre cut-off (>100 TRU/mL).

ROC analysis was performed for relative MxA gene expression assay, BAB ELISA, and NAB by MxA induction assay (Fig). For BAB, using ELISA-based MxA protein induction assay (titre >100 TRU/mL) to define the presence of NAB, the area under curve (AUC) was 0.81. At the clinical cut-off of 30 BTU, the sensitivity was 100% and specificity was 38%. For relative MxA gene expression assay and NAB (MxA protein induction assay >100 TRU/mL), the AUC was 0.79. At a cut-off of 0.20, the sensitivity was 90% and the specificity was 64%. MxA gene expression has a better performance as a screening assay when compared to BAB ELISA.

Using luciferase assay as internal reference benchmark for the presence of NAB, the ROC of NABs levels between luciferase assay and ELISA-based MxA induction assay had AUC of 0.92 (Fig). At a cut-off of 20 TRU/mL, the sensitivity was 90% and specificity was 83%. At a cut-off of 100 TRU/mL, the sensitivity was 90% and specificity was 88%. For external validation using Mayo Clinic CPE assay, our in-house MxA protein induction assay had 67% (8/12) concordant results low titre cut-off (20

TABLE 2. Association between clinical predictors and presence of neutralising antibodies (NAB) by MxA protein induction assay

	NAB positive (n=22)	NAB negative (n=56)	P value
Age, y	35.8±10.1	35.5±10.5	0.90
Expanded Disability Status Scale score	2.3±1.5	1.8±1.4	0.24
Disease duration, y	4.6±4.4	5.4±4.3	0.48
Duration of interferon use, y	3.2±3.0	3.9±3.1	0.38
Female	16 (73)	46 (82)	0.37
Types of Interferon used			0.68
Rebif	16 (73)	36 (64)	
Avonex	2 (9)	9 (16)	
Betaferon	4 (18)	11 (20)	
Magnetic resonance imaging changes			
New T2-hyperintense lesions	9 (41)	13 (23)	0.06
Contrast-enhancing lesions	7 (32)	11 (20)	0.14
Clinical relapse after interferon use (any)	12 (55)	18 (32)	0.08
No. of clinical relapse after interferon use	1.9±3.2	0.6±1.1	0.01
Clinical status			0.029
Doing well	10 (46)	38 (68)	
Intermediate disease activity	2 (9)	7 (13)	
Doing poorly	10 (46)	11 (20)	

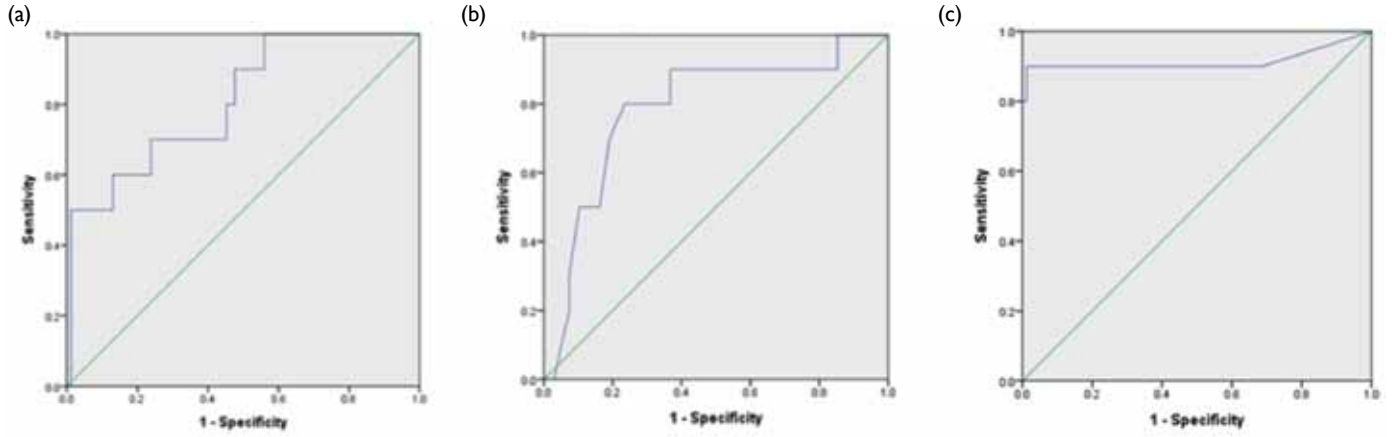

FIG. Receiver operating characteristic curves for (a) the presence of binding antibodies (BAB) and neutralising antibodies (NAB) using ELISA-based MxA protein induction assay, (b) relative MxA gene expression and NAB by ELISA-based MxA protein induction assay, and (c) NAB titres by ELISA-based MxA protein induction assay and luciferase assay

TRU/mL) and 100% concordant results with high titre (100 TRU/mL) cut-off. These results confirmed the reliability of MxA protein induction assay as a confirmatory test for NAB.

Discussion

We evaluated the performance, convenience, and reproducibility of assays for BAB and NAB to IFN-beta in a cohort of 78 Chinese patients with MS. BAB was present in 78% patients and NAB was found in 28% patients. Patients with high titres of NAB were six times more likely to have poor clinical outcome. This is the first study to determine the prevalence of NAB to IFN-beta in Chinese patients, and substantiate the need for routine clinical testing of NAB in patients treated with IFN-beta.

The prevalence of both BAB (78%) and NABs (28%) were comparable to those reported elsewhere. There were differences between NAB-

positive and NAB-negative patients in terms of clinical and MRI outcomes, especially in patients with high titres of NAB. This is consistent with the proposed detrimental effects of NABs on treatment efficacy, and thus a recommendation to switch therapy should be made. The subcutaneous preparations of IFN-beta had higher proportion (27%-31%) than intramuscular preparation (18%) although did not reach significance. High titres of BAB or very low levels of MxA relative gene expression are strongly associated with presence of NAB.[3] The immunological-clinical correlation is less pronounced for patients with low titres of NAB (20-100 TRU/mL). However, this cross-sectional study was unable to evaluate the changes of NABs over time, as low-titre NABs may disappear over time. Nevertheless, it is important to monitor the presence of NAB in patients with MS receiving IFN-beta, as presence of NAB strongly increases the risk of poor clinical response, and recommendation to switch therapy should be considered.

Conclusions

NABs are found in Chinese patients with MS and are associated with poor clinical outcome. MxA relative gene expression and protein induction assays are reliable and complimentary assays to test for NABs. Routine testing for NABs should be implemented to identify poor responders early in Chinese patients with MS using IFN-beta. The results can be used to develop cost-effective treatment algorithms to identify appropriate patients with MS for second-line therapies.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120486). We thank the neurologists and immunologists from different hospitals in Hong Kong for helping to complete this study. We also thank our research assistants, research nurses, technicians, and many others.

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