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ORIGINAL ARTICLE

High-level ambient particulate matter before influenza attack with increased incidence of *Aspergillus* antigenemia in Southern Taiwan, 2016

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Abstract We found significant correlation between the incidence of severe influenza and *Aspergillus* antigenemia among medical intensive care unit patients for 7-month observation (coefficient $\gamma = 0.976$, $p < 0.001$). High-level ambient pollution was noticed for 2 months before the epidemic, highlighting that influenza patients might coinfect with aspergillosis in the community.

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Introduction

Invasive pulmonary aspergillosis (IPA) was identified in 23–29% of critically ill patients with severe influenza A (H1N1)-related pneumonia.[1,2] However, Martin-Loeches and Valles[3] raised the query of possible overdiagnosis based on *Aspergillus* galactomannan (GM) antigen in the serum of patients with severe influenza. Martin-Loeches et al[4] isolated *Aspergillus* spp. as a pathogen of hospital-acquired pneumonia in 8.7% of 46 critically ill influenza A (H1N1) patients, which indicates the study that assessed the effect of early corticosteroid therapy but was not intended to diagnose IPA for influenza patients.[4] The above studies mentioned prior corticosteroid use as a risk factor for IPA in severe influenza patients.

On the contrary, Garcia-Vidal et al[5] found that high numbers of fungal spores and subsequent circulating respiratory viruses, such as influenza A, in the ambient air increase the development of IPA 2–6 weeks later, suggesting that patients might acquire IPA outside the hospital. Nevertheless, they could not explain the reasons of increased environmental spores and did not mention the coexistence of IPA in the influenza patients.[5] In fact, influenza A viral infection might predispose the previously healthy patients to *Aspergillus* superinfection by suppression of cellular immunity.[6] Patients with unusually severe influenza might hint depressed T-cell function, thus becoming vulnerable to IPA.[7] Therefore, we postulate a transmission mode of previous exposure to environmental spores promoting IPA in severe influenza patients.

In Taiwan, an influenza epidemic peaked in February 2016, which caused chaotic situations to the health care facilities and record-breaking death toll in Taiwan (http://www.chinapost.com.tw/taiwan/national/national-news/2016/03/09/460225/CDC-under.htm). To understand the incidence of IPA in severe influenza patients, we retrospectively reviewed the cases of severe influenza and positive *Aspergillus* GM antigen from the adult patients hospitalized in the medical intensive care units (ICUs) of two hospitals (A and B) in Tainan city, southern Taiwan, from August 1, 2015 through February 29, 2016. Hospital A is a tertiary referral medical center with 96 adult ICU beds. Hospital B is a regional cancer center with 63 adult ICU beds.

GM is a cell wall component released by *Aspergillus* spps. during active hyphal growth in tissues, thus detection of GM in blood reflecting a true *Aspergillus* infection rather than contamination from airway isolation.[8,9] The Food and Drug Association of the United States has approved the use of Platelia *Aspergillus* enzyme immunoassay to detect GM in blood, which allows doctors to diagnose life-threatening aspergillosis sooner. The revised definitions for invasive fungal disease of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) has accepted GM test as one of the microbiological criteria for diagnosing probable IPA.[10]

Before the influenza epidemic, high-level atmospheric fine particulate matter (PM) with a diameter of 2.5 μm (designed $PM_{2.5}$) was frequently alarmed by Taiwan Air Quality Monitoring Network. High-level $PM_{2.5}$ would cause discomfort such as sore eyes, sore throat, or cough and people should consider reducing outdoor activity. Therefore, we reviewed levels of atmospheric PM as an index of ambient air pollution in Tainan city, which might contribute to increased environmental *Aspergillus* spores.[11]

Methods

A confirmed influenza case was defined as at least one positive assay for testing influenza, such as rapid influenza diagnostic tests, real time polymerase chain reaction (PCR), and viral isolation for specimens of nasopharyngeal swab and/or lower respiratory tract aspirates. Severe influenza was regarded as those influenza patients admitted to ICUs.

Aspergillus GM was detected using Platelia *Aspergillus* Ag assay (Bio-Rad Laboratories, Marnes-La-Coquette, France) in serum with a positive cut-off value of optical density index ≥ 0.5.

Data of $PM_{2.5}$ were obtained from Taiwan Air Quality Monitoring Network (http://taqm.epa.gov.tw/taqm/en/). Above high-level $PM_{2.5}$ concentrations were defined by an index of ≥ 54 $\mu g/m^3$. The cumulative hours of above high-level $PM_{2.5}$ per month was regarded as the exposure time of people to ambient air pollution.

Proportions were compared using the $\chi 2$ test or Fisher's exact test. Pearson's correlation coefficient was used to determine the relationship between the incidences of severe influenza and *Aspergillus* antigenemia among the medical ICU patients. A p value < 0.05 indicated statistical significance (2-tailed test).

Results

Incidence of severe influenza and *Aspergillus* antigenemia

A total of 2712 hospitalized medical ICU patients (1640 in hospital A and 1072 in hospital B) were identified during the observed 7-month period. In February 2016, a total of 54 patients (32 in hospital A, 22 in hospital B) were diagnosed with severe influenza and 33 patients (24 in hospital A, 9 in hospital B) were positive for *Aspergillus* GM test (Table 1). The incidence trends of adult patients with severe influenza and/or positive *Aspergillus* antigenemia in medical ICU patients were significantly increased in February 2016 compared with those in previous 6 months in both the hospitals (Table 1 and Figure 1A). Furthermore, monthly incidences of *Aspergillus* antigenemia and severe influenza were highly positively correlated (Pearson's correlation coefficient $\gamma = 0.976$, $p < 0.001$; Figure 1B). The number of noninfluenza patients with *Aspergillus* antigenemia significantly increased in hospital A and overall patients but did not increase significantly in hospital B during the influenza epidemic (Table 1).

Types of influenza and patients with *Aspergillus* antigenemia

Among the total 54 severe influenza patients in February 2016, influenza A was identified in 45 patients, including 35 patients with influenza A(H1N1)pdm09 virus and 10 patients with influenza A virus different to (H1N1)pdm09 and H3N2.

Table 1 Distribution of atmospheric fine particulate matter at Tainan, Taipei, and Kaohsiung cities in Taiwan, and monthly case numbers by variables (definitions A to E) combing from data of two hospitals in Tainan city from August 2015 through February 2016

PM/Influenza/Aspergillus	2015 Aug	2015 Sep	2015 Oct	2015 Nov	2015 Dec	2016 Jan	2016 Feb	*p*
Tainan city								
PM_{10} ($\mu g/m^3$)								0.004[a]
Average/mo	27	40	52	65	66	55	65	
$PM_{2.5}$ ($\mu g/m^3$)								
Average/mo	12	20	28	30	33	36	34	
Hr of ≥54/mo	0	3	13	57	103	118	131	
Taipei (Zhongshan district)								
PM_{10} ($\mu g/m^3$)								0.005[a]
Average/mo	30	35	37	33	42	39	43	
$PM_{2.5}$ ($\mu g/m^3$)								
Average/mo	13	17	17	17	20	22	23	
Hrs of ≥54/mo	0	2	4	34	43	32	41	
Kaohsiung (Zuoying district)								
PM_{10} ($\mu g/m^3$)								0.002[a]
Average/mo	35	41	62	77	83	69	74	
$PM_{2.5}$ ($\mu g/m^3$)								
Average/mo	14	19	31	36	39	41	44	
Hospital A								
A	2	3	0	1	0	7	32	<0.001[b]
B	1	5	3	3	5	7	24	<0.001[c]
C	0	0	0	1	0	0	12	<0.001[d]
D	1	5	3	2	5	7	12	0.003[e]
E	210	245	206	222	228	267	262	
Hospital B								
A	1	1	1	2	2	4	22	<0.001[b]
B	4	6	0	3	1	3	9	0.025[c]
C	0	0	0	0	0	0	5	<0.001[d]
D	4	6	0	3	1	3	4	0.763[e]
E	158	150	139	132	145	175	173	
Combing two hospitals								
A	3	4	1	3	2	11	54	<0.0001[b]
B	5	11	3	6	6	10	33	<0.0001[c]
C	0	0	0	1	0	0	17	<0.0001[d]
D	5	11	3	5	6	10	16	0.0315[e]
E	368	395	345	354	373	442	435	0.0002[f]

[a] Pearson's correlation between monthly average value ($\mu g/m^3$) of PM_{10} and $PM_{2.5}$.

[b] Difference in the incidence of severe influenza among medical intensive care unit (MICU) patients (A/E) in 2016 February in comparison to previous 6 months: Hospital A: 12.2% (32/262) vs. 0.94% (13/1378), $p < 0.0001$; Hospital B: 12.7% (22/173) vs. 1.22% (11/899), $p < 0.0001$; Hospital (A + B): 12.4% (54/435) vs. 1.05% (24/2277), $p < 0.0001$.

[c] Difference in the incidence of *Aspergillus* antigenemia among medical intensive care units patients (B/E) in 2016 February in comparison to previous 6 months: Hospital A: 9.2% (24/262) vs. 1.74% (24/1378), $p < 0.0001$; Hospital B: 5.2% (9/173) vs. 1.89% (17/899), $p = 0.025$; Hospital (A + B): 7.6% (33/435) vs. 1.80% (41/2277), $p < 0.0001$.

[d] Difference in the incidence of severe influenza patients with *Aspergillus* antigenemia among MICU patients (C/E) in 2016 February in comparison to previous 6 months: Hospital A: 4.58% (12/262) vs. 0.07% (1/1378), $p < 0.0001$; Hospital B: 2.89% (5/173) vs. 0.00% (0/899), $p = 0.0001$; Hospital (A + B): 3.9% (17/435) vs. 0.04% (1/2277), $p < 0.0001$.

[e] Difference in the incidence of noninfluenza patients with *Aspergillus* antigenemia among MICU patients (D/E) in 2016 February in comparison to previous 6 months: Hospital A: 4.58% (12/262) vs. 1.67% (23/1378), $p = 0.0028$; Hospital B: 2.31% (4/173) vs. 1.89% (17/899), $p = 0.763$; Hospital (A + B): 3.7% (16/435) vs. 1.93% (44/2277), $p = 0.0315$.

[f] Pearson's correlation between incidence of severe influenza (A/E) and incidence of *Aspergillus* antigenemia (B/E) among MICU patients of Hospital (A + B).

A = number of confirmed cases of severe influenza reported to Taiwan Centers of Disease Control and Prevention (positive for influenza tests); B = number of cases positive for *Aspergillus* galactomannan antigen index for serum; C = number of severe influenza cases positive for *Aspergillus* galactomannan antigen; D = number of noninfluenza cases positive for *Aspergillus* galactomannan antigen; E = number of cases admitted to medical intensive care units; PM = particulate matter.

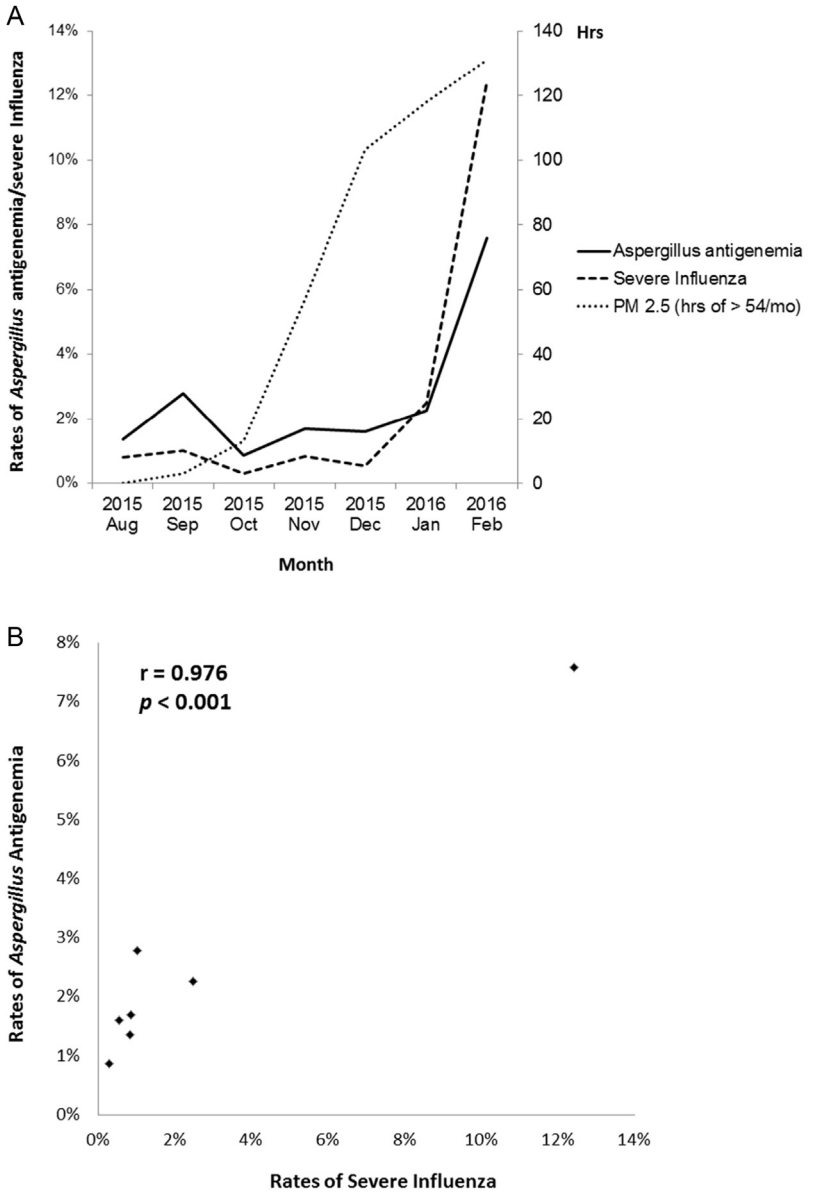

Figure 1. (A) After combining patient data of hospitals A and B, the plot shows the incidences of *Aspergillus* antigenemia and severe influenza among medical ICU patients per month (left vertical axis) and cumulative hours of high-level $PM_{2.5}$ concentrations per month (right vertical axis) in Tainan city, southern Taiwan. (B) Highly positive correlation between the incidence of *Aspergillus* antigenemia (vertical axis) and incidence of severe influenza (horizontal axis) in medical ICU patients is identified. ICU — intensive care unit; PM = particulate matter.

Meanwhile, 17 (31.5%) patients (12 in hospital A and 5 in hospital B) with severe influenza concurrently had *Aspergillus* antigenemia, which was tested on a mean of 8th day (4 patients $\leq$2 days) after admission (Table 2). Among them, nine patients were infected by influenza A(H1N1pdm) virus, eight patients were infected by influenza A (non-H1N1pdm/H3N2) virus. Only six patients received piperacillin-tazobactam before GM testing. Repeated positive *Aspergillus* antigenemia was found in seven patients. Only Patient 10 had positive H1N1 virus culture. Isolation of *Aspergillus* spps. from respiratory specimens was identified in Patients 7 and 15 (Table 2). None of the patients underwent lung biopsy for pathological diagnosis.

Five patients had other coinfections. Patient 5 had pneumonia caused by *Staphylococcus aureus* and *Klebsiella pneumoniae* with concurrent *K. pneumoniae* bacteremia. Patient 8 had pneumonia by *K. pneumoniae*. Others included *Mycobacteriun avium* complex (Patient 1), Herpes simplex virus type-1 (Patient 6), and legionellosis (Patient 13). Five patients had respiratory *Candida* colonization at early presentation (Table 2).

Host factors risk for IPA

With respect to host factors, 10 (58.8%) of 17 patients with severe influenza and IPA received sepsis doses of hydrocortisone (>20 mg/day prednisone equivalent) for a mean of 5 days (range, 2—13 days) prior to GM testing. Of the remaining seven patients, six had immunomodulation

Table 2 Demographic data, host factor, rapid influenza diagnostic tests, polymerase chain reaction assay and viral isolation from throat swab, *Aspergillus* and bacterial isolation from respiratory secretion, blood galactomannan antigen data, and clinical outcome of 17 severe influenza patients concurrently with positive *Aspergillus* antigenemia

Patient	Age / sex	RIDT for Flu (A/B)	Influenza PCR	EBA-based cultures (<7 d)	Host factor (comorbidity)	Steroid use (D)	Pip/Taz use (D)	Isolation of *Aspergillus* from BAL	*Aspergillus* galactomannan index (d)[a]	Death
1	81/F	(−/−)	Other FluA	CA, MAC	Hypertension	No	Yes (5)	Not tested	1.00 (5), 3.18	No
2	64/F	(A/−)	(H1N1)pdm09	No growth	DM	Yes (2)	No	No growth	0.81 (2), 2.46	Yes
3	39/F	(−/−)	Other FluA	CA	No	Yes (13)	No	Not tested	0.60 (24)	No[b]
4	54/F	(A/−)	(H1N1)pdm09	Normal flora	Cervical cancer	Yes (6)	No	Not tested	0.48, 0.68 (6)	Yes
5	52/F	(A/−)	(H1N1)pdm09	KP, SA	ESRD	No	No	Not tested	0.56 (2)	Yes
6	57/M	(−/−)	(H1N1)pdm09[c]	Normal flora	Hepatitis B	Yes (1)	No	Not tested	0.25, 0.50 (10)	No
7	53/M	(−/−)	Other FluA	*Aspergillus spp.*	Liver cirrhosis	No	No	Not tested	>4.43 (1), 3.35	Yes
8	61/M	(−/−)	Other FluA	KP	Prostate cancer	No	No	Not tested	0.70 (7), 0.67	Yes[b]
9	62/F	(A/−)	Other FluA	No growth	DM	Yes (4)	Yes (3)	Not tested	0.95 (6), 0.52	Yes
10	54/M	(A/−)	(H1N1)pdm09[d]	No growth	ESRD	No	Yes (3)	Not tested	0.88 (4), 0.27	No[b]
11	57/M	(−/−)	Other FluA	Normal flora	DM	Yes (5)	Yes (5)	Not tested	>4.43 (7), 1.42	Yes
12	64/M	(A/−)	(H1N1)pdm09	CA	DM	No	No	Not tested	0.88 (1)	Yes
13	44/F	(−/−)	Other FluA[e]	CP	Alcoholism	Yes (6)	No	Not tested	1.36 (8)	Yes
14	70/M	(−/−)	(H1N1)pdm09	CA	COPD	Yes (4)	No	No growth	1.24 (9)	Yes
15	77/F	(−/−)	(H1N1)pdm09	Normal flora	Aplastic anemia	No	Yes (5)	*Aspergillus spp.*	0.50 (9), 1.32	No[b]
16	41/M	(A/B)[f]	Other FluA	No growth	DM	Yes (3)	No	No growth	0.78 (14)	No[b]
17	56/M	(A/−)	(H1N1)pdm09	Normal flora	Tongue cancer	Yes (5)	Yes (11)	No growth	0.55 (14), 0.18	No[b]

[a] Numbers in parenthesis indicate days after admission prior to the first positive data of galactomannan antigen test.
[b] More than 8-week hospitalized days.
[c] Herpes simplex virus type-1 was isolated in nasopharyngeal swab virus culture.
[d] Influenza virus was isolated in nasopharyngeal swab virus culture.
[e] *Legionella* antigen test in a urine sample was positive.
[f] Flu A and Flu B antigen tests were both positive, but only Flu-A PCR was positive.
Patients 1—12: from hospital A; Patients 13—17: from hospital B.
BAL = bronchoalveolar lavage; CA = *Candida albicans*; COPD = chronic obstructive pulmonary disease; CP = *Candida parapsilosis*; D = days of usage prior to performance of positive *Aspergillus* galactomannan antigen test; d = days after admission; DM = diabetes mellitus; EBA: endobronchial aspirates; ESRD = end-stage renal disease; KP = *Klebsiella pneumoniae*; MAC = *Mycobacteriun avium* complex; Other Flu A = influenza A virus different to (H1N1)pdm09 and H3N2; PCR = polymerase chain reaction; Pip/Taz: piperacillin-tazobactam; SA = *Staphylococcus aureus*; RIDT: rapid influenza diagnostic test for Flu A and Flu B antigens.

comorbidities like prostate cancer, end-stage renal disease, decompensated liver cirrhosis, diabetes mellitus, and aplastic anemia (Table 2). The only patient without steroid use and host risk factor was an 81-year-old woman with initial respiratory colonization of *C. albicans* and *M. avium* complex, also indicating the status of immunomodulation (Patient 1).

The noninfluenza patients with IPA were almost hospitalized in medical ICUs, with vary underlying comorbidities, such as hematologic malignancies, solitary cancers, transplant recipients, and chronic obstructive pulmonary disorder. The surgical ICU patients rarely had IPA in the study period.

Clinical features of influenza with *Aspergillus* antigenemia

All 17 patients experienced worsening respiratory failure despite oesltamivir and extensive antibiotic therapy, thus requiring ventilator support. All had abnormal infiltrates on chest X-ray films, and 15 (88.2%) patients manifested with acute respiratory distress syndrome (ARDS). The overall mortality rate was 58.8%. Only four patients did not receive voriconazole therapy (Patients 3, 5, 6, and 13). Five of the seven survived patients required hospitalization for more than 8 weeks, also indicating severe morbidities (Table 2).

Ambient air pollution

Monthly average of ambient air PM_{10} in Taipei (northern Taiwan) was lower than that in Tainan and Kaohsiung (southern Taiwan), with significant correlation with $PM_{2.5}$. The monthly average concentrations of $PM_{2.5}$ in winter were three times higher than those in summer in Tainan and Kaohsiung cities (Table 1). Exposure of high-level $PM_{2.5}$ represented by cumulative hours of $PM_{2.5} \geq 54$ $\mu g/m^3$ per month sharply increased within 2 months prior to the epidemic in Tainan city (Figure 1A).

Hospital architectural construction/renovation

There were no works of architectural construction/renovation at hospitals A and B during the study period. Some architecture constructional works in hospital A started beyond the peak epidemic after March 1, 2016. Besides, the patients in hospital A with dual infections of influenza and IPA were hospitalized in four medical ICUs located at different floors (3F, 5F, 6F, and 8F) without specific predisposition to an environmental area.

Discussion

The EORTC/MSG host criteria were limited to severe immunodeficiency patients but not necessarily to critically ill influenza patients,[10] who, nonetheless, may develop IPA.[1,2] We found 17 severe influenza patients with *Aspergillus* antigenemia, who could be regarded as those with "probable" IPA, if host criteria were redefined in a more expanded way like immunomodulation. They had characteristics of: (1) low isolation rates of influenza virus and *Aspergillus*; (2) higher rate of ARDS and (3) higher proportion of non-H1N1 influenza than previous reports limited to influenza A (H1N1).[1–4]

Atmospheric *Aspergillus* spores significantly increased in the high-ambient PM levels during the Asian sandstorm days in the influenza season at Tainan city.[11] Wu et al[11] confirmed increasing small *Penicillium*/*Aspergillus* particles (2–5 μm in diameter) from a mean of 246.93 spores/m^3 in background days to 974.58 spores/m^3 in sandstorm stays ($p < 0.01$). The lower humidity in sandstorm stays also increased the dispersion of spores in the air when intercontinental dust clouds were coming from China. Furthermore, the concentrations of ambient PM_{10}, $PM_{2.5}$, and influenza A virus were significantly higher during the Asian sandstorm days than during the background days in northern Taiwan.[12] The health effects of PM exposure on Taiwan people with subsequent influenza and IPA have not yet been determined. As four of our patients were positive for *Aspergillus* GM within 2 days of admission, they probably acquired *Aspergillus* and influenza infections outside the hospital. Besides, both the hospitals A and B did not conduct architectural construction/renovation during the study period. These findings might hint physicians to change clinical practice in testing GM as early as in the emergency room for unusually severe influenza patients.

In accordance with our proposed disease transmission model, modified from the description of Garcia-Vidal et al,[5] concurrent severe influenza and *Aspergillus* antigenemia developed after the exposure of people to above high-level $PM_{2.5}$ for more than 100 hours/month in previous 2 months in Tainan. The small *Aspergillus* spores in fine $PM_{2.5}$ air might go directly into the distal lung tissue and can penetrate into the gas-exchange region to cause angioinvasion, thus explaining the difficulty in isolating the *Aspergillus* from upper respiratory secretions, whereas GM could be detected in the blood. This theory might also explain the increasing GM detection in noninfluenza patients after PM exposure. They commonly had other immunocompromised status.

There were few limitations of the study. First, all the probable IPA patients did not undergo lung biopsy as concerning potential pneumothorax in the lung with ARDS. Second, the surveillance culture of environment was not performed. Further air sampling of fungus cultures at the ICUs of hospitals A and B might be conducted to clarify whether there would be possible confounding of environmental contamination or colonization.

In conclusion, we highlight a high-level PM exposure prior to influenza attack with increasing incidence of *Aspergillus* antigenemia. The causal relationship between $PM_{2.5}/PM_{10}$ and patients with influenza/IPA remains weak. Nonetheless, a possible link with recent air pollution might hint physicians to list aspergillosis in the differential diagnosis of pneumonia for the severe influenza patients, especially with life-threatening ARDS, as suggested in a recent report.[13]

Conflicts of interest

All authors declare no potential conflicts of interest or financial support.

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