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Resolution of Coronavirus Disease 2019 Infection and Pulmonary Pathology With Nebulized DAS181: A Pilot Study

Jennifer Hui-Chun Ho, MD, PhD[1]; Yang Zhao, MD[2]; Zhenlian Liu, MS[2]; Xiaoyang Zhou, MD, PhD[3]; Xiaobei Chen, MD, PhD[4]; Yunyan Xianyu, PhD[5]; Stanley Lewis, MD, MPH[1]; Liya Fan, BA[1]; Yuan Tian, PhD[6]; Nancy Chang, PhD[1]; Zuojiong Gong, MD, PhD[4]; Ke Hu, MD[2]

Objectives: Severe acute respiratory syndrome coronavirus 2 infections commonly lead to respiratory failure and potentially fatal systemic inflammation and organ failure. Nebulized DAS181, a host-directed biologics with sialidase activity, is an investigational drug with antiviral activities on parainfluenza and influenza under phase 3 and phase 2 development. The objective of this study (NCT04324489) is to investigate the safety and effects of nebulized DAS181 on hypoxic coronavirus disease 2019 patients.

Design: Single-center, prospective, open-label, compassionate use.

Setting: Renmin Hospital of Wuhan University, Department of Respiratory and Critical Care Medicine and Department of Infectious Diseases.

Subjects: Patients 18 to 70 years old who met Chinese criteria for severe coronavirus disease 2019 pneumonia and required supplemental oxygen but not on mechanical ventilator at screening.

Interventions: Nebulized DAS181 (4.5 mg) twice a day for 10 days.

Measurements and Main Results: Three male coronavirus disease 2019 hypoxic patients with bilateral lung involvement completed DAS181 treatment for 10 days. By day 14, all achieved return to room air (primary endpoint) and their nasopharyngeal swabs were negative for severe acute respiratory syndrome coronavirus 2. Clinical severity improved from severe coronavirus disease 2019 at baseline to moderate or mild disease by day 5, consistent with rapid reduction of inflammatory cytokines by days 2–3 and radiologic improvement by days 5–10. No DAS181-related adverse events were reported.

Conclusions: Inhalation of DAS181 was well tolerated and potential clinical benefit of DAS181 on hypoxic coronavirus disease 2019 is the reduction of supplemental oxygen need. Efficacy and safety, including pharmacokinetics and viral studies of DAS181 in severe, hypoxic coronavirus disease 2019, should be examined by a double-blind, randomized controlled study.

Key Words: coronavirus disease 2019; DAS181; inhalation; pneumonia; pulmonary function

Effective antiviral therapies are urgently needed to manage coronavirus disease 2019 (COVID-19) pneumonia, specifically in its severe form, which places patients at risk of progression from hypoxia to acute respiratory distress, multiple organ failure, and death (1). In addition to clinical severity, prognostic factors associated with COVID-19 mortality include chest CT (CCT) signs (1), as well as inflammatory markers, notably the cytokines tumor necrosis factor (TNF)–α and interleukin (IL)–1 (2), which serve as independent predictors of mortality and disease severity.

DAS181 is a fusion protein carrying a sialidase catalytic domain linked to a glycosaminoglycan binding tag. A global phase 3 study on parainfluenza (PIV) (NCT03808922) and a phase 2b study on severe influenza (NCT04298060) are ongoing patient enrollment. The U.S. Food and Drug Administration has designated DAS181 as a breakthrough therapy based on resolution of supplemental oxygen need (return to room air [RTRA]) in hypoxic patients with lower respiratory tract PIV infection. Inhalation of nebulized DAS181 leads to sialic acid cleavage from glycoproteins and glycolipids in the lung epithelium and cells in the microenvironment that interferes with the life cycle of viruses targeting sialic acid glycoconjugates on their host cells (3). Severe acute respiratory

[1]Division of Clinical Development, Ansun Biopharma, San Diego, CA.

[2]Department of Respiratory and Critical Care Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.

[3]Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.

[4]Department of Infectious Diseases, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.

[5]Nursing Department, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.

[6]Wuhan University, Wuhan, Hubei, China.

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syndrome coronavirus 2 (SARS-CoV-2) may be sensitive to this host cell surface alteration because angiotensin-converting enzyme 2 (ACE2) is heavily sialylated on N- and O-linked sugar chains (4), and recent research disclosed that an evolutionary adaptation of SARS-CoV-2 spike glycoprotein reciprocal interaction with host surface sialosides to infect host cells with wide tissue tropism (5), suggesting a high barrier to resistance with this therapeutic strategy.

The aim of this compassionate use is to observe safety and potential efficacy of DAS181 on patients with severe, hypoxic COVID-19 by RTRA on day 14.

MATERIALS AND METHODS

This study (NCT04324489) was approved by the Clinical Research Ethics Committee of Renmin Hospital, Wuhan University (WDRY2020-K086). Patients 18 to 70 years old could be included if they required oxygen supplementation and met Chinese criteria for severe COVID-19 pneumonia (respiratory rate ≥ 30/min, oxygen saturation (Spo_2) ≤ 93%, Pao_2/Fio_2 ≤ 300 mm Hg, or ≥ 50% radiologic progression of lung lesions within 48 hr) (6). Patients requiring mechanical ventilation or experiencing shock or multiple organ failure were excluded. DAS181 (Ansun Biopharma, San Diego, CA) was supplied as single-dose 10 mL glass vial containing lyophilized DAS181. Before treatment, DAS181 was reconstituted with sterile water and then administered via mouthpiece by a vibrating mesh nebulizer with one-way exhalation valve filter (7) (Aeroneb Solo; Aerogen, Chicago, IL). Patients received 4.5 mg DAS181 bid for 10 days, as an add-on to other therapeutics (**Supplemental Appendix 1**, http://links.lww.com/CCX/A386). Co-primary endpoints were RTRA and improved clinical severity defined by mild, moderate, severe, or critical COVID-19 (6) by day 14. Secondary endpoints included time to undetectable SARS-CoV-2 RNA and hospital discharge. Safety outcomes included clinical and laboratory adverse events.

Imaging was performed by high-resolution CT (Optima CT680 series; GE Healthcare, Chicago, IL) without contrast. Images were acquired during a single inspiratory breath-hold, with patients in a supine position. Oxygen saturation and supplemental oxygen use were recorded daily. Clinical severity and laboratory variables were assessed at baseline, days 5, 10, and 14, and hospital discharge. Nasopharyngeal swabs (NPSs) and other samples were assayed for viral RNA by reverse transcriptase-coupled polymerase chain reaction directed to the SARS-CoV-2 ORF1ab and nucleocapsid genes (GeneoDx Biotech, Shanghai, China).

RESULTS

In March 2020, three hypoxic patients with severe COVID-19 pneumonia provided informed consent.

First patient was a 61-year-old man experiencing intermittent pyrexia for 4 days and productive cough for 2 days and placed on supplemental oxygen due to hypoxia at admission. Bilateral COVID-19 pneumonia was confirmed by RT-PCR and CCT image (**Fig. 1*B***). Elevation of D-dimer at baseline was noted. Severe COVID-19 pneumonia with continuous pyrexia and hypoxia for 16 days prior to initiation of DAS181 treatment. By day 5, the patient's Spo_2 and Pao_2 values reached normal or near-normal levels while on supplemental oxygen, and severity of COVID-19 was reduced to moderate (**Table 1**). Compared with baseline CCT, images on day 10 showed bilateral resolution of lung lesions. The patient achieved RTRA on day 14. Although NPS were negative for SARS-CoV-2 starting from day 6, a positive sputum test on day 14 suggested viral clearance remained incomplete. Sequential samples (days 22–24) tested negative, and the patient was discharged on day 25.

Patient 2, a 62-year-old man was admitted after experiencing night sweats for 8 days and aggravated cough and wheezing for 6 days, along with headache and pyrexia. SARS-CoV-2 infection and bilateral pneumonia were confirmed. Within 4 days, CCT showed extensive bilateral ground-glass opacities and consolidation, and the patient's disease met criteria for severe COVID-19 infection. He received supplemental oxygen for persistent hypoxia, progressing to a requirement for high-flow oxygen (30–50 L/min). Anticoagulants (IV heparin sodium and ulinastatin) were introduced at the time of transition to high-flow oxygen. DAS181 treatment was initiated 10 days after progression to high-flow oxygen. The patient's hypoxia improved rapidly. On day 2, he transitioned to low-flow oxygen (2–5 L/min) for the remainder of treatment, achieving RTRA on day 14. Disease severity on day 5 was moderate. Follow-up CT on day 9 indicated that lung lesions were substantially improved (Fig. 1*B*). Elevation of TNF-α and D-dimer at baseline and returned to the normal range by day 2 (**Fig. 1*A***) and after 10-day treatment, respectively. The patient's interferon-γ level was highly elevated at all times before and after the treatment period. NPS were SARS-CoV-2 negative by day 6 (Table 1). The patient was discharged on day 28.

The third patient was a 29-year-old man with fever, cough, and headache was placed on supplemental oxygen. CCT identified bilateral pulmonary inflammation with flame-shaped, dot-like, and ground-glass opacities. NPS was positive for SARS-CoV-2 RNA, and the patient began treatment with leflunomide and azithromycin. The following day, he initiated DAS181, as well as gamma globulin and, subsequently, methylprednisolone. CCT on day 5 showed reduced inflammation, consistent with a clinical assessment of mild disease severity. IL-6 and TNF-α, both elevated at baseline, declined to the normal range by day 3 (Fig. 1*A*). NPS were SARS-CoV-2 RNA-negative by day 4 and remained negative thereafter. However, the patient's body temperature rose to 38.3°C on day 9 associated with localized inflammation in the left lung. Pyrexia resolved by day 11 with IV cefoperazone/tazobactam. On day 14, radiologic signs had substantially resolved (Fig. 1*B*) and IL-6 level, which had been elevated in conjunction with the apparent bacterial infection, returned to normal. The patient achieved RTRA on day 14 and was released on day 23.

All adverse events were not deemed treatment-related. One experienced pyrexia was associated with bacterial infection and resolved by day 14 following reintroduction of IV antibiotics and methylprednisolone (patient 3). One event rise in liver enzymes (patient 3), which investigators attributed to treatment with azithromycin. In addition, patient 1 and patient 2 experienced mild, transient increased blood alkaline phosphatase (ALP) and

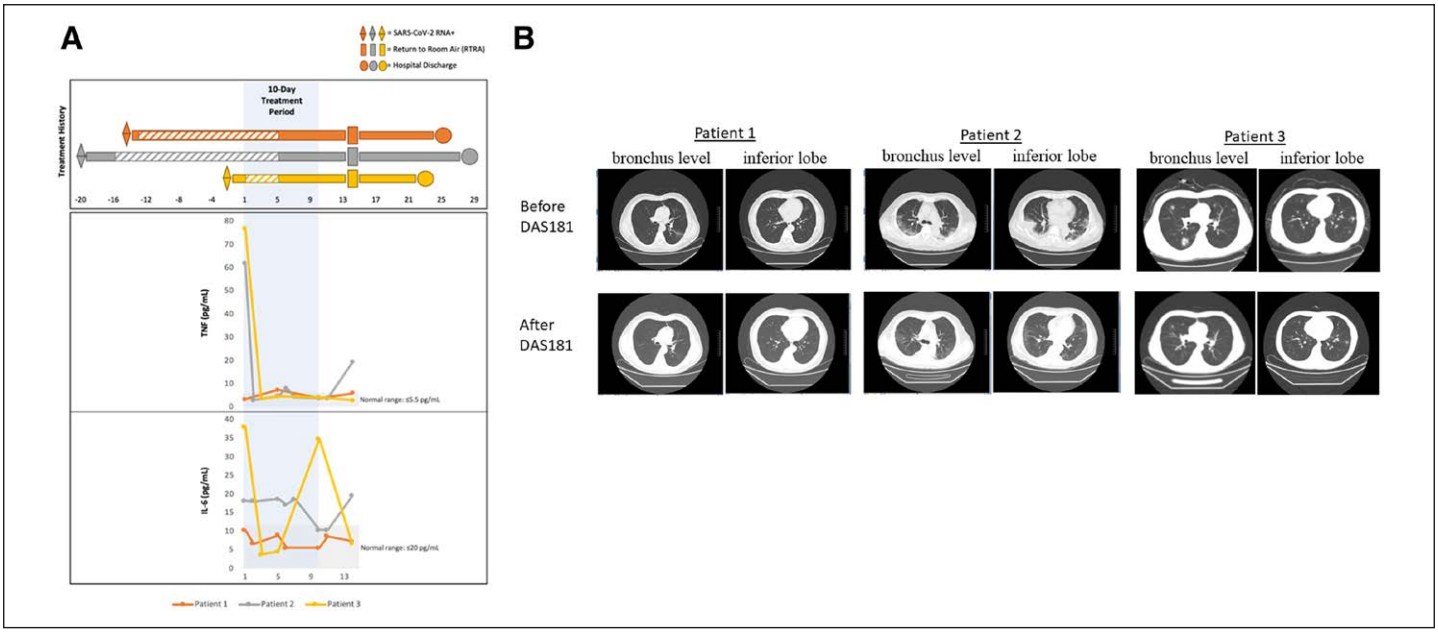

Figure 1. Change on inflammatory cytokines and chest image with DAS181. **A**, Treatment history and changes in inflammatory cytokine levels in three patients treated with DAS181 for severe coronavirus disease 2019 pneumonia. Hatch marks indicate the period of severe disease for each patient. **B**, Images showing axial views of the pulmonary window at bronchus level and at the inferior lobe were taken at baseline (*upper row*) and days 9–10 (*lower row*) of DAS181 treatment. Note significant absorption of bilateral ground-glass opacities (patient 1), bilateral ground-glass opacities and consolidation (patient 2), and bilateral multiple infiltrating shadows (patient 3). The scanning variables were as follows: 120 kV tube voltage; automatic tube current modulation; detector collimation, 64 × 0.625 mm; rotation time, 500 ms; and pitch, 1.375. The scanning range was from the apex of lung to costophrenic angle, with slice thickness 0.625 mm. IL-6 = interleukin-6, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, TNF = tumor necrosis factor.

extension of activated partial thromboplastin time with no clinical impact, which was also observed in previous DAS181 studies, back to normal range following treatment cessation.

DISCUSSION AND CONCLUSIONS

To our knowledge, this is the first reported use of a host-directed antiviral to treat severe, hypoxic COVID-19. With DAS181, laboratory and radiologic outcomes improved rapidly following treatment. CCT improved at the earliest time point observed, that is, days 5–10 of DAS181 treatment. NPS samples were negative by day 14, although one patient had detectable viral RNA in the sputum, which required additional days to clear. Clinical severity was mild or moderate by day 5; by day 14, all achieved RTRA. By comparison, in another Chinese clinical trial of severe COVID-19 pneumonia, median time to clinical improvement was greater than 20 days, with 36% of patients in the placebo arm achieving RTRA by day 14 of treatment (8). Similar to what have been observed from patients with lower respiratory tract PIV infection, duration of hypoxic is not related to the selection of potential responder to DAS181 on resolution of supplemental oxygen need. As shown in this study, two subjects (patient 1 and patient 2), who were inflicted for a longer period (> 14 d), were still response to nebulized DAS181.

Because the extreme virulence of SARS-CoV-2 may be due in part to efficient repression of antiviral responses (9), host-directed antiviral therapies such as DAS181 could be powerful tools for controlling COVID-19 pneumonia. Our in vitro study revealed that DAS181 with antiviral ability against SARS-CoV-2 infection at 1–10 μM and a trend to upregulate type I interferon response at 1 nM (manuscript in preparation). Furthermore, Siglecs are a group of sialic-acid binding lectins and Siglec-sialic acid signal can be an enhancer or an suppressor on innate immune function depends on type of immune cells and type of Siglecs in the microenvironment (10). In this study, two patients exhibiting baseline elevation of TNF-α, normalization of TNF-α occurred by days 2–3 after DAS181 treatment. In vitro study suggested that DAS181 down-regulates TNF-α expression in human lung epithelial cells and IL-1β expression in M1 macrophage at concentration 1 nM and 100 nM, respectively (manuscript in preparation).

In addition to ACE2, substrates for the DAS181 sialidase may include circulating glycoproteins, notably the liver enzyme ALP. Because desialylation of this enzyme delays its clearance (11), blood ALP in treated patients is an unreliable marker of pathogenic processes but may serve as a marker of DAS181's biochemical activity.

Limitations of this pilot study include small size and absence of controls, along with the patients' diverse clinical and treatment histories. Nevertheless, the rapid resolution of hypoxia and improvement of clinical and inflammatory variables observed in these individuals justifies further exploration of DAS181 in severe COVID-19 pneumonia and other pulmonary viral infections.

Taken together, observation from this is a single, initial pilot of a small case series of patients suggested that inhalation of DAS181 was well tolerated and potential clinical benefit of DAS181 on hypoxic COVID-19 is contributed by resolution of supplemental oxygen need. Whether DAS181 can significantly accelerate clinical recovery or biochemical events associated with regulation of inflammatory markers as well as pharmacokinetics and viral studies should be investigated in a double-blind, randomized controlled study.

TABLE 1. Clinical and Laboratory Profile After DAS181 Treatment

Patient No.	Case 1: 61-yr-Old Male				Case 2: 62-yr-Old Male				Case 3: 29-yr-Old Male			
Pre-baseline duration of COVID-19 hypoxia	15 d				20 d				2 d			
Day	Baseline	Day 5	Day 10	Day 14	Baseline	Day 5	Day 10	Day 14	Baseline	Day 5	Day 10	Day 14
	DAS181 treatment period			Follow up	DAS181 treatment period			Follow up	DAS181 treatment period			Follow up
Clinical status												
Clinical severity[a]	Severe	Moderate	Moderate	Mild	Severe	Moderate	Moderate	Moderate	Severe	Mild	Mild	Mild
Vital signs	Fever, dyspnea			Stable	Dyspnea			Stable	Mild fever		Fever	Stable
Chest CT scan	Bilateral lesions		Lesions resolved		Bilateral lesions		Significantly improved[e]		Bilateral lesions	Improved	Slight progression	Significantly improved
Oxygen saturation (≥ 95%)[b]	92 on O_2	96 on O_2	97 on O_2	98 in room air	96 on O_2	96 on O_2	99 on O_2	98 in room air	93 on O_2	97 on O_2	97 on O_2	98 in room air
Pao_2 (80–100 mm Hg)[b]	78 on O_2	99 on O_2	82 on O_2		58 on O_2	115 on O_2	80 on O_2		99 on O_2		100 on O_2	83 in room air
Supplemental O_2, if any (L/min)	3	2.5	2	None (RTRA)	30[c]	3	2	None (RTRA)	3	3	2	None (RTRA)
Hematology[b]												
WBC (3.5–9.5 × 10^9/L)	8.1	5.9	5.5	5	8.1	4.5	3.8	3.4	6.9	4.3	5.3	5.1
Lymphocytes (1.1–3.2 × 10^9/L)	0.98	1.25	0.89	1.08	2.01	0.96	1.05	1.2	0.77	0.92	0.67	0.85
Platelets (125–350 × 10^9/L)	285	213	183	214	157	109	102	79	204	264	244	183
Biochemistry[b]												
C-reactive protein (0–10 mg/L)	5	5	5	5	7.5	5	5	5	18.3	13.8	2.28	15.7
Alanine aminotransferase (7–40 U/L)	18	29	97	129	16	17	28	25	23	261	316	186
Aspartate aminotransferase (13–35 U/L)	8	22	79	90	15	16	33	32	22	202	261	126
Alkaline phosphatase (50–135 U/L)	71	146	186	175	96	162	197	269	70	393	902	317
Coagulation variables[b]												
D-dimer (0–0.55 mg/L)	2.7	3.26	1.57	0.43	7.85	2.2	6.08	0.37	0.12	0.24	0.29	0.52
Fibrin/fibrinogen degradation products (0–5.0 g/L)	8.29	6.11	5.08	1.58	19.6	6.7	16.9	1.4	0.53	0.19	0.7	1.93
Fibrinogen (2.0–4.0 g/L)	2.21	2.9	0.94	3.79	2.79	3.27	0.88	2.48	5.12	6.03	5.36	5.36
Prothrombin time (9–13 s)	10.8	10.1	9.8	10.1	12.4	11.8	11.6	12.2	12	11.3	11.9	13
Activated partial thromboplastin time (25–31.3 s)	24.2	25	30.1	39.7	27	38.7	53	60.6	29.3	47.6	77.2	51.8

(*Continued*)

TABLE 1. (*Continued*). Clinical and Laboratory Profile After DAS181 Treatment

Patient No.	Case 1: 61-yr-Old Male				Case 2: 62-yr-Old Male				Case 3: 29-yr-Old Male			
International normalized ratio (0.76–1.24)	0.92	0.86	0.83	0.86	1.06	1.01	0.99	1.05	1.03	0.96	1.02	1.12
Cytokines[b]												
IL-6 (≤ 20.0 pg/mL)	10.22	8.83	5.35	7.18	18.14	18.58	10.22	19.48	38.02	4.36	34.82	6.59
IL-10 (≤ 5.9 pg/mL)	7.50	6.05	6.33	7.01	7.25	8.65	8.19	7.66	6.05	5.2	8.28	5.77
Tumor necrosis factor-α (≤ 5.5 pg/mL)	3.04	7.06	3.71	5.83	61.56	4.54	3.4	19.12	76.49	4.25	3.88	2.72
Interferon-γ (≤ 18 pg/mL)	2.97	3.07	4.13	3.24	252.34	222.96	159.02	225.32	4.22	3.45	3.97	3.31
Immune cell count[b]												
CD4 (404–1,612/μL)	399	409	291	234	783	448	500	491	295	357	238	364
CD8 (220–1,129/μL)	191	344	272	184	275	230	282	386	148	165	117	146
CD19 (80–616/μL)	168	145	65	48	497	119	78	97	96	126	51	107
CD16/56 (84–724/uL)	177	358	656	247	64	96	154	151	89	101	150	80
COVID-19 RNA and immune response												
Viral N gene RT-PCR	NPS+[f]	NPS−[g]		NPS−, sputum+		NPS−[g]		NPS−	NPS+		NPS−	NPS−
Viral ORF1ab RT-PCR	NPS+[f]	NPS−[g]	NPS−	NPS−, sputum−		NPS−[g]	NPS−	NPS−	NPS+		NPS−	NPS−
Immunoglobulin M (AU/mL)				47			8[h]	65	BLD[d]		BLD[d]	BLD[d]
Immunoglobulin G (AU/mL)				131			528[h]	561	BLD[d]		BLD[d]	BLD[d]

BLD = below the limit of detection, COVID-19 = coronavirus disease 2019, IL = interleukin, NPS = nasopharyngeal swab, RT-PCR = reverse transcriptase-coupled polymerase chain reaction, RTRA = return to room air.

[a]Severity assessed per Chinese Guidelines as follows: Mild = mild clinical symptoms with no radiologic evidence of pneumonia; Moderate = fever and respiratory symptoms with signs of pneumonia; and Severe = any of the following: respiratory rate ≥ 30/min, oxygen saturation ≤ 93% at rest, Pao_2/Fio_2 ≤ 300 mm Hg, or ≥ 50% radiologic progression of lung lesions within 48 hr (A fourth category, Critical, includes patients in shock, with organ failure, or requiring mechanical ventilation; Critical status was not observed in this study).

[b]Values in parentheses indicate normal range.

[c]High-flow oxygen, Fio_2 33%, and Pao_2/Fio_2 175 mm Hg.

[d]Limit of detection = 10 AU/mL.

[e]Assessed on day 9.

[f]Assessed on day −2.

[g]Assessed on day 6.

[h]Assessed on day 11.

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Drs. Gong and Hu were co-corresponding authors and contributed equally to this article.

Drs. Gong and Hu designed the protocol, enrolled and treated patients, and interpreted patient data. Drs. Zhao, Liu, Zhou, Chen, and Xianyu performed medical care and collected patient data. Dr. Tian participated study design and proofread manuscript. Dr. Ho participated in study design, manuscript writing, and medical monitoring of this study. Drs. Lewis, Fan, and Chang performed data analysis and proofread the manuscript.

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For information regarding this article, E-mail: zjgong@163.com; huke-rmhospital@163.com

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