Original Article

Severe Novel Influenza A (H1N1) in Shanghai: Clinical Features, Therapeutic Management and Risk Factors for Mortality

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Objective To analyze the clinical features, therapeutic management and risk factors for mortality of patients with severe novel A (H1N1) influenza in Shanghai, China.

Methods All patients were diagnosed by influenza A (H1N1) virus mRNA detection. Chest CT scan, routine blood, hepatic function, humoral and cellular immunity, sputum smears, and sputum cultures were performed. Logistic analysis was applied to identify risk factors for mortality.

Results Total of 68 patients were enrolled in this study, the primary clinical symptoms including cough (66, 97.1%), expectoration (41, 60.3%), and polypnea (41, 60.3%). Altogether, 37 (54.4%) and 11 (16.2%) patients were infected with bacterial and fungal, respectively. CT scan demonstrated that 67 (98.6%) patients had pneumonia. Oxygen therapy, oseltamivir, antibiotic and antifungal drugs were performed in 68 (100%), 66 (97.1%), 39 (57.4%), and 11 (16.2%) patients, respectively. Finally, 4 of 68 patients died. Logistic analysis demonstrated that there was a significant correlation between the percentage of neutrophils and mortality before therapy and direct bilirubin content and mortality after therapy, respectively.

Conclusions Patients with severe H1N1 influenza were susceptible to bacterial and/or fungal infection. The risk factors for mortality may be associated with pre-therapeutic neutrophil percentage and post-therapeutic direct bilirubin content.

Key words: Novel A (H1N1) influenza; Treatment; Risk factor; Mortality; Acute respiratory distress syndrome

A novel influenza A (H1N1) virus emerged in Mexico and the United States between late March and mid-April of 2009, and rapidly spread worldwide. On August 10, 2010, the WHO announced that the pandemic H1N1 influenza was over and the virus had moved into the post-pandemic period.¹ Even in the post-pandemic period, local outbreaks show significant levels of H1N1 transmission. Mainland of China was in high-incidence season of influenza at present. According to official news, a total of 3833 cases of H1N1 influenza A, among which 15 died, had been reported in January 2011, which reaching up to more than 50% of those reported in 2010. This study retrospectively analyzed the clinical features, disease course, hospitalization period and negative seroconversion duration of viral nucleic acid following antiviral treatment, and risk factors for mortality of 68

confirmed pandemic influenza H1N1 2009 severe/critical patients hospitalized in Shanghai Public Health Clinical Center between November 2009 and July 2010.

METHODS

Diagnostic criteria

In addition to positive novel H1N1 influenza virus nucleic acid testing, patients who met any one of the following clinical situations were defined as severe cases: (1) persistent fever > 3 days; (2) severe cough, purulent sputum, bloody sputum or chest pain; (3) polypnea/tachypnea, dyspnea, cyanosis of lips; (4) consciousness changes: unresponsive, drowsiness, restlessness, convulsions; (5) severe vomiting, diarrhea, dehydration performance; (6) imaging examinations showing signs of pneumonia; (7) the level of creatine kinase (CK), creatine kinase-MB (CK-MB) and other enzyme increased rapidly; (8) aggravation of underlying diseases. Patients who met any one of the following clinical situations were defined as critical cases: (1) respiratory failure; (2) toxic shock syndrome

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caused by infection; (3) multi-organ dysfunction; (4) other severe clinical conditions in need of monitoring and urgent treatment.

Sixty-eight patients were enrolled in the study, 54 of whom were severe and 14 critical. All 68 patients were diagnosed by influenza A (H1N1) virus RNA detection at their first visit to the Center for Disease Control and Prevention (CDC) of Shanghai. RNA was assessed by quantitative reverse transcription polymerase chain reaction (RT-PCR). Primers and oligonucleotide probes: Forward, 5'-GTA CTA TAA ACA CCA GCC TYC CA-3'; Reverse, 5'-CGG GAT TTA CCT TAA TCC TGT RGC-3'; Probe, 5'-CA GAA TAT ACA TCC RGT CAC AAT TGG ARA A-3'; Y = C or T; R = A or G.

Chest CT scan

All patients underwent a chest CT scan immediately after hospital admission, and once more during the treatment procedure or before discharge in order to evaluate the reduction of lung-tissue inflammation.

Table 1. Demographic information for the patients

	n (%)		n (%)
Genders		Underlying diseases	
Male	51 (75.00)	Obesity	30 (44.10)
Female	17 (25.00)	Hypertension	16 (23.50)
Male to female ratio	3 : 1	Diabetes	4 (5.90)
Age (years)		Hyperlipemia	2 (2.90)
17-40	31 (45.60)	Steatohepatitis	5 (7.40)
41-60	33 (48.50)	Chronic hepatitis B	4 (5.90)
>60	4 (5.90)	Post-cholecystectomy	3 (4.40)
Mean age	40.80 ± 15.20	Post-appendectomy	2 (2.90)
Clinical manifestations		Chronic gastritis	1 (1.50)
Cough	66 (97.10)	Post-subtotal gastrectomy	1 (1.50)
Expectoration	41 (60.30)	Peptic ulcer	1 (1.50)
Polypnea	41 (60.30)	Upper gastrointestinal bleeding	1 (1.50)
Poor appetite	38 (55.90)	Congenital cardiac disease	1 (1.50)
Fever	20 (29.40)	Viral myocarditis	1 (1.50)
Myalgia	12 (17.60)	Arrhythmia	1 (1.50)
Cough with pink frothy sputum	10 (14.70)	Epilepsy	1 (1.50)
Sore throat	9 (13.20)	Cerebral infarction	1 (1.50)
Chills	7 (10.30)	Gout	1 (1.50)
Diarrhea	3 (4.40)	Ankylosing spondylitis	1 (1.50)
Rhinorrhea	2 (2.90)	Lymphoma	1 (1.50)
Nasal obstruction	1 (1.50)	Thyroid adenoma	1 (1.50)
Headache	1 (1.50)	Necrosis of the femoral head	1 (1.50)
Signs		Sleep apnea syndrome	1 (1.50)
Temperature (°C, armpit)		Anemia	1 (1.50)
Normal (<37.30)	48 (70.60)	Average delay time from symptom onset to antiviral treatment	6.93 ± 2.84
Mild fever (37.30–38.00)	13 (19.10)	Negative duration of the nucleic acid (days)	3.83 ± 2.17
Medium fever (38.10–39.00)	3 (4.40)	Hospitalization period (days)	10.85 ± 5.37
High fever (39.10–41.00)	4 (5.90)		
Super high fever (>41.00)	0 (0.00)		
Congestion of the throat	26 (38.20)		
Cyanotic lips	16 (23.50)		
Difficulty breathing	2 (2.90)		
Moist rales in lungs	47 (69.10)		
Rough breathing sounds	39 (57.40)		

Laboratory tests

Routine blood tests, hepatic function tests, tests of humoral and cellular immunity, sputum smears, sputum cultures, etc., were all performed at the medical laboratory of the Shanghai Public Health Clinical Center affiliated to Fudan University, Shanghai, China.

Statistical methods

All results were expressed as means ± SD. Statistical analysis was performed by Student's *t*-test ($P < 0.05$ was considered significant). Logistic analysis was applied to identify risk factors for mortality (First, single factor logistic analysis was applied to screen meaningless variables, then the significant variables were put into multivariate logistic analysis).

RESULTS

Personal data

Among the 68 cases, 51 were male and 17 were female,

yielding a sex ratio of 3 : 1 (M : F). The average age was 40.8 ± 15.2 years (range: 17-77 years), with 45.6% (31/68) cases between 17 and 40 years old, 48.5% (33/68) between 41 and 60 years old and 5.9% (4/68) older than 60 years old. Thirty-eight patients (55.9%) had one or more underlying diseases, including 23.5% (16/68) of the patients were overweight ($24 \leq \text{BMI} < 28$) and 20.6% (14/68) were obese ($\text{BMI} \geq 28$) (according to the prevention and control guidelines of overweight and obesity of Chinese adults), and 23.5% (16/68) had a history of hypertension, etc. (Table 1).

Clinical manifestations

As shown in Table 1, the main clinical manifestations included cough (66/68, 97.1%), expectoration (41/68, 60.3%), polypnea (41/68, 60.3%), and poor appetite (38/68, 55.9%), which could be accompanied by fever, myalgia, cough with a pink frothy sputum, sore throat, chills, rhinorrhea, nasal obstruction, headache and diarrhea.

Signs

A total of 47 (69.1%) patients presented moist rales and 39 (57.4%) with rough breathing sounds in the lungs. Other signs such as throat congestion, lip cyanosis and shortness of breath were also observed in some cases. The average patient temperature was 37.1 ± 0.84 °C (37.0 - 39.8 °C). Forty-eight patients (70.6%) had normal temperature (< 37.3 °C), 13 (19.1%) had low-grade fever (37.3 - 38.0 °C), 3 (4.4%) had mid-grade fever (38.1 - 39.0 °C), and 4 (5.9%) had high-grade fever (39.1 - 41.0 °C) (Table 1).

Blood routine test

The routine blood tests of pre-therapy and post-therapy both showed that white blood cell (WBC) counts, the percentages of neutrophils (N), lymphocytes (L), and platelet (PLT) counts were within normal limits. The count of WBC and PLT were increased significantly after treatment ($P < 0.05$ and $P < 0.01$, respectively), while the percentage of neutrophils decreased significantly ($P < 0.05$) (Table 2). In addition, the

numbers of pre-therapy patients with WBC counts and the percentage of lymphocytes below the lower limits of normal range were 33 (48.5%) and 27 (39.7%), respectively, while both of them became 6 (8.8%) after treatment.

Imaging findings

Up to 67 (98.6%) patients were found typical lung inflammation by chest CT scanning, and 11 (16.2%) patients accompanied by pleural effusion. Fifty-four (79.4%) patients achieved improvement in pneumonia while 4 (5.9%) patients still had pleural effusion after treatment. Minimal change were found in 11 (16.2%) patients and 2 patients (2.9%) died of aggravated pneumonia.

Sputum tests

Sputum smear and culture tests were performed in all patients. Smear results detected Gram-positive bacteria, Gram-negative bacteria, or both in the sputum specimens from 39 (57.4%) patients before therapy, and 37 (54.4%) remained infected after therapy. Fungal spores were detected in the sputum specimens from 11 (16.2%) patients before therapy, and 6 (8.8%) still had spores after therapy.

Sputum culture results showed that 4 (5.9%) patients were infected with *candida albicans* before treatment, and 3 remained infected after treatment. The sputum culture also detected *aspergillus*, *acinetobacter baumannii*, and *pseudomonas aeruginosa* concurrent with *acinetobacter baumannii* infection in 3 patients, respectively, and the patient infected with *pseudomonas aeruginosa* concurrent with *acinetobacter baumannii* died during the study period.

Hepatic functions in severe/critical patients

As shown in Table 3, the average serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) all exceeded the upper limits of normal ranges before therapy, while the levels of total bilirubin (TBil) and prealbumin (P-A) after therapy increased significantly

Table 2. Routine blood tests ($\bar{x} \pm s$)

	n	WBC ($\times 10^9$)	N (%)	L (%)	PLT ($\times 10^9$)
Before treatment	68	5.53 ± 3.71	60.42 ± 15.15	24.87 ± 12.56	146.21 ± 54.92
After treatment	64	6.80 ± 3.24^a	55.10 ± 13.45^a	29.15 ± 12.97	261.32 ± 114.5^b

Note: ^a $P < 0.05$; ^b $P < 0.01$, compared to pre-therapy.

Table 3. Impact of influenza A (H1N1) virus on hepatic function ($\bar{x} \pm s$)

	n	ALT (U/L)	AST (U/L)	TBil ($\mu\text{mol/L}$)	DBil ($\mu\text{mol/L}$)	GGT (U/L)	P-A (mg/L)	ALB (g/L)
Before treatment	68	64.56 ± 66.56	68.74 ± 51.66	11.27 ± 5.47	3.80 ± 2.14	92.59 ± 87.50	276.37 ± 97.99	36.00 ± 4.89
After treatment	64	75.90 ± 65.71	50.02 ± 112.05	14.07 ± 7.89^a	4.21 ± 3.63	129.68 ± 200.72	405.25 ± 193.22^b	36.29 ± 4.09

Note: ^a $P < 0.05$; ^b $P < 0.01$, compared to pre-therapy.

Table 4. Impact of influenza A (H1N1) virus on immune system ($\bar{x} \pm s$)

	n	CRP (mg/l)	CD3 (cell/ml)	CD4 (cell/ml)	CD8 (cell/ml)	CD45 (cell/ml)	CD4/CD8
Before treatment	68	52.48 $\pm$ 64.33	597.21 $\pm$ 368.08	335.28 $\pm$ 240.97	241.89 $\pm$ 150.32	1045.41 $\pm$ 541.67	1.54 $\pm$ 0.83
After treatment	64	39.47 $\pm$ 50.62	925.27 $\pm$ 320.88 ^b	596.00 $\pm$ 244.99 ^b	308.00 $\pm$ 124.47	1526.33 $\pm$ 644.68 ^b	2.09 $\pm$ 0.85 ^a

Note:^a $P < 0.05$; ^b $P < 0.01$, compared to pre-therapy.

compared with those before therapy ($P < 0.05$ and $P < 0.01$, respectively).

Immune test in severe/critical patients

Although C-reactive protein levels were above the upper limit (5 mg/L) of normal range before therapy and declined after treatment, no significant difference was found ($P > 0.05$). The pre- and post-therapy levels of CD3, CD4, CD8, CD45 and CD4/CD8 were all within normal limits, which increased significantly after treatment ($P < 0.01$, $P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively) (Table 4).

Treatment and efficacy

The patients received neither oseltamivir nor zanamavir except for antibiotics including macrolide antibiotics (azithromycin), fluoroquinolone antibiotics (levofloxacin and moxifloxacin), and cephalosporin antibiotics (cefuroxime axetil and ceftriaxone sodium) before admission. In addition, 50% (34/68) patients had been treated with glucocorticoids, and no patient had been treated with antifungal drugs before admission.

After admission, all patients were administered by oxygen therapy and 97.1% (66/68) patients were administered by oseltamivir with 2 patients being negative of influenza A (H1N1) virus nucleic acid after admission. Total of 74.4% (29/39) patients with bacterial infection were administered by carbapenems antibiotics including meropenem or imipenem, and another 25.6% (10/39) patients were administered by cefoperazone/sulbactam. About 16.2% (11/68) patients with fungal infection were administered by caspofungin or itraconazole. About 29.4% (20/68) patients were treated with ulinastatin, while only 22.1% (15/68) patients received low doses of glucocorticoids. Fifty-four patients with severe type A (H1N1) influenza were cured, and 28.6% (4/14) patients with critical type A influenza died eventually.

Analysis on the cause and risk factors for mortality

Four deaths aged 50-60 years, 3 of whom had a history of hypertension and/or diabetes. Two had secondary fungal infection, one of whom was infected with *pseudomonas aeruginosa* and *acinetobacter baumannii*. The average time from symptom onset to antiviral treatment for the 68 patients was 6.9 ± 2.8 days, and 9.5 ± 3.4 days for the 4 deaths. Laboratory tests indicated that the major characteristics for the 4 deaths

were serious uncontrollable infections (percentage of neutrophils remained at high levels while the percentage of lymphocytes remained at low levels before and after therapy), progressive deterioration of hepatic function (DBil and GGT levels increased continuously or progressively, and ALB level decreased continuously in all 4 died patients; ALT and TBil levels increased progressively in 3 of them). The analysis on cause of death indicated that ARDS secondary to severe pneumonia were the common features of all 4 deaths. Other diseases and complications included septicemia and septic shock in case 1, type II respiratory failure, pyopneumothorax, pyemia and septic shock in case 2, type I respiratory failure and cerebral apoplexy in case 3 and pulmonary embolism and multiple organ failure in case 4 (Table 5).

In order to determine the risk factors for mortality, logistic regression was adopted for correlation analysis with mortality. The independent variables included the following 14 factors: age, obesity, WBC, N, L, PLT, P-A, ALT, AST, ALB, TBil, DBil, GGT and CRP. First single factor logistic analysis was applied to screen meaningless variables, then the significant variables were put into multivariate logistic analysis. Results indicated that there was a significant correlation between mortality and neutrophil levels before therapy, and there was a significant correlation between mortality and DBil after therapy.

DISCUSSION

The risk factors for severe pandemic (H1N1) 2009 virus infection might be concomitant chronic cardiac, pulmonary, renal or hepatic diseases accompanied by pregnancy, cancer and immunosuppression.²⁻⁵ Whether obesity or morbid obesity being an independent risk factor for severe complications of influenza A (H1N1) virus infection remained unknown.⁶ Chest radiographs for patients presenting with severe pneumonia revealed large pleural effusion, including nodular alveolar and multifocal infiltrates. The clinical course of these patients rapidly progressed to ARDS and renal or multi-organ failure, the median time from symptom onset to death was 10 days (range: 2-33 days). In this study, four patients died of severe pneumonia and ARDS, and the median time from symptom onset to death was 20.5 days (range 10-23 days).

In severe/critical influenza patients, nearly half of

Table 5. Sputum smear/culture, blood cell count, liver function, chest CT, underlying diseases and causes of the deaths before and after treatment

Cases	Sputum tests		Routine blood tests			Hepatic function tests						
	smear	culture	WBC ($\times 10^9$)	N (%)	L (%)	PLT ($\times 10^9$)	ALT (U/L)	AST (U/L)	TBil ($\mu\text{mol/L}$)	DBil ($\mu\text{mol/L}$)	GGT (U/L)	ALB (g/L)
Case 1 (F, 50 Y)												
Before treatment	G+	Fungal spores	10.91 $\uparrow$	89.1 $\uparrow$	6.43 $\downarrow$	209	40.0	49.0	6.7	2.7	93 $\uparrow$	34.7 $\downarrow$
After treatment	G-	/	24.91 $\uparrow$	95.0 $\uparrow$	2.0 $\downarrow$	32 $\downarrow$	401.0 $\uparrow$	885.0 $\uparrow$	23.3 $\uparrow$	8.3 $\uparrow$	101 $\uparrow$	23.9 $\downarrow$
Case 2 (M, 56 Y)												
Before treatment	/	/	10.09 $\uparrow$	83.6 $\uparrow$	6.7 $\downarrow$	128	59.0 $\uparrow$	58.0 $\uparrow$	13.5	5.1 $\uparrow$	288 $\uparrow$	28.6 $\downarrow$
After treatment	/	<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter baumannii</i>	10.62 $\uparrow$	82.4 $\uparrow$	2.0 $\downarrow$	88 $\downarrow$	40.0	26.0	12.2	7.7 $\uparrow$	131 $\uparrow$	29.1 $\downarrow$
Case 3 (M, 56 Y)												
Before treatment	G+	/	5.19	74.4 $\uparrow$	12.5 $\downarrow$	117	45.0	73.0 $\uparrow$	13.7	4.6 $\uparrow$	67 $\uparrow$	33.7 $\downarrow$
After treatment	/	/	7.61	83.5 $\uparrow$	16.1 $\downarrow$	77 $\downarrow$	163.0 $\uparrow$	52.0 $\uparrow$	34.7 $\uparrow$	15.1 $\uparrow$	245 $\uparrow$	32.6 $\downarrow$
Case 4 (M, 59 Y)												
Before treatment	/	/	11.51 $\uparrow$	79.7 $\uparrow$	9.8 $\downarrow$	250	41.0	47.0	36.5 $\uparrow$	11.4 $\uparrow$	37	37.0
After treatment	G-	Fungal spores	6.92	76.0 $\uparrow$	15.7 $\downarrow$	202	164.0 $\uparrow$	79.0 $\uparrow$	42.1 $\uparrow$	20.9 $\uparrow$	130 $\uparrow$	33.4 $\downarrow$

Cases	Chest CT scan	Underlying diseases	Course of disease (d)	Causes of death
Case 1 (F, 50 Y)				
Before treatment	Inflammation of both lungs, pleural effusion	Hypertension,	19	Severe pneumonia,
After treatment	Inflammation of both lungs,	Diabetes		ARDS,
				Septicemia,
				Septic shock
Case 2 (M, 56 Y)				
Before treatment	Inflammation of both lungs,	Hypertension	23	Severe pneumonia,
After treatment	Inflammation of both lungs, Increased lung lesions, Right Pneumothorax			ARDS,
				Type II respiratory failure,
				Pus pneumothorax,
				Sepsis,
				Septic shock
Case 3 (M, 56 Y)				
Before treatment	Inflammation of both lungs,	/	10	Severe pneumonia,
After treatment	Inflammation of both lungs, Increased lung lesions			ARDS,
				Type I respiratory failure,
				Stroke
Case 4 (M, 59 Y)				
Before treatment	Inflammation of both lungs, left pleural effusion	Diabetes	22	Severe pneumonia,
				ARDS,
				Pulmonary embolism,
				Multiple organ failure

Note: M: male, F:female, Y: years old.

the patients presented with decreased WBC count. Although only 25% patients showed increased neutrophils level, it remained high levels in 4 deaths after treatment. Logistic regression analysis demonstrated that there was a significant correlation between mortality and the percentage of neutrophils before treatment. This was not consistent with previous reports in literature that indicated a correlation between obesity or post-therapy percentage of lymphocytes and mortality.⁷ This result suggest that more attention should be paid to the pre-therapy percentages of neutrophils for patients with severe/critical the novel influenza A (H1N1) virus.

It had been proved that the H1N1 virus could infect multiple organs.⁸ This study showed that the spectrum

of severe/critical disease caused by H1N1 virus was not limited to the upper-respiratory tract. In fact, 98.6% patients experienced typical pneumonia. The virus also caused hepatic function impairment, with increased ALT, AST and GGT levels, and a decrease in ALB level. In addition, there was a significant correlation between mortality and level of direct bilirubin after treatment, this liver damage may result from viral activation of Kupffer cells in the liver.⁹ Furthermore, the virus could impact the immune system, leading to increased levels of CRP, decreased levels of CD3, CD4, CD8, CD45 and the ratio of CD4/CD8.

Primary viral pneumonia was the most common finding in severe cases with influenza A (H1N1), and secondary bacterial infections had been found in

approximately 30% fatal cases. The results showed that respiratory failure and refractory shock were the most common causes of death, as confirmed by the pathological findings in patients and foregoing work in experimental animals.¹⁰ In all patients in this study, secondary bacterial and fungal infections were found in 57.2% and 16.2% cases, respectively. There were 2 cases with secondary fungal infections and 1 case with *pseudomonas aeruginosa* and *acinetobacter baumannii* infections in the 4 deaths. These results indicated that secondary bacterial and/or fungal infections might be one of critical causes of aggravation or death, confirming the necessary of broad-spectrum of relatively strong antibiotics treatment in addition to antiviral and oxygen therapy.

Corticosteroids should be used with caution, since prolonged use of systemic high-dose corticosteroids could result in serious adverse events in influenza virus-infected patients.^{11,12} However, low doses corticosteroids might be considered for patients with septic shock. Ulinastatin, a broad-spectrum enzyme inhibitor, could protect pulmonary functions from being harmed by a variety of factors from extracorporeal circulation.¹³ In addition, the morbidities of stress ulcer, hyperglycemia, and fungal infection would be low after intravenous injection of ulinastatin. Furthermore, ulinastatin had shown promise in replacing glucocorticoid for clinical management of ARDS.¹⁴ Therefore, ulinastatin might be considered as an alternative treatment of glucocorticoid for patients with severe/critical H1N1 infection.

Overall, the severe diseases caused by H1N1 virus were complicated and progressed rapidly. Patients often experienced pulmonary injury and ARDS, and the rate of mortality was high. Fortunately, appropriate combined therapy including antiviral treatment with oseltamivir, oxygen therapy and antibiotics had been proved effective.

Although low to moderate dose corticosteroids improved pulmonary and multi-organ dysfunction significantly in ARDS patients with or without H1N1 virus infection,¹² it was recommended that ulinastatin be used as substitute in order to reduce side-effects. Furthermore, the level of neutrophils pre-therapy and level of direct bilirubin post-therapy should be measured and recorded for the evaluation of prognosis.

Acknowledgements

We would like to thank Hong-Zhou Lu, and Xiao-rong Chen for identifying cases; Yun-fei Lu and Bo-zong Tang for facilitating data collection; Zong-guo Yang for performing data entry and analysis.

Grants

This study was supported by a grant-in-aid from the

state administration of Traditional Chinese Medicine of China for the infectious disease prophylaxis and treatment through grant number 200907001-2.

Conflict of interest

No conflict of interest exists in the submission of this manuscript.

Ethical approval

The clinical research protocol was approved by the Medical Ethics Committee of Shanghai Public Health Clinical Center affiliated to the Fudan University.

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