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Interpretation Criteria for Standardized Western Blot for the Predominant Species of *Borrelia Burgdorferi Sensu Lato* in China[1]

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Objective Western blotting (WB; immunoblotting) is a widely used tool for the serodiagnosis of Lyme borreliosis (LB), but so far, no generally accepted criteria for its performance and interpretation have been established in China. The present study was designed to determine the criteria for standardized Western blot for the predominant species of Borrelia burgdorferi sensu lato in China, in which WB was produced with strain PD_{91} as the representative strain attributed to predominant genospecies Borrelia garinii of *Borrelia burgdorferi sensu lato*. **Methods** Approximately 13 bands between 14 and 100 kD were differentiated for strain PD_{91} by using Gel-Pro analysis software. In a study with 631 serum samples (taken from 127 patients with Lyme borreliosis and 504 controls), all observed bands were documented. To establish criteria for a positive WB result for strain PD_{91}, receiver operating characteristic (ROC) curves were used. **Results** The following interpretation criteria were recommended: for IgG, at least one band of P83/100, P58, P39, P30, OspC, P17, P66, and OspA; for IgM, at least one band of P83/100, P58, OspA, P30, OspC, P17 or P41. In addition, syphilis, leptospirosis and other related diseases should be excluded when the positive band is P41 in IgM. For IgG criteria, the sensitivity is 73.2%, the specificity is 99.4% and Youden index is 0.726; for IgM criteria, the sensitivity is 50.6%, the specificity is 93.1% and Youden index is 0.437. **Conclusion** Standardization of WB assays is necessary for comparison of results from different laboratories. Moreover, the criteria of other genospecies of *Borrelia burgdorferi sensu lato* should be determined in the future to complete the criteria of WB for the diagnosis of the Lyme disease in China.

Key words: Criteria; Western blot; *Borrelia burgdorferi sensu lato*

INTRODUCTION

The Lyme disease, or Lyme borreliosis, is one of the most common tick-borne diseases and caused by different species of the *Borrelia burgdorferi senso lato*. The disorder develops in stages and with different manifestations involving mainly the skin, the nervous system, the joints, etc. The diagnosis of the Lyme disease is based on recognition of typical clinical signs and assisted by laboratory tests, especially when clinical symptoms are not emblematical. The only sign that could ensure a reliable clinical diagnosis of the Lyme borreliosis is erythema migrans (EM). Other features of some diagnostic value are earlobe lymphocytoma, meningoradiculoneuritis (Garin-Bujadoux-Bannwarth syndrome), and acrodermatitis chronica atrophicans (ACA).

Since culture or visualization of *B. burgdorferi* from patient specimens is difficult[1], the diagnosis has depended on recognition of a characteristic clinical picture with serologic confirmation. Serologic tests currently available for use in this disorder include screening tests like enzyme-linked immunosorbent assays (ELISA)[2-3], indirect immunofluorescence assays (IFA)[4-5] and confirmation tests like Western blots (WBs) or immunoblotting[6]. These tests are partially hampered by the occurrence of cross-reacting antibodies, leading to false-positive results, and patients may still be seronegative in early stages of infection. Furthermore, serological assays for the disease diagnosis have not been standardized so far, resulting in tests with various levels of sensitivity and specificity.

Immunological and molecular biological investigations have revealed and characterized a variety of borrelial antigens, including p83/100[7-10], p66[11], p41[12-13], p39[14-15], p17[16] and the outer surface proteins (Osp), such as OspA[17-18], OspB[19],

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OspC[20-23], OspD[24], OspE, OspF[25]. Heat shock proteins (Hsp) of the Hsp60 and Hsp70 families have also been analyzed[26-29].

The Western blot has been used by investigators to study the antibody response to *B. burgdorferi* infection with variable outcomes. This test has been reported to be more sensitive than ELISA for immunoglobulin M (IgM) detection[30] and can identify false ELISA reactions[31]. Standardization of the methodology and interpretations of Western blot is necessary for effective use of this assay in the serodiagnosis of the Lyme disease. In 1995 and in 1997, the United States[32-33] and Europe[34] separately established criteria for a positive WB result according to their different genomic species. In China, generally accepted criteria have not been established so far. The purpose of the current study was to establish criteria of Western blot for the diagnosis of the Lyme disease in China.

The interpretation criteria were evaluated for strain $PD_{91,}$ which is the representative strain attributed to predominant genospecies Borrelia garinii in China, and the specificities, sensitivities and Youden indexes for the most favourable bands-combination were compared. Criteria for interpretation of the IgM and IgG immunoblots for the serodiagnosis of the Lyme disease were proposed.

MATERIALS AND METHODS

Bacterial Culture and Antigen Preparation

Borrelial strain PD_{91} isolated from the blood of a patient with neuroborreliosis in Inner Mongolia in 1991 was used for the antigen preparation. PD_{91} was grown in Barbour-Stoenner-Kelly (BSK) medium at 33 ℃ for 4 to 5 days to reach a cell density of 10^7/mL. Low-passage PD_{91} (approximately 25 passages) was used for the Western blot. Cells were harvested by centrifugation at 12 000×g for 20 min at 4 ℃ and washed four times with 0.01 mol/L, pH 7.2 phosphate buffered saline (PBS). The protein concentrations of the final suspensions were estimated by the Bradford protein assay. The preparations were stored at -20 ℃.

Serum Samples

Serum samples from the following study groups comprising patients with the Lyme disease (*n*=127) and control (*n*=504) were investigated.

Forty serum samples from untreated patients with EM which had been collected for a former therapy study were obtained from a dermatologist. The median time period between the appearance of EM and the collection of serum samples was 3 weeks (ranging from 1 day to 31 weeks). The neuroborreliosis (NB) group (*n*=47) included 20 patients (designated group NB I) from whom *B. burgdorferi sensu lato* was isolated from the cerebrospinal fluid (CSF) and the other 27 patients (designated group NB II) with typical signs of acute NB, CSF, and CSF/serum antibody indices ⩾2.0. Cultures of CSF from 13 patients of the NB II group were negative. The serum and the CSF samples from the patient with NB were obtained on the same day. The median duration of neurological symptoms was 3 weeks (ranging from 3 days to 1 year) in group NB I and 4 weeks (ranging from 10 days to 6 months) in group NB II. The group with the late Lyme borreliosis (*n*=40) comprised 21 patients with ACA diagnosed by a dermatologist and 19 patients with Lyme arthritis. Possible differential diagnoses had been excluded. Serum samples from 200 healthy blood donors, 196 patients with syphilis, 68 patients with Leptospirosis and 40 patients with Rheumatoid arthritis (RA) served as controls. The healthy blood donors had no history of frequent tick bites and/or going to or living in epidemic areas, and interrelated symptoms, such as erythemas, neurological symptoms, or joint disorders.

SDS-PAGE and WBs

The prepared antigen was dissolved in an equal volume of lysing buffer (0.01 mol/L Tris hydrochloride, 10% glycerol, 2% sodium phenol blue, pH8), and heated for 5 min at 95 ℃. For preparative gels, the protein antigens (350 to 400 mg of protein/gel) of strain PD_{91} were electrophoresed separately by the protocol of Laemmli. Polyacrylamide gels (12.5%; acrylamide/bisacrylamide ratio, 39:1; 12 cm by 16 cm by 0.75 mm) and run at 4.0 to 4.5 mA for 24 h at room temperature. The proteins in the gel were transferred to nitrocellulose membrane for 3h at 24 V and 200 mA. Staining with Ponceau S served to control efficient and homogeneous protein transfer. After unspecific binding sites were blocked with 5% non-fat dried milk diluted with PBS (pH 7.2) for 24h at 4 ℃, they were cut into strips of 3mm in width, dried, numbered, and stored at 4 ℃.

Strips were incubated overnight at 4 ℃ in sera diluted with PBST (PBS, 0.01 mol/L, PH 7.4): Tween20=2000:1) by 1:25 for IgG WB and IgM WB, washed five times for more than 10 min each time with PBST, and then incubated with horseradish peroxidase-conjugated rabbit antihuman IgG and IgM antibodies, respectively (dilutions, 1:800 for IgG and 1:500 for IgM in the same diluent as the sera). After five washes of 10 min each, colour was developed by adding 4-Chloro-1-Naphthol and H_2O_2. The colour development was stopped when the strips of the reference serum sample reached a defined intensity (after approximately 5 min). To avoid biases due to

limitations of reproducibility, WB strips obtained from the same gel were always incubated with sera from subjects in the different study groups. Incubation was always done in parallel with one strip of each antigen in the same well. For high accuracy, strips were always fixed in the original position (as they were cut from the membrane) for documentation and analyses.

Analyses of WBs

The positions of bands (apparent molecular masses [apparent MMs]) were calculated by interpolation between MM marker lanes. Therefore, the molecular weights of the proteins were determined by using the Gel-Pro analysis software, version 3.0 (Pharmacia Biotech, Freiburg, Germany). In our 12.5% polyacrylamide gel system, another strategy was used. WB strips were incubated in the serum of a rabbit immunized by PD_{91}, which also served as an IgG-positive control and were used as MM markers. For this purpose, analytical gels and MM markers (Gibco BRL) in every fourth lane were blotted, the membranes were stained with Ponceau S, and the positions of the marker proteins were traced with a ballpoint pen. Data were imported into a data bank for further analyses (see definition of interpretation criteria for a positive WB result in the results section).

Statistics

Whenever appropriate, results were analyzed by Fisher's exact test (independent proportions) and McNemar's χ^2 test (paired proportions). All analyses were performed in a two-sided manner. In the study, receiver operating characteristic (ROC) curves, as a plot of sensitivity versus specificity, were constructed for the most common IgG bands and IgM bands in various phages of the Lyme disease. The ROC area was then determined for each curve and the optimal ROC curves marked the greatest ROC area.

RESULTS

Identification of Bands

It was a prerequisite for all further analyses to establish an unequivocal description of all bands. The WB patterns of different strains varied considerably, and homologous antigens of different strains migrated differently. Therefore, identification and designation of bands had to be performed separately for each strain.

As shown in Fig. 1A, two different strains had different protein antigens. PD_{91} strain was attributed to *Borrelia garinii* while FP_1 strain to *Borrelia afzelii*. It was almost homologous in protein antigens with a molecular weight above 36 kD in the two genospecies. However, OspA, OspB, and proteins with a low molecular weight, such as PC protein, manifested a high variety.

By using the Gel-Pro software, the following bands were identified as common bands in PD_{91} strain: P83/100, P75, P66, P60, P58, P43, P41, P39, OspB, OspA, P30, OspC, and P17 as shown in Fig. 1 B.

Fig. 2 clearly shows antigen proteins of PD_{91} strain in positive reference with the WB assay.

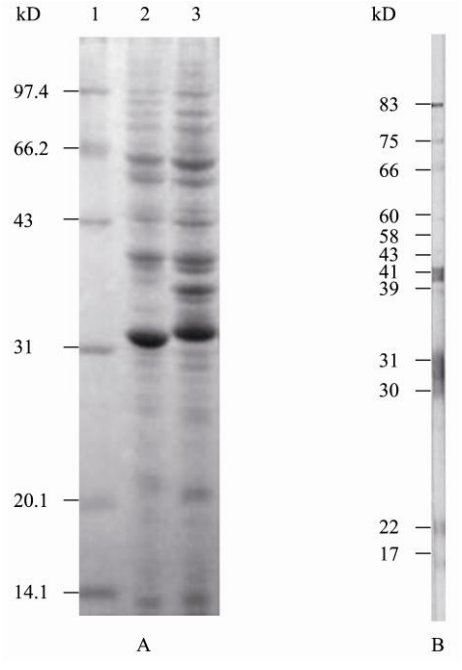

FIG. 1. (A) Coomassie brilliant blue-stained SDS- polyacryla mide gel of the antigen preparations used for this study. Lane 1, SDS-PAGE molecular weight markers for proteins; lane 2, strain PD_{91}; lane 3, strain FP_1. (B) Immublots of PD_{91} strain with positive rabbit serum. The molecular weight of the antigens were manifested on the left.

Immunblot

Table 1 summarizes the frequency of recognition of the bands in WB assay. The frequencies of band recognition in each study group with LB in comparison to those in the control group were analyzed statistically. Bands showing highly significant differences between patients and controls are boxed (P<0.05).

The antigens in *Borrelia garnii* in which cross-reaction occurred between Lyme disease patients and syphilis patients were 75 kD, 60 kD, 43 kD, 41 kD. The antigens in *Borrelia garnii* in which

cross-reaction occurred between Lyme disease patients and leptospirosis patients were 75 kD, 60 kD, 41 kD (Fig. 3).

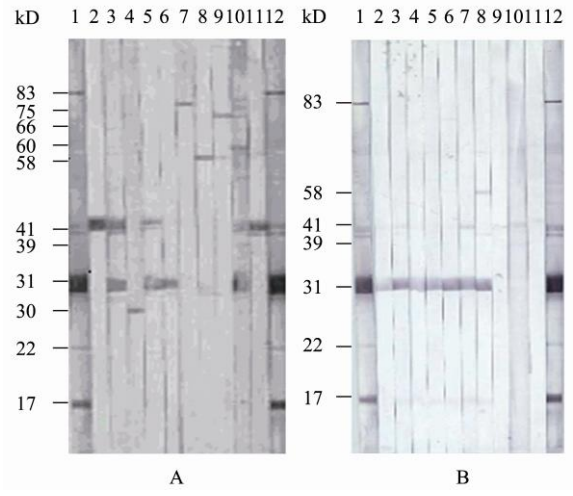

FIG. 2. Representative WBs of strain PD_{91} with sera from patients with various types of LB.A: IgG WB. Lanes 1 and 12, positive control; lanes 2-4, EM; lanes 5-8, NB; lanes 9-11, late Lyme borreliosis, B: IgM WB. Lanes 1 and 12, positive control; lanes 2-6, EM; lanes 7-11, NB.

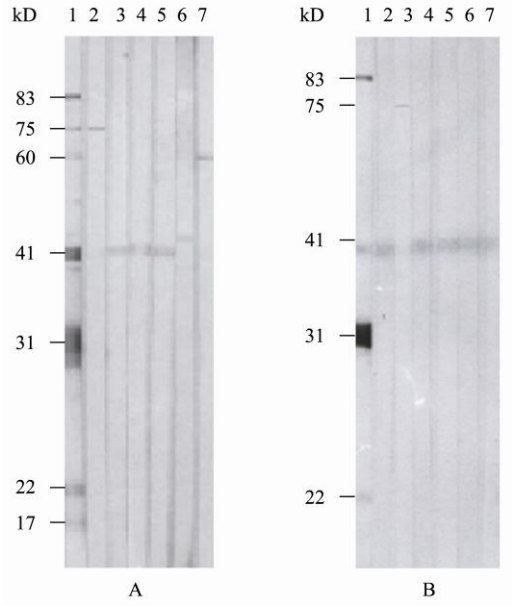

FIG. 3. (A) Cross-reaction between syphilis sera and *Borrelia burgdorferi* antigens with WB. 1, positive control; 2, 3, 6,7 IgG test; 4, 5 IgM test. (B) Cross-reaction between leptospirosis sera and *Borrelia burgdorferi* antigens with WB. 1, positive control; 2, 3, 4 IgM test; 5, 6, 7 IgG test.

TABLE 1

Frequency of Recognition of Proteins of Strain PD_{91}

Protein Band	Molecular weight (kD)	% Sera with IgG Reactivity to Protein Band[a]							% Sera with IgM Reactivity to Protein Band[a]					
		LB Sera			Control Group Sera				LB Sera[b]		Control Group Sera			
		EM	NB	Late	Syphilis	Health	Leptospi-rosis	RA	EM	NB	Syphilis	Health	Leptospi-rosis	RA
		n=40	*n*=47	*n*=40	*n*=196	*n*=200	*n*=68	*n*=40	*n*=40	*n*=47	*n*=196	*n*=200	*n*=68	*n*=40
P83/100	97	3	6	9					2	7				
	83	7	4	9					3	15				
P75	75	4	3	5	16		1		4	5	15		6	1
P66	66	3	4	6				1	1					1
P60	60	3	5	7	40	1	2	2	4	2	20	1	1	3
P58	58	4	4	13					2	3				
P43	43	3	4		18				3		17			
P41	41	22	17	25	34	5	9	1	20	8	19	3	12	1
P39	39	2	1	7					2	1				
OspB	35													
OspA	31	17	16	21		2			6	7				
P30	30	6	1	1					4	1				
OspC	22		1	2					1					
P17	17	5	2	8						1				

Note.[a]Frequencies of band recognition of sera from patients with LB versus those of sera from the total control group (*n*=504) were analyzed by Fisher's exact test. If $P<0.001$, frequencies were boxed. Zeros were not shown to improve clarity. Sera[b] from patients with late-stage LB were not tested for IgM.

Definition of Interpretation Criteria for A Positive WB Result

By means of database queries in which a subset of data was selected from the database, criteria consisting of combinations of reactive bands were evaluated systematically for their discriminating abilities. Since it was not possible to prove all conceivable combinations, the following strategy was used. Combinations of bands showing no false-positive reactions were evaluated first. Then, significant bands (P<0.05) obtained through testing the results for patients with EM or NB versus the results for the whole control group were added stepwise to the putative criteria. More than 20 band combinations were evaluated for IgG tests and 3 band combinations were evaluated for IgM tests.

ROC Curve

To establish criteria for positive Western blots, we constructed a ROC curve for each combination.

Table 2 and Table 3 summarized areas under ROC curves of different band combinations.

The following interpretation criteria were recommended: for IgG, at least one band of P83/100, P58, P39, P30, OspC, P17, P66, and OspA; and for IgM, at least one band of P83/100, P58, OspA, P30, OspC, P17, or P41. Statistically, the criteria are practicable in WB assays for the Lyme disease. However, it is inapplicable to a patient with a syphilis or leptospirosis which has a high cross-reaction with the Lyme disease. There will be a high false-positive rate according to the IgM criteria when the patient is affected with syphilis or leptospirosis, as a high cross-reaction takes place between the Lyme disease and syphilis or leptospirosis in 41 kD. So the criteria should be completed: for IgM, if P41 was positive, the patient could be diagnosed with the Lyme disease after syphilis and leptospirosis or other related diseases were excluded.

TABLE 2

Areas under ROC Curves for Different Combinations for IgG Test

Band Combination	Area under ROC Curve	SE	95%Confidence Interval
P83/100,P58,P39,P30,OspC,P17	0.752	0.029	0.695-0.809
P83/100,P58,P39,P30, OspC,P17,P75	0.752	0.029	0.695-0.808
P83/100,P58,P39,P30, OspC,P17,P66	0.779	0.028	0.723-0.834
P83/100,P58,P39,P30, OspC,P17,P60	0.784	0.028	0.735-0.847
P83/100,P58,P39,P30, OspC,P17,P41	0.788	0.027	0.735-0.842
P83/100,P58,P39,P30, OspC,P17,OspA	0.849	0.025	0.800-0.898
P83/100,P58,P39,P30, OspC,P17,P75,P66	0.772	0.028	0.716-0.827
P83/100,P58,P39,P30, OspC,P17,P75,P60	0.736	0.028	0.680-0.791
P83/100,P58,P39,P30, OspC,P17,P75,P41	0.789	0.027	0.736-0.843
P83/100,P58,P39,P30, OspC,P17,P75,OspA	0.848	0.024	0.800-0.896
P83/100,P58,P39,P30, OspC,P17,P66,P60	0.755	0.028	0.700-0.810
P83/100,P58,P39,P30, OspC,P17,P66,P41	0.809	0.026	0.757-0.860
P83/100,P58,P39,P30, OspC,P17,P66,OspA*	0.864	0.024	0.817-0.911
P83/100,P58,P39,P30, OspC,P17,P60,P41	0.781	0.027	0.729-0.834
P83/100,P58,P39,P30, OspC,P17,P60,OspA	0.823	0.025	0.773-0.872
P83/100,P58,P39,P30, OspC,P17,P41,OspA	0.800	0.027	0.749-0.870
P83/100,P58,P39,P30, OspC,P17,P75,P66,P60	0.753	0.028	0.698-0.807
P83/100,P58,P39,P30, OspC,P17,P75,P66,P41	0.803	0.027	0.751-0.855
P83/100,P58,P39,P30, OspC,P17,P75,P66,OspA	0.861	0.024	0.814-0.907
P83/100,P58,P39,P30, OspC,P17,P66,P60,P41	0.798	0.026	0.748-0.849
P83/100,P58,P39,P30, OspC,P17,P66,P60,OspA	0.837	0.025	0.788-0.885
P83/100,P58,P39,P30, OspC,P17,P75,P66,P60,P41	0.783	0.022	0.735-0.822
P83/100,P58,P39,P30, OspC,P17,P75,P66,P60,OspA	0.835	0.024	0.787-0.882

Note. *The largest area under ROC curve of band combination in IgG test.

TABLE 3

Areas under ROC Curves for Different Combinations for IgM Test

Band Combination	Area under ROC Curve	SE	95%Confidence Interval
P83/100,P58, OspA, P30, OspC, P17	0.672	0.037	0.600-0.744
P83/100,P58, OspA, P30, OspC, P17, P60	0.655	0.036	0.584-0.726
P83/100,P58, OspA, P30, OspC,P17,P41*	0.724	0.035	0.655-0.792

Note. *The largest area under ROC curve of band combination in IgM test.

TABLE 4

Evaluation of Various Interpretation Criteria for Positive WB Result

Ig Class	Bands Required	Sensitivity (%)	Specificity (%)	Youden Index
IgG	⩾1of P83/100,P58,P39,P30,OspC,P17	50.4	100	0.504
	⩾1of P83/100,P58,P39,P30, OspC,P17,P75	53.5	96.6	0.501
	⩾1of P83/100,P58,P39,P30, OspC,P17,P66	55.9	99.8	0.557
	⩾1of P83/100,P58,P39,P30, OspC,P17,OspA	70.1	99.6	0.697
	⩾1of P83/100,P58,P39,P30, OspC,P17,P75,P66	57.5	96.4	0.539
	⩾1of P83/100,P58,P39,P30, OspC,P17,P75,OspA	72.4	96.2	0.686
	⩾1of P83/100,P58,P39,P30, OspC,P17,P66,P60	59.1	90.9	0.5
	⩾1of P83/100,P58,P39,P30, OspC,P17,P66,OspA	73.2	99.4	0.726
	⩾1of P83/100,P58,P39,P30, OspC,P17,P75,P66,OspA	74.8	96	0.708
IgM	⩾1 of P83/100,P58,OspA,P30, OspC,P17	34.5	100	0.345
	⩾1of P83/100,P58,OspA,P30, OspC,P17,P60	35.6	95	0.306
	⩾1of P83/100,P58,OspA,P30, OspC,P17,P41	50.6	93.1	0.437

Note. Recommended interpretation criteria are boxed.

TABLE.5

Comparison of Different Criteria

Criteria	Strain	IgG		IgM	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
China	PD_{91}	73.2	99.4	50.6	93.1
	PKa2	50.8	96.5	36.9	97.2
Europe	Pko	56.1	97.9	42.3	98.6
	Pbi	56.1	97.2	40.3	97.9
America	B_{31}	83.0	95.0	58.5	93.0

DISCUSSION

Related researches have demonstrated that there are more than 10 different genospecies in *Borrelia burgdorferi*. Of the 10 different genospecies of *Borrelia burgdorferi*, only *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, and *Borrelia afzelii* have been identified as being pathogenic to humans[35]. It has been found that these three genomic species exist in China and *Borrelia garinii* is the predominant genospecies (68%), *Borrelia afzelii* comes second and *Borrelia burgdorferi sensu stricto* is rare. In this study, we chose the representative strain PD_{91} attributed to the predominant genospecies *Borrelia garinii* in China. Strain PD_{91} was separated from a typical patient with neuroborreliosis by scholars from the Institute of Infectious Disease Control & Prevention and the Chinese Center of Disease Control & Prevention (CDC). Strain PD_{91} has a stable passage and low variation, and its protein maps are comparatively clear and complete, which is convenient for sera diagnosis and statistics analyses.

For the diagnosis of the Lyme disease with WB, the following interpretation criteria were

recommended to be used in China: for IgG, at least one band of P83/100, P58, P39, P30, OspC, P17, P66, and OspA; and for IgM, at least one band of P83/100, P58, OspA, P30, OspC, P17, or P41. In addition, syphilis, leptospirosis and other related diseases should be excluded when P41 is a positive band in IgM.

WB as a potential confirmation test should be able to differentiate between true-positive and false-positive results and therefore needs a high specificity. These criteria accordingly have a high specificity (with 99.4%for IgG and 93.1% for IgM). In principle, an interpretation rule that requires at least two reactive bands among a given combination of bands will be more reliable than a rule that requires only one reactive band[36]. However, a criterion of at least two bands would lead to considerable loss of sensitivity (see the section of Results). Usually, sera from patients at a late stage LB show a broad band pattern on WB[37], and thus, stricter interpretation rules could be used for such samples. However, for routine purposes, use of different criteria for sera from patients at early and late stages of the disease does not seem to be reasonable. In this study, band intensity is little considered. More extended evaluation of different band intensities was performed in the previous study, but seemed not so beneficial[34, 37].

In the study, 97 kD protein and 83 kD protein are attributed to the protein P83/100. Since comparison of the P83/100 molecule with sequence from protein databases should show similarities in characteristics of eukaryotic cell structures, the P83/100 might mimic these structures and may, therefore, be involved in the immune escape mechanism of the pathogenic agent of the Lyme disease. The recombinant P83/100 protein can produce IgG and IgM and be used as a specific antigen in the sera diagnosis, as shown in the criteria[9]. In terms of the molecular weight, OspA is 31 kD, OspB 35 kD, and OspC 22 kD. The outer surface proteins are only rarely detected by antibodies in the sera and consequently could not contribute to the sensitivity of the blots. However, in this study, OspA was strongly reacted, probably due to different strains used and thus, served as a major antigen in the criteria. Some proteins in the range from 66 000 to 73 000 were found to possess homologies to a known heat shock protein. A 60 kD protein was recognized by cross-reactive antibodies to many other related and nonrelated bacterial species, as also reported by Bruckbauer *et al*.[38]. In the study, cross-reactions occurred between the Lyme disease and syphilis and leptospirosis in the antigens of 75 kD and 60 kD. The deduced amino acid sequences of P66 in different Lyme disease borreliae were 92%-94% identical and had no horologes in the database. Database searches revealed that the P66 gene sequence was homologous to a previously reported gene fragment of unknown functions[10]. The P41 flagellin of *B. burgdorferi* is the most common antigen recognized by the serum of patients with Lyme borreliosis. This antigen shares amino acid homology, particularly in the amino and carboxy termini, with periflagellar antigens found in other microorganisms, including Treponema pallidum. The cross-reaction occurred in 41 kD between the Lyme disease and the control group as shown in the results. The 41 kD protein flagellin has a high immune quality. In patients with *B. burgdorferi* infection, the first detectable immune responses were directed to the flagellin and this antigen was important for sero-diagnosis, especially in the early Lyme disease. Few researches have reported on the cross-reaction in 43 kD and so further studies will be largely needed.

When the criteria were used, the positive rate of patients with the Lyme disease was high and false-positive rates of controls were comparatively low. The largest area under the ROC curve for IgG and for IgM was 0.864 and 0.724 respectively, and when that was between 0.7-0.9, the accuracy of the criteria was comparatively moderate. The Youden index was also the highest in the different combinations of most common bands, and so it may be suited to diagnose the Lyme disease in China.

In 1995 and in 1997, the United States and Europe separately established criteria for a positive WB result according to their different genomic species. In China, genomic species of *Borrelia burgdorferi* are different from those in other countries. It seems that the immune response of patients with LB in China is more restricted than those in North America and in Europe. Furthermore, the main protein antigens are also different. For these reasons, the interpretation criteria for positive WB results recommended by scholars in North America and in Europe cannot be used in China. Direct comparison of studies by different investigators is not possible, since a variety of strains and different WB protocols have been used.

Several technical problems of Western blot with *B. burgdorferi* should be stressed. First, the molecular weight of the same protein may be somewhat different depending on the strain of the spirochete or the conditions of the assay. Second, multiple proteins may simultaneously migrate to the same area. Third, the number of bands apparent in the blot is influenced by the concentration of reagents, and the results of Western blot are observer-dependent. Therefore, care must be taken to read the correct molecular weights of the bands, and faint bands may pose interpretation difficulties. Video densitometry may help with

resolution of this problem, but it is not suitable for reading miniblots or for reading bands that are close together, which is a common problem in the Lyme disease. Although the use of recombinant borrelial proteins may improve specificity, sensitivity may not be enhanced in this way.

In conclusion, with clearly defined interpretation criteria, Western blot is considered a useful method for the confirmation of positive results obtained through standard assays. The criteria of other genospecies of *Borrelia burgdorferi* should be determined in the future to complete the criteria of WB for the diagnosis of the Lyme disease in China.

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